

Copyright is owned by the Author of the thesis. Permission is given for a copy to be downloaded by an individual for the purpose of research and private study only. The thesis may not be reproduced elsewhere without the permission of the Author.

**Strigolactones and hormonal interaction in control of
branching in *Zantedeschia* and other horticultural
species**

A thesis presented in partial fulfillment of the requirements
for the degree of

Doctor of Philosophy in Plant Science

at Massey University, Palmerston North,
New Zealand



Sarina Manandhar

2016

Abstract

Shoot branching that involves development of lateral buds into shoots is one of the important factors influencing crop productivity. Strigolactones have recently been found to be involved in the control of branching, but the actual bioactive compound/s that inhibits bud outgrowth is still unknown. A germination assay utilizing the seeds of a parasitic weed (*Orobanche minor*), detected strigolactones within the xylem exudates of different horticultural crop species; the strigolactone concentration negatively correlated with branching of cultivars or mutants. In *Zantedeschia* grown *in vivo*, the concentration of strigolactones was independent on the volume of guttation fluid (xylem exudates) suggesting the difference in concentration of strigolactones in high and low branched cultivars was due to the difference in potential of producing strigolactones between these cultivars and not due to differences in volume of guttation fluid. While identifying a bioactive compound using germination and branching assays in combination with liquid chromatography and mass spectrometry, compounds containing 'N' were detected in the low branched wild-type *Petunia*, but not in the highly branched mutant, suggesting the possibility of such compounds being SL-conjugates which may be associated with bud outgrowth inhibition.

In *Zantedeschia* (*in vitro*) and pea stems, strigolactone reduced the axillary shoot number stimulated by the cytokinin suggesting an antagonistic interaction between these two hormones on bud release. However, as cytokinin may stimulate subsequent growth of released buds by increasing the auxin transport out of the bud, strigolactone may have reduced subsequent growth by reducing auxin transport. Since GA₃ enhanced subsequent growth of buds in pea stems, but not the release, an antagonistic interaction between strigolactone and gibberellins on subsequent growth is possible. Interestingly, strigolactone successfully reduced adventitious bud formation in *Zantedeschia* grown *in vitro*, adding a new role for strigolactones in plant development.

Despite correlation between strigolactone and branching inhibition in different horticultural crops such as apple, kiwifruit, *Zantedeschia* and *Acer*, further studies relating to strigolactone and its interaction with other hormones on branching of these crops could be performed using *in vitro* techniques for a clear understanding of strigolactones' role on branching inhibition. More importantly, quantification of strigolactones using the germination assay may have significant implications in horticultural crop breeding for obtaining desired shoot branching.

Extended abstract

Shoot branching, one of the important factors influencing crop productivity, involves development of lateral buds into shoots on an actively growing primary shoot. Recently, a new hormone, which may be a strigolactone, has been found that inhibits bud outgrowth, however, the precise chemical identity of the bioactive compound(s) is unknown. A bioassay based on the germination of a parasitic weed (*Orobanche minor*) was optimized to detect strigolactones. Although there has been controversy in the literature related to whether or not strigolactones are present in xylem exudates, in this thesis strigolactones were found in xylem exudates of a range of horticultural species. The strigolactone concentration correlated with branching of cultivars or mutants mainly at the stage of the growth cycle before the branches were visually evident.

As the germination assay detects all/most strigolactones, not necessarily specifically those associated with branching, a more specific bioassay based on branching was developed. This bioassay was combined with liquid chromatography and mass spectrometry in an attempt to identify a specific branching hormone, whether or not this was a strigolactone. In *Petunia*, four compounds containing 'N' were detected in the xylem sap of the wild-type, low branched, V26, but not in highly branched *dad3* mutant, suggesting the possibility of such compounds being SL-conjugates which may be associated with branching inhibition. In *Zantedeschia* grown *in vivo*, since the concentration of strigolactones was independent of the volume of guttation fluid, it was suggested that difference in concentration of strigolactones in high and low branched cultivar was due to the difference in potential of producing strigolactones between these cultivars and not due to differences in volume of guttation fluid.

The synthetic strigolactone GR24 (0.1 or 1 mg L⁻¹) was able to reduce axillary shoot number stimulated by cytokinin in un-decapitated pea stems, and *Zantedeschia* grown *in vitro*, suggesting an antagonistic interaction between these two hormones on bud release, as opposed to subsequent growth. Likewise, strigolactone (1 mg L⁻¹) reduced decapitation-induced bud release, supporting the hypothesis that strigolactone may have interacted with endogenous cytokinin and/or sucrose. Strigolactone was also able to

reduce subsequent growth of the shoot, but the effect was stronger in buds of pea stems orientated horizontally, compared to those orientated vertically. Such disparity was possibly due to the fact that, in addition to vascular stream, the buds of horizontally orientated stems received strigolactones directly. Although exogenously applied cytokinin appeared to enhance subsequent growth of the released bud, the effect of cytokinin on subsequent growth may be via increasing the auxin transport out of the bud. Hence, rather than interacting with cytokinin, strigolactone may have reduced subsequent growth of the buds by reducing auxin transport. As GA₃ enhanced the subsequent growth of buds in pea stems, but not the release, an antagonistic interaction between strigolactone and gibberellins on subsequent growth is considered highly likely. Interestingly, strigolactone successfully reduced cytokinin-stimulated adventitious bud formation in *Zantedeschia* grown *in vitro*. Interaction studies of strigolactone with cytokinin, and probably ethylene, is recommended within highly branched cultivars of *Zantedeschia* spp. or other species, such as gentians and kiwifruit, to further explore the role of strigolactone in adventitious bud formation and development in order to obtain desirable shoots for commercial purposes.

Although strigolactone correlated with branching inhibition in different horticultural crops such as apple, kiwifruit, *Zantedeschia* and *Acer*, further studies for answering the direct role of strigolactone on bud outgrowth in these crops as well as its interaction with other hormones can be performed using *in vitro* techniques. More importantly, quantification of strigolactones using the germination assay may have significant implications in horticultural crop breeding for obtaining desired shoot branching. Since guttation fluid from *Zantedeschia* was found to give a true estimate of the concentration of strigolactones present in the xylem of the shoot system, future experiments may benefit through the use of guttation fluid for hormonal analysis and/or interaction studies *in vivo*. Consideration of the stages of shoot branching during such studies would be valuable for a clear understanding of the shoot branching mechanism and help modify the branching of commercially important crops.

Acknowledgments

Foremost, I would like to express my sincere gratitude to my chief supervisor **Dr David Woolley** for the continuous support of my PhD study and research; for his patience, motivation, enthusiasm, and immense knowledge. His guidance helped me during the research and writing of this thesis. His unending encouragement, constructive criticism throughout the course of my PhD as well as his kindness to understand me is highly appreciated.

Thanks to my co-supervisor Dr Keith Funnell for helping me learn technical things right from the beginning of my research until end of my thesis write-up. Your remarkable quality of planning, organizing and implementing things is really appreciated. Your constructive feedback and suggestions helped me drive towards achieving perfection.

I would like to extend my thanks to staff of the IAE, as well as at the Plant Growth Unit; especially Chris, Kay, James and Lesley for their technical help and moral support. Kay, also thank you for your advice when dealing with the health of my son.

I wish to acknowledge the staff of Plant & Food Research, especially Andrew Mullan for helping me prepare media during tissue culture experiments, Maree Debenham for teaching me skills associated with tissue culture, and Duncan Hedderley for statistical help while analyzing data. I am also grateful to Revel Drummond for the supply of petunia seed and helping me learn sample extraction in petunia plants. My sincere thanks go to Dr Tony McGhie and Dr Daryl Rowan for assisting me in learning LC/MS and also thanks to Dr. Janney Cooney and her team for analyzing cytokinins and strigolactones through LC/MS.

I would particularly like to acknowledge the financial support from a Massey University Doctoral Scholarship, without which I my PhD would not have run as smoothly. I am also grateful for the financial support covering the research costs provided by The New Zealand Institute for Plant & Food Research Limited Core funding: 1198 – “Fashionable Plants for the Ornamentals Industry”, 1972 – “Breeding Technology Development”, as well as both the Helen E. Akers PhD scholarship and Johannes Anderson PhD scholarship.

Special thanks to Marzieh, Fadhil and my senior colleagues for their help, sharing ideas and creating a friendly environment during the period of study. This thesis would be incomplete if I do not mention the help and support of dear friends Rachael, Mahesh, Bhawana, Rajiv, Srijan and my entire circle of friends in Palmerston North. Thank you so much my dear sisters Jamuna and Saraswati for looking after my son during such a busy time.

Most importantly, I would like to express my deep gratitude to my husband Bishad for your many sacrifices, constant support and encouragement. My son Aaron, his innocent words and funny activities were a tonic to recover from having a frustrated mind. My heart-felt thanks to both of you boys. At last, my mother, brothers, sisters, and in-laws, who are far from me; thank you so much for your constant support.

Table of contents

Abstract.....	i
Extended abstract.....	iii
Acknowledgments.....	v
List of Figures.....	xv
List of Tables.....	xxvi
List of Abbreviations.....	xxviii
1 General Introduction	1
1.1 Importance of research on architecture of horticultural crops	1
1.2 Shoot branching.....	3
1.3 Strigolactones	5
1.3.1 Strigolactones and branching	6
1.3.2 Biosynthetic and transduction pathway of strigolactones.....	11
1.3.3 Relationship between strigolactones and auxin	13
1.3.3.1 Auxin is required for biosynthesis of SLs.....	13
1.3.3.2 Auxin may not be involved in action of SLs.....	13
1.4 Theories related to the mechanism of apical dominance and/or shoot branching.....	13
1.4.1 Nutritive theory	14
1.4.2 Direct auxin action theory.....	14
1.4.3 Indirect auxin action theory.....	15
1.4.3.1 Hormonal interaction theory	15
1.4.3.2 Auxin transport theory	23
1.4.4 Non hormonal / sucrose theory	27
1.5 General morphology and growth habit of different horticultural plants in relation to branching	28
1.5.1 <i>Zantedeschia</i>	29
1.5.2 <i>Petunia</i>	30

1.5.3	Woody perennials.....	32
1.5.3.1	Apple.....	33
1.5.3.2	Kiwifruit.....	33
1.5.3.3	Japanese Maple	34
1.6	<i>Orobanch</i> e: Life cycle and host interaction	35
1.6.1	Life cycle of <i>Orobanch</i> e spp.....	36
1.6.2	Germination stimulants	37
1.7	Summary, rationale and thesis objectives	40
2	Germination assays using seeds of <i>Orobanch</i>e <i>minor</i> for detection of strigolactones	43
2.1	Introduction.....	43
2.2	Materials and methods	46
2.2.1	General methods - germination assay	47
2.2.2	Experiment One- Dose response of <i>O. minor</i> seeds to GR24.....	48
2.2.2.1	Preparation of GR24 solutions.....	48
2.2.2.2	GR24 treatments	48
2.2.2.3	Microscopic observations and data analysis	49
2.2.3	Experiment Two- Existence of strigolactones in different horticultural species	49
2.2.3.1	Plant selection	49
2.2.3.2	Plant growing conditions and sample collection.....	50
2.2.3.3	Treatments and experimental design.....	55
2.2.3.4	Microscopic observations and data analysis	56
2.2.3.5	Quantification of endogenous SLs in <i>Zantedeschia</i> (Year 1 samples)	56
2.3	Results and discussion.....	56
2.3.1	Standardized dose response curve.....	56
2.3.2	Strigolactones in different horticultural species.....	58

2.3.2.1	<i>Zantedeschia</i>	58
2.3.2.2	<i>Petunia</i>	62
2.3.2.3	<i>Acer</i> , apple and kiwifruit.....	64
2.3.3	Radicle length.....	69
2.4	Conclusion.....	72
3	Development of methods to detect a specific branch inhibiting hormone	73
3.1	Introduction	73
3.2	Materials and methods	75
3.2.1	Experiment One - Dose response of a branching assay of pea seedlings to GR24.....	75
3.2.1.1	Plant material and growing conditions.....	76
3.2.1.2	Growth condition for pea seedlings and GR24 application	76
3.2.1.3	Treatments and experimental design.....	77
3.2.2	Experiment Two - Bioassays and LC/MS analysis to detect a branch inhibiting hormone.....	77
3.2.2.1	Preliminary purification of plant samples using Sep-Pak [®] C ₁₈ columns.....	79
3.2.2.2	Separation of fractions having SLs detected by the <i>O. minor</i> germination assay.....	79
3.2.2.3	HPLC purification of the selected Sep-Pak fraction for branching assay.....	79
3.2.2.4	Branching assay on HPLC fractions and experimental design	80
3.2.2.5	Preparation of samples for LC/MS	80
3.2.2.6	Analysis of HPLC fraction by LC/MS.....	81
3.2.3	Data collection and analysis.....	83
3.3	Results and discussion.....	84
3.3.1	Effect of GR24 on axillary bud release and shoot length	84
3.3.2	Strigolactones (SLs) in different Sep-Pak fractions.....	89
3.3.3	Axillary shoot length in different HPLC fractions.....	90

3.3.4	Detection of unknown compounds in V26.....	92
3.4	Conclusion.....	95
4	Transport of strigolactones in guttation fluid of <i>Zantedeschia</i>	96
4.1	Introduction	96
4.2	Materials and methods	99
4.2.1	General growing conditions and treatment methodology	99
4.2.2	Experiment One- Determination of lag period between transpiration and guttation.....	100
4.2.2.1	Plant material	100
4.2.2.2	Treatments and experimental layout	100
4.2.2.3	Irrigation schedule treatment	100
4.2.2.4	Transpiration and guttation rate	101
4.2.2.5	Water content of the growing medium	102
4.2.2.6	Relative water content (RWC).....	103
4.2.2.7	Measurement of leaf area	104
4.2.3	Experiment Two- Relationship between concentration of SLs and guttation volume	104
4.2.3.1	Plant materials.....	104
4.2.3.2	Treatments and experimental layout	104
4.2.3.3	Irrigation schedule treatment	104
4.2.3.4	Germination assay.....	106
4.2.3.5	Variables recorded and data analysis	106
4.3	Results	106
4.3.1	Transpiration and guttation rate of <i>Zantedeschia</i> plants	106
4.3.2	Measures of water content	108
4.3.3	Guttation fluid and water content of the medium	110
4.3.4	Strigolactones in <i>Zantedeschia</i> cultivars.....	111

4.3.5 Correlation between strigolactone content and volume of guttation fluid.....	112
4.3.6 Correlation between guttation fluid and leaf area.....	112
4.4 Discussion.....	114
4.4.1 Guttation in <i>Zantedeschia</i>	114
4.4.2 Guttation fluid and strigolactones concentration.....	115
4.4.3 Dynamics of xylem sap flow and transport of SLs.....	117
4.5 Conclusion.....	120
5 Interaction between strigolactone and cytokinin in shoot branching of <i>Zantedeschia</i> grown <i>in vitro</i>.....	121
5.1 Introduction.....	121
5.2 Materials and methods.....	124
5.2.1 General methods.....	124
5.2.2 Experiment One - Concentration response relationship of <i>Zantedeschia</i> to GR24.....	125
5.2.2.1 Plant material.....	125
5.2.2.2 Treatments and experimental design.....	126
5.2.3 Experiment Two - Concentration response relationship of <i>Zantedeschia</i> to BAP.....	126
5.2.3.1 Plant material.....	126
5.2.3.2 Treatments and experimental design.....	126
5.2.4 Experiment Three - Interaction between cytokinin and strigolactone.....	127
5.2.4.1 Plant material.....	127
5.2.4.2 Treatments and experimental design.....	127
5.2.5 Variables recorded and data analysis.....	127
5.3 Results.....	128
5.3.1 Experiment One - Concentration response relationship of <i>Zantedeschia</i> to GR24.....	128
5.3.1.1 Axillary shoot number.....	128

5.3.1.2	Adventitious shoots.....	131
5.3.2	Experiment Two - Concentration response relationship of <i>Zantedeschia</i> to BAP.....	133
5.3.2.1	Influence BAP on axillary shoot number.....	133
5.3.2.2	Effect of BAP on adventitious shoot number and primary roots.....	134
5.3.3	Experiment Three - Interaction between cytokinin and strigolactone.....	135
5.3.3.1	Axillary shoot numbers.....	135
5.3.3.2	Adventitious shoot number.....	138
5.3.3.3	Number of nodes.....	141
5.4	Discussion.....	143
5.4.1	Influence of BAP on axillary and adventitious shoot formation.....	143
5.4.2	Influence of GR24 on axillary and adventitious shoot formation.....	145
5.4.3	Interaction between strigolactone and cytokinin in shoot branching.....	148
5.5	Conclusion.....	150
6	Hormonal interaction influencing shoot branching of pea plants.....	151
6.1	Introduction.....	151
6.2	Materials and methods.....	154
6.2.1	General methods.....	154
6.2.2	Preparation of hormonal solutions.....	155
6.2.3	Experiment One - Horizontal orientation.....	156
6.2.3.1	Treatment application.....	156
6.2.3.2	Treatments and experimental design.....	156
6.2.4	Experiment Two- Vertical orientation.....	157
6.2.4.1	Treatment application.....	157
6.2.4.2	Treatments and experimental design.....	157
6.2.5	Experiment Three- Application of GA ₃	158
6.2.6	Variables recorded and data analysis.....	158
6.3	Results.....	158

6.3.1	Experiment One- Horizontal orientation.....	158
6.3.1.1	Axillary shoot length and number at node 2	158
6.3.1.2	Axillary shoot length and number at node 3	160
6.3.1.3	Number of nodes on main stem	162
6.3.1.4	Root growth.....	162
6.3.2	Experiment Two-Vertical orientation	163
6.3.2.1	Axillary shoot length and number at node 2	163
6.3.2.2	Axillary shoot length and number at node 3	164
6.3.2.3	Number of nodes on main stem	165
6.3.2.4	Root growth.....	166
6.3.3	Experiment Three- Application of GA ₃	166
6.4	Discussion	167
6.4.1	Hormonal influences and their interaction on bud release and subsequent growth.....	167
6.4.2	Impact on proposed model of shoot branching.....	174
6.5	Conclusion.....	175
7	General discussion.....	176
7.1	Introduction	176
7.2	Detection of strigolactones and its correlation with branching inhibition	176
7.3	Is strigolactone a branching inhibitor?	179
7.4	Hormonal mechanism of axillary bud outgrowth	182
7.4.1	Bud release versus subsequent growth.....	182
7.4.2	Interaction between hormones on bud release and subsequent growth	185
7.5	Strigolactones and the formation of adventitious shoots	189
7.6	Proposed model of axillary bud outgrowth	190
7.6.1	Bud release	190
7.6.2	Subsequent growth.....	190
7.7	Implication of this research and recommendations for future work	192

Appendices.....	195
Appendix I. Effect of storage during preconditioning and age of seeds on germination in response to different concentration of GR24.....	195
Appendix II: Composition within medium and fertilizer used for growing <i>Zantedeschia</i>	198
Composition of structural components (in % volume) of the bark-based medium used for growing <i>Zantedeschia</i>	198
Appendix III: A dose response curve of GR24 on germination of seeds of <i>O. minor</i> for use while quantifying endogenous SLs in different cultivars of <i>Zantedeschia</i>	199
Appendix IV. LC/MS analysis of cytokinins and strigolactones within the samples of high and low branched cultivars of <i>Zantedeschia</i> and <i>Acer</i>	200
Appendix VI. Effect of externally applied cytokinin on axillary or adventitious shoots number of low branched <i>Zantedeschia</i> cultivar Best Gold grown <i>in vitro</i>	205
Appendix VII Dose response of un-decapitated pea stems to BAP	206
Appendix VIII Axillary shoot growth of intact pea stems laid horizontally in a Petri dish in response to BAP with or without lanolin paste at stem base.....	207
References	208

List of Figures

Figure 1.1 Developmental stages of apical dominance before and after axillary bud release caused by decapitation of the shoot apex (Cline, 1997).	4
Figure 1.2 Wild-type and branching mutant phenotypes of ‘Garden pea’ (A) and <i>Arabidopsis</i> (B). Mutant-induced branches on these plants are indicated by arrows (Dun, Brewer et al., 2009).....	7
Figure 1.3 Root exudates of <i>ccd8</i> mutant of pea is deficient in SLs (B) compared to wild-type (A) and <i>rms4</i> plants (B). Characteristic transitions for orobanchyl acetate (grey line) and the second strigolactone (black line) were monitored in the MRM mode (Gomez-Roldan et al., 2008). Grey lines in Figures A and B are magnified.....	8
Figure 1.4 Effect of GR24 on growth of axillary bud/shoot of; (A) strigolactone deficient mutant <i>rms1-10</i> and response mutant <i>rms4-3</i> of pea seedlings, (B) strigolactone deficient mutant <i>max4-1 (ccd8)</i> and wild-type (WT) of <i>Arabidopsis</i> . GR24 reduced the shoot length/number of branches of <i>ccd8</i> plants but not of <i>rms4-3</i> of pea plants and wild-type of <i>Arabidopsis</i> . GR24 was applied directly to the bud at node 4 of pea plants or to the rosette axillary buds and leaf axils of <i>Arabidopsis</i> . Observations were taken 10 days and 25 days after GR24 application in pea seedlings and <i>Arabidopsis</i> , respectively (Gomez-Roldan et al., 2008).	9
Figure 1.5 Schematic representation of results of reciprocal grafting between mutant and wild type-pea plants. (a) branching in self grafts of the wild type, (b) branching phenotype in self grafts of the mutant, (c) mutant grafted over wild type stock, (d) wild type grafted over mutant stock and, (e) mutant scion with wild type stem segment (i.e. interstock) and mutant stock (Leyser, 2003).....	10
Figure 1.6 Proposed biosynthetic and transduction pathway of SLs to inhibit bud outgrowth (Foo & Reid, 2013). β -carotene isomerase and carotenoid cleavage dioxygenases (CCD7 and CCD8) are required for biosynthesis of SLs, cytochrome 450 that encodes the apocarpetenous enzyme for SLs biosynthesis and downstream signaling, while the F-box protein and α/β hydrolase are required for responding to SLs. BRC1 that encodes for a transcriptional factor, is regulated by SLs.	12
Figure 1.7 Potential points of control of CK biosynthesis by auxin (Coenen & Lomax, 1997). Open-shaped arrows showing the points where auxin could regulate enzyme activity to inhibit the formation of active CKs.	17

Figure 1.8 Effect of GR24 on polar auxin transport. (A) Dose-response of basipetal indole-3-acetic acid (IAA) transport to GR24. (B) The effect of 10 μ M GR24 on basipetal IAA transport in wild-type, max4 and max2 stems (Crawford et al., 2010).	25
Figure 1.9 Flowering sized tuber of <i>Zantedeschia</i> with dominant and axillary buds. Dominant buds are contributed by the previous season (at centre of a tuber) as well as the current season (at periphery within a tuber). Figure contributed by Keith Funnell.....	29
Figure 1.10 Growth habit of different mutants of <i>Petunia</i> including wild-type. Wild-type <i>Petunia</i> showing high apical dominance while the rest; dad1, dad2 and dad3, are showing a profuse branching phenotype (Simons et al., 2007).	32
Figure 1.11 Life cycle of <i>Orobancha</i> spp. and <i>Striga</i> spp. (a) the seeds germination is stimulated by host-derived strigolactones, (b) seed radicle attached to host root via haustoria, (c-d) the seedling develops into a flowering shoot, (e) the weed produces a large number of seeds which get dispersed (Xie et al., 2010).	36
Figure 1.12 Chemical structure of different forms of Strigolactones (Yoneyama et al. 2010).	39
Figure 1.13 Thesis outline presenting chapters and experiments with associated research targets	42
Figure 2.1 Inflorescence of <i>Orobancha minor</i> (inside the red oval) associated with the host (red clover) at Castlepoint, New Zealand. Seeds of this weed species were collected for the series of experiments presented in this thesis.....	47
Figure 2.2 Different stages of annual growth cycle of <i>Zantedeschia</i> (A-D) at which guttation fluid was collected	52
Figure 2.3 Equipment set-up for extraction of root xylem sap of container-grown plants of <i>Petunia</i> (A), and at right a magnified view of section within figure A highlighted within red circle (B). A syringe fitted over the mouth of the cut stump of the primary shoot at the base where a small silicon tube was used to connect the stem and the syringe ensuring an air tight condition was achieved... ..	53
Figure 2.4 Equipment set-up for xylem sap extraction from shoots, illustrating section of a kiwifruit shoot inserted into a vial, held within an airtight Buchner flask (front) connected to a vacuum pump (back) (red circle indicates vial placed in the Buchner flask.....	55

Figure 2.5 A standardized dose response curve of germination of <i>O. minor</i> seeds to different concentrations of GR24. Vertical bars represent standard errors (n=3).....	57
Figure 2.6 Germination of <i>O. minor</i> seeds as affected by root xylem sap (non diluted and 10 times diluted) of wild-type ‘V26’ and different dad-mutants of <i>Petunia</i> . Water was used as a control.....	63
Figure 2.7 Germination of <i>O. minor</i> seeds as affected by shoot xylem sap of cultivars Red Emperor (low branched) and Sango Kaku (highly branched) of <i>Acer palmatum</i> Thunb. (Japanese maple) collected in mid-June, 2010 (A) and mid-August, 2013 (B) and late December 2010 (C). Water was used as a Control. Within Figure A, germination % was obtained with the sap diluted 5 times; data with non diluted sap was not obtained due to the contamination in a Petridish containing <i>O. minor</i> seeds treated with non diluted sap.	65
Figure 2.8 Highly branched (Sango Kaku) and low branched (Red Emperor) cultivars of <i>Acer palmatum</i> Thunb. (Japanese maple). Pictures were by provided by Keith A. Funnell.	66
Figure 2.9 Germination of <i>O. minor</i> seeds as affected by shoot xylem sap of cultivars M9 (dwarfing growth) and Royal Gala (vigorous growth) of <i>Malus domestica</i> Borkh (Apple). Water was used as a Control. The graph represents results of two assays using non diluted or diluted 5 times. Control did not produce any germinated seeds when non diluted sap samples were tested whereas about 3% germination occurred in the Control in the assay that used diluted saps.....	67
Figure 2.10 Germination of <i>O. minor</i> seeds as affected by shoot xylem sap of cultivars Hort 16A and Hayward of <i>Actinidia spp.</i> (Kiwifruit). Water was used as a control.	68
Figure 2.11 Radicle length of germinating seeds of <i>O. minor</i> as affected by different concentrations of GR24; (A) control (water) with medium radical length, (B) 0.00001 mg L ⁻¹ with long radicles, (C) 0.01 mg L ⁻¹ with medium length radicle, and (D) 100 mg L ⁻¹ with short radicles (red circle in each figure indicates a seed with a typical radicle for that treatment).....	69
Figure 3.1 Seeds of pea var Ashton spread over moist germination paper (A) and germinated pea seeds with radicles (B). For germination, seeds were wrapped within the paper and allowed to germinate in darkness for 3 days.	76

Figure 3.2 Sequential steps of methods applied to detect bioactive compound(s) that inhibit bud outgrowth using <i>Petunia</i> samples.....	78
Figure 3.3 Base peak chromatograms (BPC) of GR24 (10 μ M, top, blue traces) [magnified] or (1 μ M, bottom, pink traces). The peak detected within each chromatogram trace (i.e., top or bottom) at 14 minutes retention time represents GR24, whereas the other peaks are the contaminant (or background) compounds. Brown traces within GR24 10 μ M or 1 μ M are the extraction ion chromatograms (EIC) for m/z 299.0914.....	82
Figure 3.4 Mass chromatograms of a compound detected at 14 minutes retention time within base peak chromatograms (BPC) of GR24 (10 μ M), see Figure 3.3. Only four ions with m/z 97.0318 (M + H) ⁺ , 299.0945 (M + H) ⁺ , 321.0767 (M + Na) ⁺ and 619.1620 (2M + Na) ⁺ derived from GR24, whereas the other ions are derived from the contaminant (or background) compounds. Out of four ions, the ion with m/z 97.0318 corresponds to the daughter fragment of GR24 for the D ring moiety, and the other three to the GR24 molecule (GR24 molecular mass is 298).....	83
Figure 3.5 Percentage of buds released on Day One and Day Ten at node 2 of decapitated pea seedlings (seven days old), var Ashton, as affected by different concentrations of GR24. Seedlings were treated with the GR24 solutions immediately after decapitation. Nodes were numbered acropetally, with node 1 being the first above the cotyledons. For each parameter, means with the same letters do not differ significantly at $P \leq 0.05$ based on LSD. Vertical bars represent standard errors (n=3)	84
Figure 3.6 Number of buds released from Day Zero to Day Ten at node 2 of decapitated pea seedlings (seven days old), var Ashton, as affected by different concentrations of GR24. Seedlings were treated with the GR24 solutions (mgL^{-1}) immediately after decapitation. Nodes were numbered acropetally, with node 1 being the first above the cotyledons. Numbers of buds recorded were out of a total of seven seedlings.	85
Figure 3.7 Effect of different concentration of GR24 on the length of the axillary shoot at node 2 of decapitated pea seedlings (seven days old), var Ashton, observed at 3 days (A) and 10 days (B) after treatment. Seedlings were treated with the GR24 solutions immediately after decapitation. Nodes were numbered acropetally, with node 1 being the first above the cotyledons. Vertical bars represent standard errors (n=2 or 3). In each graph, mean values with the same letters do not differ significantly at $P \leq 0.05$ based on LSD.....	87

Figure 3.8 Axillary shoot length at node 2 of decapitated pea seedlings (seven days old), var Ashton, as affected by different concentrations of GR24 observed from Day 0 to Day 10 after treatment. Seedlings were treated with the GR24 solutions immediately after decapitation. Nodes were numbered acropetally, with node 1 being the first above the cotyledons. Vertical bars represent standard errors (n=2 or 3).	87
Figure 3.9 Axillary shoot growth of decapitated pea seedlings (seven days old), var 'Ashton', 10 days after decapitation following treatment with; (a) 0 (control) with magnified view of the part within red oval, (b) 0.001, (c) 0.01, (d) 0.1 and (e) 1 mgL ⁻¹ GR24. Seedlings were treated with the GR24 solutions immediately after decapitation. N – node (values indicate node number), AX - axillary shoot, C – cotyledon and, DS – Decapitated stump.	88
Figure 3.10 Germination percentage of <i>O. minor</i> seeds as affected by different Sep-Pak methanol fractions of <i>Petunia</i> (V26 and dad3).	89
Figure 3.11 Germination percentage of <i>O. minor</i> seeds as affected by different Sep-Pak methanol fractions of the low branched <i>Zantedeschia</i> cultivars Best Gold and GE45.	90
Figure 3.12 LC/MS traces or base peak chromatogram (BPC) of HPLC fractions derived from either <i>Petunia</i> V26 or dad3 root xylem sap collected between 11-16 minutes. Green coloured peaks represent LC/MS trace of V26 (low branched) and red peaks represent dad3 (highly branched). Four distinct peaks were observed, indicated by the numbers (1-4), in V26 but not in dad3. Among these peaks, two were detected in between 9-10 minutes and 12-13 minutes and the other two at 14-16 minutes.	93
Figure 3.13 Mass spectrum of four compounds derived from the sample from V26 ([1] – [4]) (refer Figure 3.12), with their respective elemental compositions within small brackets and <i>m/z</i> values. MS conditions: positive mode; capillary voltage, –4000 V; end plate offset, –500 V; source temperature, 200°C; drying gas flow, 4 L/min.	94
Figure 4.1 Illustration of methodology for use of weighing balance in calculating transpirational water loss from plants of <i>Zantedeschia</i> . The planter bag was sealed within a plastic bag to eliminate water loss due to evaporation (A). Collection of guttation fluid from the <i>Zantedeschia</i> plant using a zip-lock plastic bag. The bag was positioned to cover the distal half of the leaf for collecting guttation fluid (B). The magnified image within the red circle illustrates the accumulated guttation fluid during one such period.	102

Figure 4.2 Physical arrangement of plants of <i>Zantedeschia</i> ‘Best Gold’ and ‘Goldilocks’ in terms of the three treatments of differing periods of water withheld (i.e. 0, 1 and 2 days). Two plants of both cultivars, as indicated by the spheres without a number, were allocated to each period of water withheld for obtaining samples from the medium to measure water content.....	105
Figure 4.3 Transpiration (Water lost) per cm ² leaf (µl/cm ²) at different hours of a day in plants of <i>Zantedeschia</i> ‘Goldilocks’ that were either water withheld or well watered. Well watered plants received irrigation once daily prior to the experiment and twice on the day of experiment during the morning and evening whereas water withheld plants did not receive irrigation 3 days prior to the collection of guttation fluid. Shaded area within the graph represents the dark period. Vertical bars represent standard errors (n=3) ...	107
Figure 4.4 Guttation fluid per unit leaf area (µl/cm ²) collected every three hours from leaves of well watered plants of <i>Zantedeschia</i> ‘Goldilocks’. Water withheld plants did not produce guttation fluid except a single drop from one of the water withheld plant. Shaded area within the graph represents the dark period. Vertical bars indicate standard errors (n=3)	108
Figure 4.5 Water content (%) of the growing medium of plants of <i>Zantedeschia</i> ‘Goldilocks’ that were either water withheld or well watered, at three measurement times of a 24 hr period. Means with same letters do not differ significantly at $P \leq 0.05$ based on DMRT. Bars represent standard error (n=3).....	109
Figure 4.6 Relative water content (RWC) of leaves of ‘Goldilocks’ plants that were either water withheld or well watered at different times of a 24 hour period. Means with same letters do not differ significantly at $P \leq 0.05$ based on DMRT. Bars represent standard error (n=3)	110
Figure 4.7 Germination of <i>O. minor</i> seeds treated with guttation fluid of highly branched (Goldilocks) and low branched (Best Gold) <i>Zantedeschia</i> cultivars at three different levels of water withheld i.e., 0, 1 or 2 days of water withheld. Means with different letters between two cultivars differ significantly at $P \leq 0.05$ based on DMRT. Vertical bars represent standard errors of means between each level of water withheld within each cultivar	111
Figure 4.8 Correlation analysis between germination percentage of <i>O. minor</i> seeds and amount of guttation fluid (µl/cm ²) for individual plants of the highly branched Goldilocks and low branched Best Gold <i>Zantedecshia</i> cultivar	112

Figure 4.9 Correlation analysis between amount of guttation fluid (ml) per leaf and leaf area per leaf (cm ²) at different levels of water withheld in two <i>Zantedeschia</i> cultivars Goldilocks (GL) and Best Gold (BG).	113
Figure 4.10 Exudation of guttation fluid; (A) at the tip of a leaf of a <i>Zantedeschia</i> plant (present work) and, (B) around the margin of leaves of an unidentified plant (Konning, 1994).	115
Figure 4.11 Dynamics of xylem sap flow during day and night or period of light and dark (Stages A-D) and transport of SLs from root (R) to shoot (S) (within a red circle). Red arrows indicate water coming out through stomata during transpiration and the blue arrows indicate root exudates due to root pressure. Green solid line represents stomata closure whereas green dotted line indicates open stomata.	119
Figure 5.1 Leaf emergence and axillary shoot development on <i>Zantedeschia</i> grown <i>in vitro</i> . Shoot within figure (A) represents a diagrammatic representation of the primary shoot in figure (B) inserted into the media within a culture vessel. Structures labelled 1-4 represent leaves, with 3-4 presenting newly emerged leaves. Axillary shoot development occurring at node 2 is highlighted within the red oval.	125
Figure 5.2 Influence of GR24 on number of axillary shoots per culture vessel (eight plants in each vessel) of highly branched cultivars (GE49, GE4120, Goldilocks and GE20/4) of <i>Zantedeschia</i> grown <i>in vitro</i> observed four weeks (A) and eight weeks (B) after treatment. Bars represent standard errors (n=3). Mean values within each graph with the same letter do not differ significantly at $P \leq 0.05$ based on DMRT.	129
Figure 5.3 Influence of GR24 on the number of axillary shoots per culture vessel (eight plants in each vessel) of highly branched cultivars of <i>Zantedeschia</i> grown <i>in vitro</i> observed at weekly intervals up to eight weeks after the treatment. Fitted lines for each concentration of GR24 are separate for 0-4 weeks and 4-8 weeks.	130
Figure 5.4 Slopes of fitted lines for the effect of different concentrations of GR24 on increase in axillary bud numbers obtained from 0-4 weeks (A) and 4-8 weeks (B). Bars represent standard errors (n=3).	130
Figure 5.5 Influence of BAP on number of axillary shoots per plant of low branched <i>Zantedeschia</i> cultivar Best Gold grown <i>in vitro</i> observed at eight weeks after treatment. Vertical bars represent standard errors (n=4).	133

Figure 5.6 Influence of BAP concentration on number of adventitious shoots, root number and total root length per plant of *Zantedeschia* ‘Best Gold’ grown *in vitro* eight weeks after treatment commenced. For number of adventitious shoots and root length, the number associated with each column presents the square root mean value, with y-axis presenting back-transformed values so as to enable interpretation. Means with same letters of same growth parameter do not differ significantly at $P \leq 0.05$, based on DMRT.134

Figure 5.7 Shoots of *Zantedeschia* ‘Best Gold’ grown *in vitro* that received (A) 0 mgL⁻¹, (B) 0.3 mgL⁻¹, (C) 0.9 mgL⁻¹ and (D) 3 mgL⁻¹ of BAP observed eight weeks after treatment. A part of shoot within red circle in Figure C is magnified showing axillary and adventitious shoots.135

Figure 5.8 Number of axillary shoots per culture vessel (eight plants in each vessel) in three cultivars (A) Best Gold, (B) Goldilocks and (C) GE49 of *Zantedeschia* grown *in vitro* supplied with GR24 (\pm)(in mgL⁻¹) and/or BAP (\pm) observed in week four (1) and week eight (2). The vertical bars indicate SE of the mean (n=4), means within each graph with same letters do not differ significantly at $P \leq 0.05$ based on DMRT.137

Figure 5.9 Number of axillary shoots per culture vessel (eight plants in each vessel) in low and highly branched cultivars of *Zantedeschia* grown *in vitro* supplied with GR24 (\pm) and 0.3 mgL⁻¹ BAP (A), and GR24 (\pm) and 0.9 mgL⁻¹ of BAP (B) observed after 4 weeks. The vertical bars indicate SE of the mean (n=4).....138

Figure 5.10 Number of adventitious shoots per plant of *Zantedeschia* grown *in vitro* as affected by main effect of BAP observed after 4 weeks. The vertical bars indicate SE of the mean (n=4).139

Figure 5.11 Number of adventitious shoots per plant in three cultivars (A) Best Gold, (B) Goldilocks and (C) GE49 of *Zantedeschia* grown *in vitro* supplied with GR24 (\pm) and/or BAP (\pm) (in mgL⁻¹) observed after eight weeks. The vertical bars indicate SE of the mean (n=4). For each cultivar, means with same letters do not differ significantly at $P \leq 0.05$ based on DMRT.140

Figure 5.12 Adventitious shoots (within red circles) of *Zantedeschia* grown *in vitro* arising from plant parts other than leaf axils.....144

Figure 6.1 Pea seedlings growing in plastic containers half filled with moist sand in a growth cabinet.....154

Figure 6.2 An excised stem of a 14 day old pea seedling var Ashton that was used for the experiments. Basal nodes (1 & 2) beared a scale leaf, while the remaining nodes beared true leaves. Plant parts above the dash line were used for the experiments.....155

Figure 6.3 Diagrammatic illustration of excised stem with an intact apex, of pea seedlings of var Ashton, orientated either; (A) horizontally in a Petri dish filled with 20 ml of treatment solution (Plan view) or, (B) vertically upright in a glass vessel filled with 4 ml of treatment solution (Side view). Grey colour in each figure represents treatment solution.....157

Figure 6.4 Effect of different combinations of GR24 on; (A) average total axillary shoot (maximum 3 shoots) length and, (B) average length of the longest shoot length at node 2 of pea stems of var Ashton that were laid horizontally. Hormones were applied on excised stems (devoid of cotyledons and node 1 of 14 day-old-pea seedlings. Data was collected 12 days after treatment. Nodes were numbered acropetally with basal node named as node 1. In each graph, means with different letters indicate significant difference at $P < 0.05$ through Fisher's mean separation test. Numbers within brackets in each graph are the transformed square root values of the mean. Vertical bars indicate standard errors (n=3).159

Figure 6.5 Effect of different combinations of GR24 and BAP concentration on average number of axillary shoots at node 2 of pea stems of var Ashton that were orientated horizontally. Hormones were applied on excised stems (devoid of cotyledons and node 1) of 14 day-old-pea seedlings. Data was collected 12 days after treatment. Nodes were numbered acropetally with basal node named as node 1. Means with different letters indicate significant difference at $P < 0.05$ through Fisher's mean separation test. Vertical bars indicate standard errors (n=3).160

Figure 6.6 Effect of different combinations of GR24 and BAP concentration on average total axillary shoot (maximum two shoots) length at node 3 of pea stems of var Ashton that were orientated horizontally. Hormones were applied on excised stems (devoid of cotyledons and node 1) of 14 day-old-pea seedlings. Data was collected 12 days after treatment. Means with different letters indicate significant difference at $P < 0.05$ through Fisher's mean separation test. Numbers within brackets are the transformed square root values of the mean. Vertical bars indicate standard errors (n=3).161

Figure 6.7 Effect of different combinations of GR24 and BAP concentration on average number of axillary shoots at node 3 of pea stems of var Ashton that were orientated

horizontally. Hormones were applied on excised stems (devoid of cotyledons and node 1) of 14 day-old seedlings. Data was collected 12 days after treatment. Nodes were numbered acropetally with basal node named as node 1. Means with different letters indicate significant difference at $P < 0.05$ through Fisher's mean separation test. Vertical bars indicate standard errors (n=3).....161

Figure 6.8 Effect of different combinations of GR24 and BAP concentration on (A) average total axillary shoot (maximum 3 shoots) length and (B) average length of the longest shoot length at node 2 of vertically oriented pea stems of var Ashton. Hormones were applied to vertical excised stems (devoid of cotyledons and node 1) of 14 day-old seedlings. Data was collected 12 days after treatment. Nodes were numbered acropetally with basal node named as node 1. In each graph, means with different letters indicate significant difference at $P < 0.05$ through Fisher's mean separation test. Vertical bars indicate standard errors (n=3).163

Figure 6.9 Effect of different combinations of GR24 and BAP concentration on average number of axillary shoots at node 2 of vertically oriented pea stems var Ashton. Hormones were applied on excised stems (devoid of cotyledons and node 1) of 14 day-old seedlings. Data was collected 12 days after treatment. Nodes were numbered acropetally with basal node named as node 1. Means with different letters indicate significant difference at $P < 0.05$ through Fisher's mean separation test. Vertical bars indicate standard errors (n=3).164

Figure 6.10 Effect of different combinations of GR24 and BAP concentration on average axillary shoot length at node 3 of vertically oriented pea stems of var Ashton. Hormones were applied on excised stems (devoid of cotyledones and node 1) of 14 day-old seedlings. Data were collected 12 days after treatment. Nodes were named acropetally with basal node named as node 1. For each variable, means with different small letters indicate significant difference at $P < 0.05$ through Fisher's mean separation test. Vertical bars indicate standard errors.164

Figure 6.11 Effect of different combinations of GR24 and BAP concentration on average number of axillary shoots at node 3 of vertically oriented pea stems of var Ashton. Hormones were applied on excised stems (devoid of cotyledones and node 1) of 14 day-old seedlings. Data were collected 12 days after treatment. Nodes were named acropetally with basal node named as node 1. For each variable, means with different small letters indicate significant difference at $P < 0.05$ through Fisher's mean separation test.....165

Figure 6.12 Effect of different combinations of GR24 and BAP concentration on average number of nodes of vertical primary shoots of pea stems of var Ashton devoid of cotyledons and node 1. Means with different letters indicate significant difference at $P < 0.05$ through Fisher's mean separation test.	165
Figure 6.13 Effects of different combination of hormones including control (water) on average axillary shoot (maximum 1 shoot) length at node 3 of vertically orientated pea stems of var Ashton. Hormones were applied on excised stems (devoid of cotyledons, node 1 & 2) of 14 day-old seedlings. Data were collected 12 days after treatment. Nodes were named acropetally with basal node named as node 1. Vertical bars indicate standard error (n=2).	167
Figure 7.1 Sequential steps utilized while identifying a bioactive compound/s that inhibit/s shoot branching.....	181
Figure 7.2 A proposed model of hormonal interaction in axillary bud release and subsequent growth of a shoot of an annual plant or a shoot produced from the bud of current season (or sylleptic shoot). The model involves the hormonal interaction for two stages of shoot branching i.e., bud release and subsequent growth. For bud release, SL and CK interact with each other via BRC1 (transcription factor). Apically derived auxin indirectly inhibits bud release by increasing SL or reducing CK level. For subsequent growth, multiple pathways of hormonal interaction occur. SL inhibits the subsequent growth either directly or indirectly by reducing the transport of auxin from the released buds. SL may also inhibit the subsequent growth by reducing the effect of GA	191

List of Tables

Table 1.1 Different forms of strigolactones present in some of the plant species noted as acting germination stimulants of <i>Orobancha</i> spp.....	38
Table 2.1 Descriptive branching and/or growth characteristics of cultivars/variety of different horticultural species used for detecting and quantitatively comparing endogenous strigolactones	50
Table 2.2 Concentration of strigolactones (ng L ⁻¹ of GR24 equivalent; calculated using dose response curve; Appendix III) present in guttation fluid of three different cultivars of <i>Zantedeschia</i> at different stages of the annual growth cycle - Year One. (Number in parenthesis is germination percentage of <i>O. minor</i> ; n = 30-40). About 20% seeds germinated in the Control (Water).....	59
Table 2.3 Germination percentage (%) ±SE of seeds of <i>O. minor</i> as affected by guttation fluid of different <i>Zantedeschia</i> cultivars at different stages of annual growth cycle – Year Two. About 1% or near to zero seeds germinated in the Control (Water)	59
Table 2.4 Radicle length ^Z of germinated <i>O. minor</i> seeds treated with guttation fluid of different <i>Zantedeschia</i> cultivars collected at different stages of the annual growth cycle.....	70
Table 2.5 Radicle length ^Z of germinated <i>O. minor</i> seeds treated with guttation fluid of different <i>Zantedeschia</i> cultivars collected at early senescence either 10 times diluted or non diluted	70
Table 2.6 Radicle length ^Z of germinated <i>O. minor</i> seeds treated either 10 times or non diluted root xylem sap of <i>Petunia</i>	71
Table 3.1 Length of axillary shoots (±SE) at each of two nodes of decapitated seedlings (nine days old) of pea var Ashton, as affected by different HPLC fractions derived from ‘dad3’ and ‘V26’ <i>Petunia</i> , obtained 10 days after treatment commenced.	91
Table 4.1 Amount of guttation fluid (µl/sq.cm) collected over overnight and water content of the medium (%) of two cultivars of <i>Zantedeschia</i> after different durations of water withheld	111
Table 5.1 Effects of different concentrations of GR24 on number of adventitious shoots (±SE) per tub (eight plants per tub) of four cultivars of <i>Zantedeschia</i> at four weeks and eight weeks after GR24 application.	132
Table 5.2 Main and interaction effects between cultivars and hormones as represented by <i>P</i> values for the number of axillary shoots in <i>Zantedeschia</i> grown <i>in vitro</i> observed in week four and week eight.....	136

Table 5.3 Main and interaction effects between cultivars and hormones as represented by <i>P</i> values for the number of adventitious shoots in <i>Zantedeschia</i> grown <i>in vitro</i> observed in week 4 and week 8.	139
Table 5.4 Main and interaction effects between cultivars and hormones as represented by <i>P</i> values for the number of nodes on the primary shoot in <i>Zantedeschia</i> grown <i>in vitro</i> observed in week 4 and week 8.	141
Table 5.5 Hormonal influence on the number of nodes on the primary shoot (\pm SE) of three <i>Zantedeschia</i> cultivars grown <i>in vitro</i> observed in week 4 and week 8.	142
Table 6.1 Effect of different combinations of BAP and GR24 concentrations on average number of roots and total length of the roots (\pm SE) in horizontally orientated pea stems var Ashton.	162
Table 6.2 Effect of different combinations of BAP and GR24 concentrations on average number of roots and total length of the roots (\pm SE) in vertically orientated pea stems var Ashton.	166

List of Abbreviations

ABA	Absisic acid
ANOVA	Analysis of variance
BAP	Benzylaminopurine
BPC	Base peak chromatogram
BRC1	Branched1
CCD	Carotenoid cleavage dioxygenases
CK	Cytokinin
CRD	Completely randomised design
D27	Dwarf27
DAD	Decreased apical dominance
DMRT	Duncan's multiple range test
DW	Dry weight
FW	Fresh weight
GA	Gibberellin
GA ₃	Gibberellic acid
GLM	General linear model
GR24	a synthetic strigolactone (3aR*,8bS*,E)-3-(((R*)-4-methyl-5-oxo-2,5-dihydrofuran-2-yloxy)methylene)-3,3a,4,8b-tetrahydro-2H-indeno[1,2-b]furan-2-one
HPLC	High performance liquid chromatography
HRMS	High resolution mass spectrometry
IAA	Indole-3-acetic acid
IPT	Isopentenyl transferase
LC/MS	Liquid chromatography/ Mass spectrometry
LSD	Least significant difference

M.9	Malling 9
MAX	More axillary shoot
MRM	Multiple reaction monitoring
NAA	Naphthalene 1-acetic acid
NMR	Nuclear magnetic resonance
NPA	1-N-Naphthylphthalamic acid
NZ	New Zealand
PAT	Polar auxin transport
PIN	Pin-Formed
PPFD	Photosynthetic photon flux density
RO	Reverse osmosis
RMS	Ramosus
RWC	Relative water content
SAM	Shoot apical meristem
SE	Standard error
SL	Strigolactone
TW	Turgid weight
V26	Velm 26
X-CK	Xylem cytokinin

