The Physiology and Control of Re-greening in Spathes of *Zantedeschia*

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Abstract

Spathe re-greening is a primary determinant limiting postharvest quality of *Zantedeschia* Spreng. as a cut flower, pot- or landscape-plant. A treatment that can be utilised by growers to delay re-greening offers potential to improve the postharvest quality and provide a marketing advantage. To achieve this, and develop an understanding of the physiological mechanism of re-greening, this project investigated the changes in colour, levels and types of pigment, and differentiation of plastids in spathe tissue durng re-greening; and how this process was controlled by various factors including fructification, light and various plant hormones (e.g. cytokinin and gibberellin).

In the hybrid 'Best Gold', spathe re-greening was initiated within three days after horticultural harvest-maturity and, within two weeks the whole abaxial surface of the spathe had re-greened. During this period, the adaxial surface did not re-green and remained yellow in colour. The change in colour of the abaxial surface primarily resulted from the accumulation of chlorophyll within the subepidermal layers, as reflected by a strong correlation between the colour coordinate hue angle (H°) and total chlorophyll content in that surface (r = 0.98). Monitoring H° can therefore, be used to evaluate the degree of re-greening for 'Best Gold' without chlorophyll analysis. The content of carotenoid (in particular lutein which was predominant) was comparatively steady during re-greening. From an ultrastructural perspective, spathe re-greening was characterized by redifferentiation of chloroplasts from chromoplasts, as compared with *de novo* synthesis of chloroplasts from proplastids. The redifferentiation of chloroplasts involved thylakoid reformation through multiple

mechanisms. In addition to *de novo* synthesis of thylakoid by invaginations of the inner-envelope membrane, it is likely that the thylakoids were either derived from primary thylakoids or plastoglobuli present in mature chromoplasts.

The occurrence of re-greening in the spathe of both *Zantedeschia aethiopica* and 'Best Gold' following the removal of the spadix prior to pollination, contradicted the hypothesis that re-greening was induced by fructification (Pais and Neves, 1982-1983). Further to this, the occurrence of re-greening in the spathe of 'Best Gold' with a spadix naturally devoid of female flowers, and the re-greening of pigmented leaves devoid of any true flower parts, also contradicted this hypothesis. The current findings therefore, indicate that fructification is not necessarily a prerequisite for induction of re-greening.

In absence of light, no chlorophyll accumulated in spathe tissue of 'Best Gold', but the initial redifferentiation of chloroplasts from chromoplasts, as characterized by the formation of double-membrane lamella, was noted. Without light however, redifferentiation of chloroplasts was not completed. These suggest spathe re-greening requires light for the process to complete, but the onset of re-greening can be induced in darkness. The application of 6-benzylaminopurine (BAP) stimulated re-greening in spathe tissue by enhancing accumulation of carotenoid and chlorophyll, and also stacking of grana. But the response to BAP was dependent on the presence/absence of light, the stage of re-greening, and which surface, i.e. abaxial or adaxial. In contrast, the application of gibberellin (GA_3) retarded formation of double-membrane lamella, and thus delayed the onset of re-greening. Hence, a synergistic effect of BAP and GA_3 in delaying the onset of re-greening was likely to be a result of co-regulation between

BAP-stimulated accumulation of carotenoid and GA_3-stimulated retardation of chloroplast redifferentiation.

By integrating both light and hormonal factors, several methods were tested on the actual horticultural commodity, i.e. flowers (peduncle, spathe and spadix) of 'Best Gold', so as to evaluate their efficacy in delaying re-greening. Pulsing flowers in darkness at 5 ºC for 24-h in a solution containing both GA_3 and BAP, was the most effective treatment in postponing re-greening, resulting in a seven-day delay in re-greening of the spathe.

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Table of Contents

ABSTRACT ..... **I**

ACKNOWLEDGEMENTS ..... **IV**

TABLE OF CONTENTS ..... **VII**

LIST OF FIGURES ..... **XIII**

LIST OF TABLES ..... **XX**

LIST OF ABBREVIATIONS ..... **XXI**

CHAPTER 1 GENERAL INTRODUCTION **1**

1.1 *Zantedeschia* **1**

1.2 Spathe re-greening of *Zantedeschia* **5**

1.3 Re-greening in organs of other plant species **8**

1.4 Greening, de-greening, re-greening and senescence **11**

1.4.1 Plastids 13

1.4.2 Greening: formation of functional chloroplasts 16

1.4.3 De-greening: conversion of chloroplasts to other forms of plastids 18

1.4.4 Re-greening: reformation of functional chloroplasts 21

1.4.5 Senescence: dismantling of plastids 24

1.5 Induction and control of re-greening **25**

1.5.1 Fructification 26

1.5.2 Cytokinins 27

1.5.3 Gibberellins 28

1.5.4 Other plant hormones 29

1.5.5 Sugars 30

1.5.6 Light 31

1.5.7 Other chemical treatments 32

1.6 Measurement of re-greening and human perception 33

1.6.1 What is colour? 33

1.6.2 How do our eyes perceive re-greening? 35

1.6.3 Colour measurement 36

1.6.4 Pigment analysis 39

1.7 Thesis goals and objectives 41

CHAPTER 2 RELATIONSHIP BETWEEN CHANGES IN COLOUR AND PIGMENT CONTENT DURING SPATHE RE-GREENING OF *ZANTEDESCHIA* 'BEST GOLD' 43

2.1 Introduction 43

2.2 Materials and Methods 46

2.2.1 Plant materials and handling 46

2.2.2 Experimental design and data analysis 47

2.2.3 Colour measurement 48

2.2.4 Pigment analysis 48

2.2.5 Histology 48

2.3 Results 49

2.3.1 Change in pigment distribution 49

2.3.2 Change in colour 50

2.3.3 Change in pigment content 50

2.3.4 Correlations between lightness, chroma and hue angle with pigment content ... 54

2.4 Discussion 60

2.5 Conclusion 65

CHAPTER 3 PATTERN OF RE-GREENING IN THE SPATHE OF *ZANTEDESCHIA* 'BEST GOLD' 66

3.1 Introduction 66

3.2 Materials and methods 68

3.2.1 Plant materials and handling 68

3.2.2 Disc versus spathe 69

3.2.3 Pattern of re-greening 69

3.2.4 Pigment analysis 70

3.2.5 Histology 75

3.2.6 Data analysis 76

3.3 Results 77

3.3.1 Disc versus spathe 77

3.3.2 Pattern of re-greening 77

3.3.3 Pigment profile 80

3.3.4 Pigment quantity 85

3.3.5 Plastid redifferentiation 87

3.4 Discussion 90

3.5 Conclusion 95

CHAPTER 4 INFLUENCE OF FRUCTIFICATION AND CYTOKININ ON SPATHE RE-GREENING: COMPARISON OF WINTER- AND SUMMER-FLOWERING SPECIES OF *ZANTEDESCHIA* 96

4.1 Introduction 96

4.2 Materials and Methods 98

4.2.1 Fructification 98

4.2.2 Application of cytokinin 101

4.2.3 Pigment analysis 102

4.2.4 Statistical analysis 102

4.3 Results 102

4.3.1 Presence / absence of spadix 102

4.3.2 Presence / absence of female flowers ('Best Gold' only) 106

4.3.3 Re-greening of yellow-pigmented leaves ('Best Gold' only) 106

4.3.4 Pigment profile 106

4.3.5 Application of cytokinin 108

4.4 Discussion 112

4.5 Conclusion 117

CHAPTER 5 PRELIMINARY SCREENING OF PLANT HORMONES AND SUCROSE ON ALTERING RE-GREENING IN *ZANTEDESCHIA* 'BEST GOLD' 119

5.1 Introduction 119

5.2 Materials and Methods 122

5.2.1 Plant materials and handling 122

5.2.2 Treatments, experimental design and statistical analysis 123

5.2.3 Preparation of treatment solutions 125

5.3 Results 126

5.3.1 Change in spathe colour in control treatment 126

5.3.2 Cytokinins and changes in spathe colour 127

5.3.3 GA_3 and changes in spathe colour 131

5.3.4 NAA and changes in spathe colour 132

5.3.5 Sucrose supplementation and changes in spathe colour 132

5.3.6 Combination of GA_3 and BAP and changes in spathe colour 133

5.4 Discussion 136

5.5 Conclusion 141

CHAPTER 6 ULTRASTRUCTURAL AND PIGMENT CHANGES RESULTING FROM CYTOKININ, GIBBERELLIN, LIGHT, AND THEIR COMBINATION, DURING SPATHE RE-GREENING OF *ZANTEDESCHIA* 143

6.1 Introduction 143

6.2 Materials and Methods 145

6.2.1 Pigment accumulation 146

6.2.2 Histology 148

6.3 Results 149

6.3.1 Colour changes 149

6.3.2 Changes of pigment content in light 154

6.3.3 Changes of pigment content in darkness 160

6.3.4 Plastid differentiation in light 162

6.3.5 Plastid differentiation in darkness 170

6.4 Discussion 178

6.4.1 Initial differentiation of chloroplasts from chromoplasts is light-independent. . 178

6.4.2 Stimulation of re-greening by BAP 180

6.4.3 Gibberellin temporarily delays re-greening 185

6.4.4 Combination of cytokinin, gibberellin and darkness offers potential in postponing re-greening 186

6.4.5 Re-greening in spathe tissue: some insights into thylakoid reorganization 189

6.5 Conclusion 196

CHAPTER 7 HORTICULTURAL EVALUATION OF TREATMENTS TO POSTPONE SPATHE RE-GREENING IN *ZANTEDESCHIA* 'BEST GOLD' 199

7.1 Introduction 199

7.2 Materials and Methods 201

7.2.1 Plant materials and handling 201

7.2.2 Treatments, experiment design and statistical analysis 202

7.3 Results 204

7.3.1 Spray versus vase solution 204

7.3.2 Pulsing versus vase solution 206

7.4 Discussion 209

7.5 Conclusion 213

CHAPTER 8 GENERAL DISCUSSION 215

8.1 What is re-greening about? 215

8.2 Why does the spathe re-green? 220

8.3 How re-greening is induced and controlled? 221

8.4 Horticultural application 226

APPENDIX I 228

APPENDIX II 242

REFERENCES 243

List of figures

Figure 1.1 Inflorescence of *Zantedeschia* 'Best Gold' (left A, C & E) and *Zantedeschia aethiopica* (B, D, & F). A & B: a leaf-like spathe encloses a spadix; C & D, female and male flowers congregate on the spadix; E: fully re-greened spathe of 'Best Gold' embraces enlarging fruits (not visible); F: fully re-greened spathe of *Z. aethiopica* with upper part of spathe withered while the lower part embraces the enlarging fruits. 4

Figure 1.2 Left to right, within approximately a five-week timeframe: five progressive developmental stages of the spathe in *Zantedeschia* 'Best Gold' from immature green bud through to fully re-greened and enrolled spathe. 7

Figure 1.3 Interrelationship between different forms of plastids in higher plants and their association with some developmental processes including: greening, de-greening, re-greening and senescence. Note that this diagram is not comprehensive, as only the forms of plastids that relate to the current research have been included.....12

Figure 1.4 CIE LAB colour space. Adapted from SpectraMagic^{TM} NX (Konica Minolta, Japan). L*: lightness; C*: chroma; H$^{\circ}$: hue angle.....38

Figure 2.1 Pigment distribution on both abaxial (right side of section) and adaxial (left side of section) surfaces of the spathe of *Zantedeschia* 'Best Gold' at, A (day 0) and B (day 7) under bright-field of light microscope; C (day 0) and D (day 7) under confocal microscope. Red colour indicates chlorophyll autofluoresence. E (day 0) and F (day 7) are enlargements of the abaxial surface. Scale bars = 100 µm. 52

Figure 2.2 Colour coordinates (lightness, chroma, and hue angle) and content of pigments (chlorophyll *a*, chlorophyll *b* and total carotenoid) of spathe tissue of *Zantedeschia* 'Best Gold' during 14 days after horticultural harvest-maturity. The line bars represent the LSD at $P < 0.05$. n=3. 53

Figure 2.3 Content of chlorophyll *a*, chlorophyll *b*, or total carotenoid (µg·g^{-1} DW) versus lightness, chroma, and hue angle of both abaxial and adaxial surfaces of the spathe of *Zantedeschia* 'Best Gold' over a 14-day period from horticultural harvest-maturity.....56

Figure 2.4 Content of chlorophyll *a*, chlorophyll *b*, or total carotenoid (μg·cm^{-2}) versus lightness, chroma, and hue angle of both abaxial and adaxial surfaces of spathes of *Zantedeschia* 'Best Gold' over a 14-day period from horticultural harvest-maturity. 57

Figure 2.5 Relationships between the content of chlorophyll *a*, chlorophyll *b* or total carotenoid with, hue angle or chroma, for the abaxial surface of the spathe of *Zantedeschia* 'Best Gold' over a 14-day period from horticultural harvest-maturity. 59

Figure 2.6 Relationship between the content of total carotenoid with chroma for the adaxial surface of the spathe of *Zantedeschia* 'Best Gold' over a 14-day period from horticultural harvest-maturity. 59

Figure 3.1 Positions on the spathe of 'Best Gold' where discs were sampled. 72

Figure 3.2 Hue angle of detached entire spathe and discs of spathe tissue of *Zantedeschia* 'Best Gold'. Measurements at the same position from the abaxial surface, i.e. central left and right (refer Figure 3.1). Each point is mean ± se of four replicates.....79

Figure 3.3 Hue angle of both abaxial (A & B) and adaxial (C & D) surfaces of discs of spathe tissue excised from left (A & C) and right (B & D) sides of the midrib at basal, central, central-outer and upper positions of spathe (positions refer Figure 3.1). Vertical bars represent LSD at $P < 0.05$ (n = 4). 79

Figure 3.4 Pigments in the abaxial surface of discs of spathe tissue of *Zantedeschia* 'Best Gold' at horticultural harvest-maturity (day 0), day 7 and day 14, as well as the mature leaf of 'Best Gold' were separated by thin layer chromatography. The putatively identified pigments were as listed in Table 3.2. 81

Figure 3.5 Pigments in the abaxial surface of spathe tissue and leaf of *Zantedeschia* 'Best Gold' were co-eluted with standards of β-carotene, lutein and zeaxanthin by thin layer chromatography. 82

Figure 3.6 HPLC chromatogram traces of pigments (right column) from discs of spathe tissue of *Zantedeschia* 'Best Gold' (left column) on day 0 (A: abaxial; C: adaxial) and day 14 (B: abaxial; D: adaxial). Peaks: 1, neoxanthin; 2, violaxanthin; 3, unknown; 4, lutein; 5, 8-apo-carotenal; 6, chlorophyll *b*; 7, α-carotene; 8, chlorophyll *a*; 9, β-carotene; 10, *cis*-β-carotene. 84

Figure 3.7 Content of pigments present in the abaxial surface of detached entire spathe or discs of spathe tissue of *Zantedeschia* 'Best Gold' during 14 days after horticultural harvest-maturity. Vertical bars represent the LSD at $P < 0.05$, n=4. 86

Figure 3.8 Electron micrographs of plastids in subepidermal cell layers of both abaxial and adaxial surfaces at different stages of spathe development in *Zantedeschia* 'Best Gold'. A) Chloroplast present in the abaxial surface of spathe at green-bud stage, showing well-stacked grana and a few plastoglobuli; B) Chromoplast appears in the abaxial surface of the yellow spathe at horticultural harvest-maturity; C) Chloroplast (right) and an intermediate stage of plastid (left) between chloroplast and chromoplast were observed in abaxial surface of the detached entire spathe at day 14; D) Amyloplast present in the adaxial surface of the spathe at green-bud stage; E) Chromoplast found in the adaxial surface of the yellow spathe at horticultural harvest-maturity; F) Plastid remain in chromoplast-form in the adaxial surface of detached entire spathe at day 14. Bar = 1 µm. *G*, grana; *P*, plastoglobuli; *S*, starch grain. 89

Figure 4.1 Re-greening of the spathe of *Zantedeschia aethiopica* (top) and *Zantedeschia* 'Best Gold' (bottom). Flowers were harvested at horticultural harvest-maturity (day 0), i.e., spathe fully open and prior to pollen release. 104

Figure 4.2 Colour coordinates of lightness, chroma, and hue angle measured at the basal and central positions of the spathes of *Zantedeschia aethiopica* (left) or at the central position of *Zantedeschia* 'Best Gold' (right) with either the spadix removed (open symbols) or attached (solid symbols). Flowers were harvested at horticultural harvest-maturity. n=4, vertical bars = Standard error. 105

Figure 4.3 Changes in pigmentation in adaxial and abaxial surfaces of yellow-pigmented leaves of *Zantedeschia* 'Best Gold'. 107

Figure 4.4 Discs of tissue of *Zantedeschia aethiopica* from basal, central and upper positions of the spathe, when treated with either RO water (control) or BAP at 10^{-4} M. 110

Figure 4.5 Influence of BAP on colour coordinates of lightness, chroma, and hue angle of the abaxial surface of discs of *Zantedeschia aethiopica* (left) and *Zantedeschia* 'Best Gold' (right). Discs were sampled from basal and central positions of the spathe for stems harvested at horticultural harvest-maturity. For *Z. aethiopica*, colour measurement

stopped when discs turned brown in the control treatment after day 5 (central) and day 7 (basal). Vertical bars represent LSD at *P* < 0.05 (n = 4). 111

Figure 5.1 Discs of spathe tissue of *Zantedeschia* 'Best Gold' treated with either BAP, zeatin, CPPU, or GA_3 at a concentration of 10^{-4} M after 11 and 21 days. Control discs were held in RO water. 128

Figure 5.2 Lightness, chroma and hue angle of the abaxial surface of the spathe of *Zantedeschia* 'Best Gold' treated with: BAP, zeatin, CPPU, or GA_3. Only the data pertaining to the control and 10^{-4} M of each compound are presented. Vertical bars represent LSD at *P* < 0.05 (n = 4). Controls were held in RO water. 129

Figure 5.3 Hue angle of the abaxial surface of the spathe of *Zantedeschia* 'Best Gold' treated with BAP at 10^{-8} M, 10^{-6} M or 10^{-4} M. Vertical bar represents LSD (1.49º) at *P* < 0.05 (n = 4). Controls were held in RO water. 130

Figure 5.4 Hue angle of the abaxial surface of spathe tissue of *Zantedeschia* 'Best Gold', treated with either RO water (control) or sucrose at 1.5×10^{-3} M. LSD = 3.59º at *P* < 0.05 (n = 4). 134

Figure 5.5 Hue angle of the abaxial surface of spathe tissue of *Zantedeschia* 'Best Gold', simultaneously treated with GA_3 and BAP at ratios of GA_3:BAP (v:v) of 1:0, 2:1, 1:2, 1:1 or 0:1. LSD = 3.59º at *P* < 0.05 (n = 4). Controls were held in RO water. 135

Figure 5.6 The abaxial surface of discs from the spathe of *Zantedeschia* 'Best Gold' treated with sucrose (1.5×10^{-3} M) or combinations of GA_3 and BAP in ratios of 1:0, 2:1, 1:2, 1:1 or 0:1 (v:v). Control discs were held in RO water. 135

Figure 6.1 Colour coordinates of lightness (A & B), chroma (C & D) and hue angle (E & F) of both abaxial (A, C & E) and adaxial (B, D & F) surfaces of *Zantedeschia* 'Best Gold' spathe tissue, treated with BAP, GA_3 or GA_3+BAP in light. Controls were held in RO water. Each point is the mean of 5 replicates. Vertical line bars are the LSD at *P* < 0.05. 152

Figure 6.2 Colour coordinates of lightness (A & B), chroma (C & D) and hue angle (E & F) of both abaxial (A, C & E) and adaxial (B, D & F) surfaces of *Zantedeschia* 'Best Gold' spathe tissue, treated with BAP, GA_3 or GA_3+BAP in darkness. Controls were held in RO water. Each point is the mean of 5 replicates. Vertical line bars are the LSD at *P* < 0.05. 153

Figure 6.3 Content of chlorophylls and carotenoids in the ABAXIAL surface of the spathe of *Zantedeschia* 'Best Gold', treated with BAP, GA_3 or GA_3+BAP in light over 14 days after horticultural harvest-maturity. Controls were kept in RO water. Each point is the mean of five replicates. Vertical line bars represent the LSD at $P < 0.05$. 158

Figure 6.4 Content of chlorophyll *a* and carotenoids in the ADAXIAL surface of the spathe of *Zantedeschia* 'Best Gold', treated with BAP, GA_3 or GA_3+BAP in light over 14 days after horticultural harvest-maturity. Controls were kept in RO water. Each point is the mean of five replicates. Vertical line bars represent the LSD at $P < 0.05$. 159

Figure 6.5 Content of carotenoids on the ABAXIAL surface of spathes of *Zantedeschia* 'Best Gold', treated with BAP and/or GA_3 in darkness over 14 days after horticultural harvest-maturity. Controls were kept in RO water. Each point is the mean of five replicates. Vertical line bars are the LSD at $P < 0.05$..... 161

Figure 6.6 Transmission electron micrographs of plastids in subepidermal cell layers of abaxial surface of *Zantedeschia* 'Best Gold' at horticultural harvest-maturity (day 0). A) An oval-shaped chromoplast showing large, irregular size of plastoglobuli and swollen (type-2) thylakoids (dashed arrow); B) round-shaped chromoplast showing both flattened-double-membrane (type-1) thylakoids (solid arrow) and type-4 thylakoid remnants (block arrow); C) round-shaped chromoplast showing a single-membrane-bound body (type-3 thylakoid; arrow head); D) shoe-shaped chromoplast showing the development of both type-1 and type-2 thylakoid membrane. P, plastoglobuli; S, starch grain. 165

Figure 6.7 Influence of GA_3 and/or BAP in light on the differentiation of plastids within the abaxial surface of spathes in *Zantedeschia* 'Best Gold'. A) day 7, control: chromoplast to chloroplast transition indicated by the various intermediate stages of plastids between chromoplasts and chloroplasts; B) day 10, control: a more developed chloroplast containing some grana stacking; C) day 14, control: a fully developed chloroplast with well-organized thylakoid system; D) day 7, GA_3: plastids mainly occupied by type-3 thylakoids (arrow head) and plastoglobuli; E) day 10, GA_3: similar to B; F) day 14, GA_3: a fully developed chloroplast; G) day 7, BAP: a developing chloroplast characterized by the formation of type-1 thylakoids (solid arrow), type-3 thylakoids and clustered plastoglobuli; H) day 10, BAP: similar to B and E; I) day 14, BAP: a fully developed

chloroplast with an intensive thylakoid membrane system; J) day 7, GA_3+BAP: chromoplasts showing an initial stage of chloroplast differentiation with formation of type-1 thylakoids (solid arrow) and retention of plastoglobuli; K) day 10, GA_3+BAP: chromoplast with a decline in plastoglobuli and increase in type-1 and type-2 thylakoids; L) day 14, GA_3+BAP: various stages of plastids between chromoplasts and chloroplasts. *P*, plastoglobuli; *G*, grana; *S*, starch. 166

Figure 6.8 Influence of BAP in light on the differentiation of plastids within the adaxial surface of spathes in *Zantedeschia* 'Best Gold'. A) day 7, control: a typical chromoplast containing large and irregular-shaped plastoglobuli; B) day 10, control: chromoplast; C) day 14, control: chromoplast; D) day 7, BAP: elongated chromoplast; E) day 10, BAP: initial conversion of chromoplast to chloroplast showing the formation of thylakoids (type-1) and grana stacking; F) day 14, BAP: plastids showing less thylakoids than those at day 10, BAP. *P*, plastoglobuli; *G*, grana..... 167

Figure 6.9 Influence of GA_3 and/or BAP in darkness on the differentiation of plastids within the ABAXIAL surface of the spathe in *Zantedeschia* 'Best Gold'. A) day 7, control: initial differentiation of chromoplasts into chloroplasts with the appearance of type-1 thylakoids (solid arrow); B) day 10, control: a further differentiated plastid from chromoplast to chloroplast containing both type-1 and type-3 thylakoids; C) day 14, control: a plastid showing signs of degradation; D) day 7, GA_3: plastids mainly occupied by type-3 thylakoids (arrow head) and plastoglobuli (*P)*; E) day 10, GA_3: appearance of small type-3 and a tubular complex of thylakoids (asterisk); F) day 14, GA_3: a partially differentiated chloroplast, but with no grana stacking; G) day 7, BAP: accumulation of large starch granules and type-3 thylakoids; H) day 10, BAP: initial differentiation of chloroplasts coexisting with a prolamellar-body-like structure (4-point star); I) day 14, BAP: similar to H; J) day 7, GA_3+BAP: plastid remains as chromoplast occupied by large starch granules; K) day 10, GA_3+BAP: chromoplast with disappearance of starch; L) day 14, GA_3+BAP: chromoplast showing less electron-dense plastoglobuli and stroma. *P*, plastoglobuli; *G*, grana; *S*, starch. 171

Figure 6.10 Ultrastructural characteristics of plastids in the abaxial surface of *Zantedeschia* 'Best Gold' spathe tissue during re-greening. A) double-membrane primary thylakoids

(type-1) showing a close connection with plastoglobuli (solid arrows). Plastoglobuli were inter-connected with each other via direct adhesion or some tubular extension (arrow heads); B) primary thylakoids (type-1 and type-2) showing continuity with inner-envelope membrane (dashed arrows); C) an enlarged image of a tubular complex, demonstrating the possible origin of type-1 thylakoids from the extension of type-3 thylakoids (asterisk); D) an enlarged image of a prolamellar-body-like structure observed only in the BAP-treated tissue in darkness; E) thylakoid remnants (type-4 thylakoids) with close contact with type-3 thylakoids and the inner-envelope membrane (block arrows); F) type-3 thylakoids were divided and fused with type-1 to form grana (arrows). *P*, plastoglobuli. 173

Figure 6.11 Diagrammatic illustration of possible processes by which type-1 thylakoids are formed during the plastid differentiation from chromoplast to chloroplast. The number indicates the process by which type-1 thylakoids are possibly formed (for descriptive details refer Section 6.4.5) 195

Figure 6.12 Model for the mechanism of light-, cytokinin-, and gibberellin-mediated spathe re-greening of *Zantedeschia* 'Best Gold'. CK: cytokinin; GA: gibberellin. 198

Figure 7.1 Colour coordinates of lightness, chroma and hue angle for the spathe of *Zantedeschia* 'Best Gold', treated with GA_3+BAP either as a spray or vase solution. Controls were held in RO water. Vertical bars represent LSD at $P < 0.05$ (n = 4). 205

Figure 7.2 Colour coordinates of lightness, chroma and hue angle for the spathe of *Zantedeschia* 'Best Gold', treated with GA_3+BAP either as a 24-h pulse in darkness or a continuous vase solution. The dark-control comprised a 24-h pulse in RO water in darkness. The control was held in RO water in light. Vertical bars represent LSD at $P < 0.05$ (n = 4). 207

Figure 7.3 Spathes of *Zantedeschia* 'Best Gold' under various treatments (as illustrated in Figure 7.2). Photos taken at day 9 (left) and day 15 (right) after harvest. 208

Figure 8.1 Conceptual model for the physiological mechanism of light-, cytokinin-, and gibberellin-mediated re-greening of mature spathes of *Zantedeschia* 'Best Gold'. CK: cytokinin; GA: gibberellin. 225

List of tables

Table 2.1 Pearson correlation coefficient (r), and probability level, between lightness (L*), chroma (C*), or hue angle (Hº) and chlorophyll a, chlorophyll b, total chlorophyll or total carotenoid, for both the abaxial and adaxial surfaces of *Zantedeschia* 'Best Gold', on both the basis of per unit dry weight (µg·g^{-1} Dry Weight) and per unit area (µg·cm^{-2}). 58

Table 3.1. Step gradient for HPLC analysis. Solvent A (Acetonitrile: Methanol: Tris-HCL buffer; 72:8:3; v:v:v); Solvent B (Methanol: Ethyl acetate; 68:32; v:v). 74

Table 3.2 Elution order and absorption characteristics of pigments from discs of spathe tissue of *Zantedeschia* 'Best Gold' encompassing the 14-day period from horticultural harvest-maturity through re-greening 83

Table 4.1 Pigment content (µg·g^{-1} dry weight) of spathe tissue in *Zantedeschia aethiopica* or *Zantedeschia* 'Best Gold' at horticultural harvest-maturity (day 0) and when re-greened (day 14). Each value is the mean of three measurements, SE = standard error. 107

List of abbreviations

ABA	Abscisic acid
ANOVA	Analysis of variance
BAP	6-benzylaminopurine
C*	Chroma
CPPU	N_1-(2-chloro-4-pyridyl)-N_3-phenylurea
cv.	Cultivar
DW	Dry weight
GA_n	$Gibberellin_n$ – denotes the number
H°	Hue angle
HPLC	High performance liquid chromatography
IAA	Indolylacetic acid
L*	Lightness
LSD	Least significant difference
NAA	1-naphthaleneacetic acid
POR	Protochlorophyllide reductase
RO	Reverse osmosis
SAS	SAS system for statistical analysis
SE	Standard error
TEM	Transmision electron microscopy
TLC	Thin layer chromatography
Type-1	Flattened, double-membrane lamella
Type-2	Swollen membrane thylakoids
Type-3	Vacuole-like, single-membrane-bound bodies

Type-4	Clusters of membrane fragments
10^{-2} M	1×10^{-2} M
10^{-4} M	1×10^{-4} M
10^{-5} M	1×10^{-5} M
10^{-6} M	1×10^{-6} M
10^{-8} M	1×10^{-8} M