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Crop Architecture: Investigating 'Strigolactones' in Different Horticultural Species having Different Branching Phenotypes

Sarina Manandhar
PhD Student
Institute of Agriculture and Environmental Science,
Massey University
New Zealand

David J. Woolley
PhD Supervisor
Institute of Agriculture and Environmental Science,
Massey University
New Zealand

Keith A. Funnell
Scientist
The New Zealand Institute for Plant & Food Research Ltd.,
New Zealand

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Athens Institute for Education and Research
8 Valaoritou Street, Kolonaki, 10671 Athens, Greece
Tel: + 30 210 3634210 Fax: + 30 210 3634209
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Sarina Manandhar,
PhD Student
Institute of Agriculture and Environmental Science,
Massey University
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David J. Woolley,
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Institute of Agriculture and Environmental Science,
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New Zealand

Keith A. Funnell
Scientist
The New Zealand Institute for Plant & Food Research Ltd.,
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Abstract

Crop architecture, which is important for crop productivity, is mainly determined by shoot branching. Recently, a new hormone has been postulated as being a strigolactone-type compound that is involved in the control of shoot branching. Strigolactones in different horticultural crop species exhibiting different branching phenotypes were investigated using a specific germination assay based on the parasitic weed seed Orobanche minor, which requires strigolactones for its germination. Strigolactones were found in all horticultural crop species studied but not in all varieties within the species. Highly branched varieties showed little or no strigolactone suggesting that strigolactones may influence branching inhibition. In Zantedeschia, a commercially important species for cut flower production, a good correlation between strigolactone content and branching was observed during leaf emergence, early in the annual growth cycle, but not in later stages: the highly branched variety also produced high levels of strigolactones in later stages of the annual growth cycle. As there are many different strigolactones a specific branching assay is being developed, in addition to physico-chemical methods like mass spectrometry, in order to identify the strigolactone or related compound responsible for branching inhibition. Production of strigolactones in later stages of development also indicates that strigolactones may have other physiological roles.

Key words: apical dominance, novel inhibitor, biological assay

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Corresponding Author:

Introduction

Strigolactones (SL), recently proposed as a branch inhibiting hormone (Gomez-Roldan et al., 2008; Umehara et al., 2008) are the carotenoid-derived compounds produced by action of CAROTENOID CLEAVAGE DIOXYGENASES, CCD7 and CCD8 (Matusova et al., 2005). These compounds are also known to trigger the germination of parasitic plant seeds of *Striga* spp. and *Orobanche* spp. (Bouwmeester et al., 2003; Matusova et al., 2005; Muller et al., 1992) and stimulate symbiotic fungi (Besserer et al., 2006). It is likely that SL are mainly synthesized in roots as one of the SL 'Orobanchol' has been detected in xylem sap (Kohlen et al., 2011).

As SLs or closely associated compounds are involved in shoot branch inhibition, study on this novel hormone is important for horticultural crops as the degree of branching are major factors determining production yield and quality (Gomez-Roldan et al., 2008). Several horticultural crops like *Actinidia* Lindl. (kiwifruit), have been considered as crops of architectural interest (Seleznyova et al., 2002) with regard to branching. Recent investigation has detected SL synthesizing genes that encode CCD8 and CCD7 in kiwifruit (Ledger et al., 2010), *Dendranthema grandiflorum* (chrysanthemum) (Liang et al., 2010) and *Solanum lycopersicum* (tomato) (Vogel et al., 2010). However, the precise physiological relationship between natural SL and shoot branching is still unknown. This paper will focus on the existence of SL in different annual and perennial horticultural plants having different branching phenotypes, using a germination assay of *Orobanche minor* Sm. In this study, a synthetic strigolactone 'GR24' has been used as a standard for developing a dose response curve in order to quantify SL present in plant samples of some species investigated.

Materials and Methods

Plant Selection

Different horticultural species were selected that included *Zantedeschia* spp. K. Spreng, *Actinidia chinensis* and *deliciosa* Lindl. (kiwifruit), *Malus domestica* Borkh (apple) and *Acer palmatum* Thunb. (Japanese maple). Selected varieties of each of these species, and descriptive growth characteristics, were:

- *Zantedeschia*: 'Goldilocks' (highly branched) and 'GE45' (less branched)
- Kiwifruit: 'Hort16A'(*A. chinensis*) and 'Hayward'(*A. deliciosa*) - both varieties have vigorous shoot growth with more proleptic shoots (shoots of previous season) and less or no sylleptic shoots (shoots that developed from current season buds)
- Apple: 'Royal Gala' ('RG') (vigorous shoot growth) and 'M9' (dwarfing growth habit)

- *Acer*: 'Red Emperor' (less branched) and 'Sango Kaku' (highly branched)

Sample Preparation and Purification
Guttation Fluid Collection - Zantedeschia

Xylem exudate collected as guttation fluid of *Zantedeschia* was obtained from ten plants per variety, and pooled together for subsequent analysis utilising a germination assay method as described by (Matusova et al., 2005). Guttation fluid was collected early in the annual growth cycle commencing 45 days after planting tubers, and on five further occasions at intervals of one month. At the first date of collection, the plants were at the leaf emergence stage with one to two fully opened leaves, by the second date they had started branching, third date plants were flowering and, during the last two dates the plants had progressed to early senescence and late senescence, respectively. For collecting samples, plastic 'zip locks' bags were used to envelop a group of two to eight leaves per plant, depending upon variety. Collection bags were put in place late afternoon, with the accumulated guttation fluid collected the following morning.

Xylem Sap Collection - Acer, Kiwifruit and Apple

For *Acer*, kiwifruit and apple, extraction of xylem sap was carried out as described by Bollard (1953) in early summer (November) for both kiwifruit and apple, both summer (December) and winter (June) for *Acer*.

One day before extraction, the plants were fully watered and sap extraction was done by inserting the proximal end of the shoot into a vial placed in a sealed Buchner flask connected to a vacuum pump (Hivac, Auckland, New Zealand). Starting from the distal end, 100 mm of shoot was removed with pruning shears every five seconds. The flow of xylem sap was allowed to drip into the vial until the entire shoot was cut.

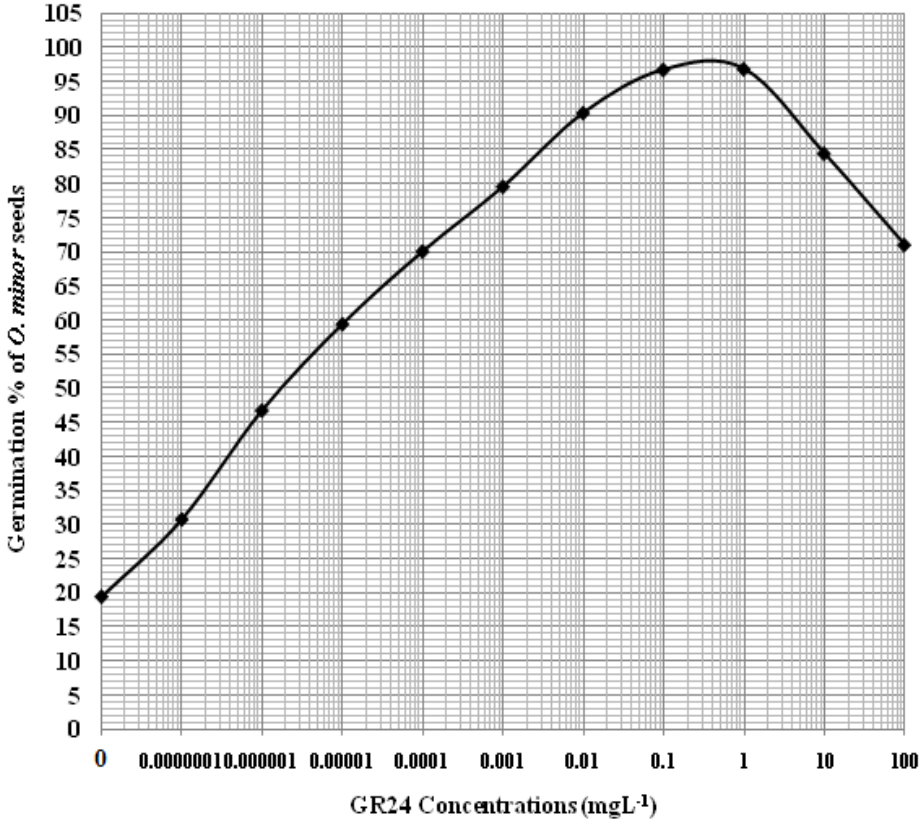
Preparation of GR24 Solutions

Ten mls of GR24 (298.29 molecular weight, Chiralix, The Netherlands) stock solution of 100 mgL^{-1} was prepared in 10% acetone and diluted with distilled water to obtain 10 mgL^{-1} to 10^{-7} mgL^{-1} final concentrations.

Germination Assay

Based on the germination assay described by Matusova et al. (2005), *Orobanche minor* seeds that were preconditioned at 21°C for 14 days were allowed to germinate at 25 °C in the dark after the seeds were treated with the samples. Distilled water was used as a control. A dose-response curve for 'GR24' was obtained over the concentration range 10^{-7} to 100 mgL^{-1} (Figure 1). For both the dose response curve and plant samples analysed, the germination assay was based on 30 seeds and the data analysed using a Chi-square test. To estimate the amount of SL present in the guttation fluid of *Zantedeschia*, the resulting germination percentage of *O. minor* was converted to GR24 equivalents from the dose-response curve (Figure 1).

Figure 1. *Dose response on germination of O. minor seeds at different concentrations of GR24*



Results and Discussion

Dose-response Curve of GR24

The dose-response curve showed maximum germination in 1 mgL^{-1} of GR24, and above this concentration the germination activity started reducing (Figure 1). The fact that higher concentrations can inhibit parasitic seed germination was supported by several other authors (Economou et al., 2007; Wigchert et al., 1999; Malik et al., 2010; Matusova et al., 2004; Joel et al., 1995). The dose- response also clearly indicated that the seed was sensitive to GR24 at concentrations as low as 0.0000001 mgL^{-1} (i.e. 10^{-7}), suggesting that GR24 is highly effective at very low concentrations. This is also supported by Malik et al. (2010) who suggested that GR24 could stimulate germination from 10^{-10} to 10^{-12} $molL^{-1}$. It also indicates that detection of SLs using a germination assay is a highly sensitive technique as the response of *Orobanche* spp. seeds to SL are at least 100 fold more sensitive for the detection of SLs, as compared with chromatography and mass spectrometry (Yoneyama et al., 2010).

Strigolactone content in guttation fluid of Zantedeschia at different stages of growth

In both varieties of *Zantedeschia*, the SL content varied between the different stages of development (Table 1), a finding which is supported by

Ledger *et al.* (2010). They found varied expression of AcCCD7 and AcCCD8 in kiwifruit plants at different stages of their annual growth cycle. At the time of first sampling when the plants were at leaf emergence, guttation fluid of 'GE45' contained about 70 times more SL concentration than 'Goldilocks', supporting the hypothesis of SL involvement in inhibition of branching. If so, it could also be predicted that higher concentration of SL may be responsible for reduced cytokinin level in *Zantedeschia* plants, as external application of cytokinin can enhance branching in the less branched variety 'Best Gold' during leaf emergence (Subbaraj et al. 2010).

In contrast, guttation fluid collected from 'Goldilocks' when branching was visibly evident seemed to have the highest SL concentration of about 100,000 fold more than that produced in leaf emergence, though this variety is characterized by naturally being highly branched (Table 1). Hence it becomes apparent that the amount of SL present in *Zantedeschia* was potentially correlated with shoot branching during leaf expansion prior to when branching was evident, but not when branching had already occurred. A high concentration of SL in 'Goldilocks' at the visible branching stage may be because of basipetal supply of a high amount of auxin generated from the higher number of leaves developed by these plants (a function of the comparatively greater number of stems and branches with this variety). This high level of auxin may lead to enhanced production of SL in roots, as the SL synthesis genes that encode CCD7 and CCD8 are positively regulated by apically derived auxin (Beveridge, 2006; Dun et al., 2006). In addition, recent studies have found several other physiological roles for SL in the plant system apart from inhibition of shoot branching (Cardoso et al., 2011), and the presence of different forms of SL (Yoneyama et al., 2009) with different biological activity have also been noted (Kisugi et al., 2013). Thus, it could be predicted that different forms of SL could have different physiological roles (Kisugi et al, 2013), though the relationships between chemical structure and function are still unknown.

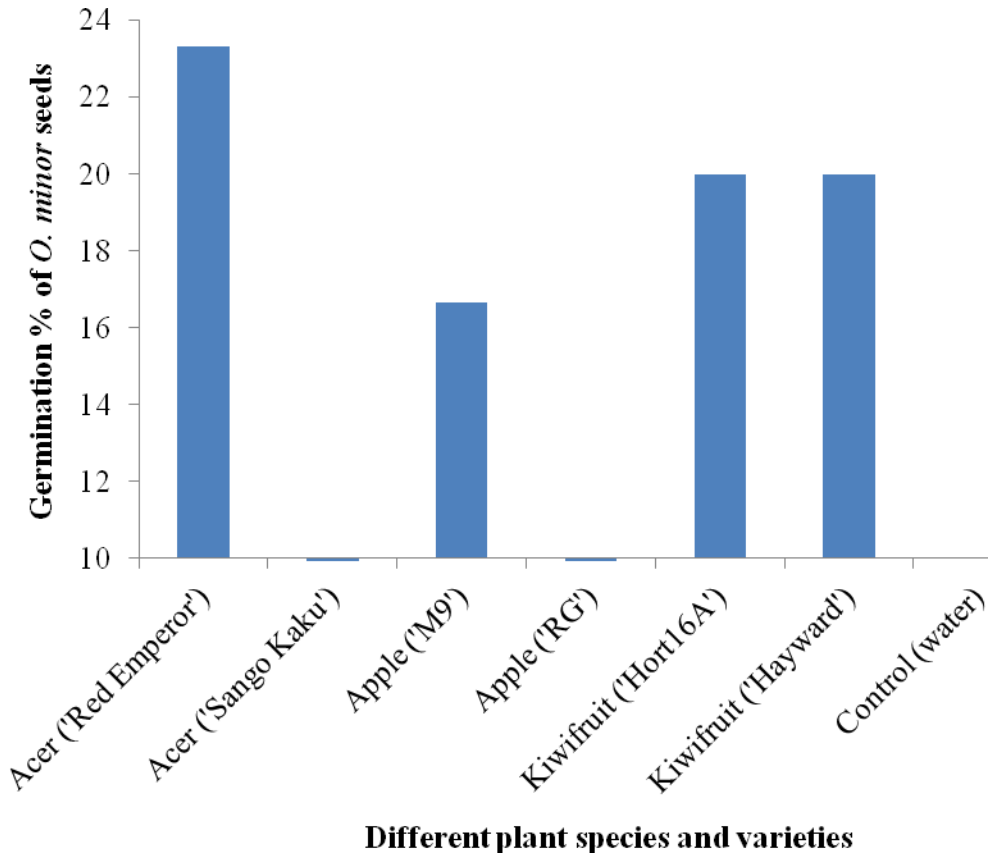
Table 1. *Calculated concentration of strigolactones (expressed as ng L^{-1} of GR24 equivalent) present in guttation fluid of three different varieties of Zantedeschia in different growth stages. (Number in parenthesis is germination percentage of O. minor)*

Varieties of *Zantedeschia*	Nov 2010 Emergence stage	Dec 2010 Branching stage	Jan 2011 Flowering stage	Feb 2011 Early Senescence	Mar 2011 Late Senescence
Goldilocks (highly branching)	0.18 (34)	20000 (93)	1.9 (50)	90 (69)	20 (63)
GE 45 (less branching)	130 (71)	0.16 (33)	100 (70)	5 (57)	0 (0)

As an extension of this emerging discussion, a specific branching assay is being developed in addition to liquid chromatography/ mass spectrometry (LC/MS) in order to identify a bio-active compound that may be either a strigolactone or its derivatives. In doing so, this acknowledges the fact that while the germination bioassay may be more sensitive, it is not necessarily specific to branching. In addition, the relationship between SL, cytokinin and auxin needs to be developed further in *Zantedeschia*, so as to better understand the hormonal influence upon the shoot branching mechanism.

The variety 'GE45' seemed to have little or no SL during leaf senescence as the germination of *O. minor* seeds was greatly reduced at this stage of development (Table 1). As bud dormancy associated with leaf senescence ensues rapidly after cessation of new leaf production (Subbaraj et al., 2010), it is possible that SL content could be high but lack of seed germination may be due to high levels of abscisic acid (ABA), produced in plants entering dormancy (Ofir & Kigel, 1998; Yamazaki et al., 2002).. This argument is supported by the fact that abscisic acid at concentrations of 10^{-5} M and 10^{-6} M has been found to reduce the germination percentage of *Striga* seeds (*Orobanche* family) when applied in the presence of germination stimulants (Yoneyama et al., 1998).

Figure 2. *Germination of O. minor seeds as affected by xylem sap of different horticultural species*



Strigolactones in xylem sap of Acer, kiwifruit and apple

Xylem sap of the *Acer* variety exhibiting less branching, i.e. 'Red Emperor', induced higher germination of *O. minor* seeds as compared to those of 'Sango Kaku' (Figure 2). However, repeated experiments using the *Acer* sap collected in summer showed that sap from 'Sango Kaku' also achieved 30% seed germination, suggesting that the production of SL could vary at different stages of development like that noted in *Zantedeschia* (Table 1) and kiwifruit (Ledger et al., 2010).

Sap of the dwarfing 'M9' apple plants also showed more germination activity than the vigorous variety 'RG' (Figure 2). This finding supports the hypothesis of a direct relationship between SL and branching inhibition, as 'M9' used as a dwarfing rootstock greatly reduces scion branching (van Hooijdonk et al., 2010, 2011). However, further investigations are required to determine whether the level of SL varies in different developmental stages of apple growth, as seen in *Zantedeschia* and *Acer*. Kiwifruit sap of both 'Hayward' and 'Hort16A' stimulated germination to a similar level (Figure 2), suggesting the existence of SL in different kiwifruit species. The result also suggests both species of kiwifruit characterised with similar shoot vigour, but lack of sylleptic branching, might contain similar levels of SLs at particular stages of development.

These overall results indicate that the germination assay can be a powerful tool to detect and quantify the level of SL. However, this method would be improved if the germination percentage can be converted into GR24 equivalents using a dose response curve as in SL quantification in *Zantedeschia* (Table 1).

In summary, branching of different horticultural crops appears to correlate with the level of SL as detected by *O. minor* germination assay, but consideration has also to be given to both the developmental stage of the plant and hormonal response to branching or the transduction pathway. Further research is required in order to establish the physiological relationship between different forms of natural SL and branching, by using a specific branching assay.

The potential exists that the level of SL can be used as a marker for early selection of plants that exhibit desirable branching phenotype. As evident with other crop species, marker assisted selection can reduce some of the limitations of the traditional population breeding and selection method based on phenotypic traits (Collard & Mackill, 2008; Foolad & Panthee, 2012).

References

Besserer, A., Puech-Pagès, V., Kiefer, P., Gomez-Roldan, V., Jauneau, A., Roy, S., . . . Séjalon-Delmas, N. (2006). Strigolactones stimulate arbuscular mycorrhizal fungi by activating mitochondria. *PLoS Biol, 4*(7), e226.

Bollard, E. G. (1953). The use of tracheal sap in the study of apple tree nutrition. *Journal of Experimental Botany, 4*, 363-368.

Bouwmeester, H. J., Matusova, R., Zhongkui, S., & Beale, M. H. (2003). Secondary metabolite signalling in host-parasitic plant interactions. *Current Opinion in Plant Biology, 6*(4), 358-364. doi: Doi: 10.1016/s1369-5266(03)00065-7

Cardoso, C., Ruyter-Spira, C., & Bouwmeester, H. J. (2011). Strigolactones and root infestation by plant-parasitic *Striga*, *Orobanche* and *Phelipanche spp*. *Plant Science, 180*(3), 414-420. doi: DOI: 10.1016/j.plantsci.2010.11.007

Collard, B. C. Y., & Mackill, D. J. (2008). Marker-assisted selection: an approach for precision plant breeding in the twenty-first century. *Philosophical Transactions of the Royal Society B: Biological Sciences, 363*(1491), 557-572. doi: 10.1098/rstb.2007.2170

Foolad, M. R., & Panthee, D. R. (2012). Marker-Assisted Selection in Tomato Breeding. *Critical Reviews in Plant Sciences, 31*(2), 93-123. doi: 10.1080/07352689.2011.616057

Gomez-Roldan, V., Fermas, S., Brewer, P. B., Puech-Pages, V., Dun, E. A., Pillot, J.-P., . . . Rochange, S. F. (2008). Strigolactone inhibition of shoot branching.

Kohlen, W., Charnikhova, T., Liu, Q., Bours, R., Domagalska, M. A., Beguerie, S., . . . Ruyter-Spira, C. (2011). Strigolactones Are Transported through the Xylem and Play a Key Role in Shoot Architectural Response to Phosphate Deficiency in Nonarbuscular Mycorrhizal Host Arabidopsis. *Plant Physiology, 155*(2), 974-987.

Ledger, S. E., Janssen, B. J., Karunairetnam, S., Wang, T., & Snowden, K. C. (2010). Modified CAROTENOID CLEAVAGE DIOXYGENASE8 expression correlates with altered branching in kiwifruit (*Actinidia chinensis*). *New Phytologist, 188*(3), 803-813.

Liang, J., Zhao, L., Challis, R., & Leyser, O. (2010). Strigolactone regulation of shoot branching in chrysanthemum (*Dendranthema grandiflorum*). *Journal of Experimental Botany, 61*(11), 3069-3078.

Malik, H., Rutjes, F. P. J. T., & Zwanenburg, B. (2010). A new efficient synthesis of GR24 and dimethyl A-ring analogues, germinating agents for seeds of the parasitic weeds *Striga* and *Orobanche spp*. *Tetrahedron, 66*(35), 7198-7203. doi: DOI: 10.1016/j.tet.2010.06.072

Matusova, R., Rani, K., Verstappen, F. W. A., Franssen, M. C. R., Beale, M. H., & Bouwmeester, H. J. (2005). The strigolactone germination stimulants of the plant-parasitic *Striga* and *Orobanche* spp.are derived from the carotenoid pathway. *Plant Physiol., 139*(2), 920-934. doi: 10.1104/pp.105.061382

Müller, S., Hauck, C., & Schildknecht, H. (1992). Germination stimulants produced by *Vigna unguiculata Walp* cv. Saunders Upright. *Journal of Plant Growth Regulation, 11*(2), 77-84. doi: 10.1007/bf00198018

Ofir, M., & Kigel, J. (1998). Abscisic acid involvement in the induction of summer-dormancy in Poa bulbosa, a grass geophyte. *Physiologia Plantarum, 102*(2), 163-170. doi: 10.1034/j.1399-3054.1998.1020202.x

Seleznyova, A. N., Thorp, T. G., Barnett, A. M., & Costes, E. (2002). Quantitative analysis of shoot development and branching patterns in *Actinidia*. *Annals of Botany, 89*(4), 471-482. doi: 10.1093/aob/mcf069

Subbaraj, A., Funnell, K., & Woolley, D. (2010). Dormancy and flowering are regulated by the reciprocal interaction between cytokinin and gibberellin in *Zantedeschia*. *Journal of Plant Growth Regulation, 29*(4), 487-499. doi: 10.1007/s00344-010-9160-1

Umehara, M., Hanada, A., Yoshida, S., Akiyama, K., Arite, T., Takeda-Kamiya, N., . . . Yamaguchi, S. (2008). Inhibition of shoot branching by new terpenoid plant

hormones. [10.1038/nature07272]. *Nature, 455*(7210), 195-200. doi: http://www.nature.com/nature/journal/v455/n7210/suppinfo/nature07272_S1.html

Vogel, J. T., Walter, M. H., Giavalisco, P., Lytovchenko, A., Kohlen, W., Charnikhova, T., . . . Klee, H. J. (2010). SlCCD7 controls strigolactone biosynthesis, shoot branching and mycorrhiza-induced apocarotenoid formation in tomato. *The Plant Journal, 61*(2), 300-311. doi: 10.1111/j.1365-313X.2009.04056.x

Yamazaki, H., Nishijima, T., Koshioka, M., & Miura, H. (2002). Gibberellins do not act against abscisic acid in the regulation of bulb dormancy of *Allium wakegi* cv. Araki. *Plant Growth Regulation, 36*(3), 223-229. doi: 10.1023/a:1016577529378

Yoneyama, K., Awad, A. A., Xie, X., Yoneyama, K., & Takeuchi, Y. (2010). Strigolactones as germination stimulants for root parasitic plants. *Plant and Cell Physiology, 51*(7), 1095-1103. doi: 10.1093/pcp/pcq055

Yoneyama, K., Takeuchi, Y., Ogasawara, M., Konnai, M., Sugimoto, Y., & Sassa, T. (1998). Cotylenins and fusicoccins stimulate seed germination of *Striga hermonthica* (Del.) *Benth* and *Orobanche minor* Smith. *Journal of Agricultural and Food Chemistry, 46*(4), 1583-1586. doi: 10.1021/jf970964i

Yoneyama, K., Xie, X., Yoneyama, K., & Takeuchi, Y. (2009). Strigolactones: structures and biological activities. *Pest Management Science, 65*(5), 467-470. doi: 10.1002/ps.1726