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Full Length Research Paper

Endogenous gibberellin levels during early fruit development of macadamia

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Gibberellins play a key role in flower and fruit development, and fruits often contain high gibberellin concentrations. This study quantified endogenous gibberellins in flowers and immature fruits of the macadamia cultivars, H2 and 246, to assist in developing growth-regulator treatments for improving fruit set. GA_1, GA_3, GA_8, GA_9 and GA_{20} was detected in flowers or fruits of both cultivars, and GA_4 were detected in flowers of cultivar 246. The timing of gibberellin accumulation was consistent between cultivars but total concentrations were very low (< 7 pmol g^{-1} fresh weight). Low gibberellin levels are consistent with the inability of the gibberellin-synthesis inhibitor, paclobutrazol, to influence fruit retention in macadamia.

Key words: Abscission, fruit drop, kernel, nut, paclobutrazol, plant growth regulators, Proteaceae.

INTRODUCTION

Macadamia (*Macadamia integrifolia*, *Macadamia tetraphylla* and hybrids) is a subtropical evergreen tree that is grown in plantations in South Africa, Australia, Hawaii and Brazil. Macadamia trees produce masses of racemes in spring, each bearing 100 to 300 flowers (Trueman and Turnbull, 1994a; Olesen et al., 2011). More than 98% of flowers and immature fruits abscise during the first 10 weeks after anthesis (Trueman and Turnbull, 1994b). Fruits reach maturity about 24 weeks post-anthesis, after which they fall to the orchard floor and are harvested and dehusked to provide the nut-in-shell and edible kernel (Trueman et al., 2000, 2002; Walton and Wallace, 2008, 2009).

Auxins, cytokinins, gibberellins and the gibberellin-synthesis inhibitor, paclobutrazol, have been tested on macadamia to reduce flower and fruit drop but none has increased final fruit set (Williams, 1980; Trueman, 2010a). The cytokinin, benzyladenine, increases fruit retention for up to 8 weeks after anthesis but does not increase fruit set beyond the major abscission period at 10 weeks post-anthesis (Trueman, 2010a). Endogenous cytokinin levels are very high in macadamia fruits around 10 weeks post-anthesis, which suggests that fruits may be, in effect, cytokinin-saturated and insensitive to applied cytokinins at this stage (Trueman, 2010b).

Gibberellins also play a key role in flower and fruit development (Wilkie et al., 2008; de Jong et al., 2009), and fruits often contain high levels of endogenous gibberellins (Zhang et al., 2007; Ayele et al., 2010). This paper describes the identification and quantification of gibberellins in developing macadamia fruits, and discusses their relationship with the effects of gibberellic acid and paclobutrazol applications on fruit retention (Trueman, 2010a). Such information is useful for developing growth regulator treatments to improve macadamia yield and kernel quality.

MATERIALS AND METHODS

Fruit samples

Fruits were collected from macadamia cultivars 'H2' ('Hinde') and '246' ('Keauhou') at Hidden Valley Plantations, Beerwah, Queensland (26°50'S 152°56'E). Racemes were selected and tagged on four trees of each cultivar, approximately 3 d pre-anthesis (28 Sep 1989). All selected racemes were pollinated using the test-tube method of Trueman and Turnbull (1994a) and Wallace et al. (1996), with cv. H2 racemes pollinated using cv. 246 pollen, and cv. 246 racemes pollinated using cv. H2 pollen. Fruit samples were collected 2, 4, 7, 11, 14, 21, 30, 36, 42, 56 and 70 days post-anthesis. Each sample consisted of all the fruits from four racemes, with one raceme from each tree. Samples at 2 to 7 days post-anthesis also included sepals that had not yet abscised from some fruits. Fresh weights of all samples were recorded, and samples

Table 1. Gibberellin concentrations (pmol g^{-1} fresh weight) in macadamia cv. H2 fruits.

	Time post-anthesis (d)										
	2	**4**	**7**	**11**	**14**	**21**	**30**	**36**	**42**	**56**	**70**
GA_1	0.78	nd	nd	nd	nd	0.40	0.29	1.26	0.52	0.57	nd
GA_3	nd	nd	nd	0.49	0.92	0.75	1.45	nd	nd	1.33	1.36
GA_4	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
GA_8	4.75	0.55	0.33	nd	nd	nd	0.91	nd	0.33	nd	nd
GA_9	nd	nd	nd	nd	nd	3.20	2.63	nd	nd	nd	nd
GA_{20}	nd	0.81	nd	nd	0.39	1.96	0.42	0.81	0.33	4.10	0.60
Total	5.53	1.36	0.33	0.49	1.31	6.31	5.70	2.07	1.18	6.00	1.96

nd' = Not detected.

were stored in liquid nitrogen. Extractions were performed on entire samples except for the 56 to 70 days samples, where only two fruits were used per extract due to the higher fresh weights of individual fruits.

Extraction procedure

Tissue samples were homogenised with an Ultra-Turrax tissue disintegrator together with 90% methanol (10 ml g^{-1} fresh weight) and 330 Bq of [1,2-^{3}H]GA_1 (1.21 x 10^{12} Bq $mmol^{-1}$). Extracts were cleared by centrifugation. Insoluble material was reextracted in 90% methanol. The combined extracts were reduced to an aqueous residue by rotary evaporation at 40°C. The extract was frozen to -20°C, then thawed and centrifuged (10000 g for 5 min) to remove precipitated lipids and chlorophyll. The extract was then passed through a column of insoluble polyvinylpolypyrrolidone (6 ml g^{-1} fresh weight) to remove phenolic compounds. Three volumes of water were then passed through the column to recover the gibberellins. The eluate was rotary evaporated to < 3 ml, adjusted to pH 2.9 with HCl, and extracted 5 times with 2/3 volume of ethyl acetate. The combined ethyl acetate fractions were washed with water to remove excess acid, rotary evaporated to dryness, dissolved in 2 ml methanol, and then desiccated. The gibberellins were methylated with ethereal diazomethane, and the sample was again rotary evaporated to dryness.

HPLC

Gibberellins were fractionated on a Shandon ODS 5 µm Hypersil column (250 × 4.6 mm) using a gradient of methanol in water at a flow rate of 1 ml min^{-1}. Samples were firstly dissolved in 900 µl of 30% methanol and centrifuged at 10000 g for 5 min to remove particulate matter prior to injection. Samples were then fractionated using the following gradient: 35% for 4 min, 35 to 47% over 7 min, 47 to 87% over 10 min, 87 to 100% over 5 min, and 100% for 5 min. Thirty-five 0.6-min fractions were collected from 6 to 27 min. All collected fractions were dried in a Savant SVC100H centrifugal sample concentrator at 45°C, and then dissolved in 200 µl water. Recoveries of [^{3}H]GA_1 were estimated from the appropriate HPLC fractions to be 6 to 73%.

Radioimmunoassay

Radioimmunoassay was performed on all HPLC fractions. An antiserum against GA_1 was used. This antiserum had a broad specificity for C_{19}-GAs, which allowed accurate simultaneous measurement of GA_1, GA_3, GA_4, GA_{20} and, at lower sensitivity, GA_8 and GA_9. All samples and standards were assayed in duplicate. Each assay tube contained 100 µl Tris-HCl buffer (0.1 M pH 7.1) plus 0.1 M NaCl, 200 µg bovine immunoglobulin and 170 Bq tracer ([^{3}H]GA_1-Me, corresponding to a concentration of 0.7 nM). To this was added 50 µl of standard (GA_1-Me, 5 – 5000 pg) or sample. Water was used for determination of B_{max}. Finally 50 µl of appropriate diluted antiserum was added (water for non-specific binding blank) and the tubes were mixed and incubated for 1 h. Next, 200 µl of saturated ammonium sulphate solution was added, thoroughly mixed and left to precipitate the antibody-antigen complexes for 20 min. Tubes were centrifuged (10000 g for 3 min) and supernatants discarded. Pellets were washed with 150 µl of 50% saturated ammonium sulphate, centrifuged as before and decanted again. Pellets were dissolved in 225 µl water, mixed thoroughly for 1 h, before 1.2 ml of Beckman ReadyValue scintillation cocktail was added. Tubes were counted on a Packard Minaxi 4000 scintillation counter for 2 min. Data was processed using a custom-written program. The assays were calibrated using a least-squares linear regression plot of logit [B/B_{max}] *v.* log [standard concentration]. Compounds were identified on the basis of immunoreactivity and HPLC retention times, compared with standard gibberellins. The lowest standard (5 pg) was regarded as the detection limit for all samples.

RESULTS

Compounds with HPLC and radioimmunoassay behaviour of GA_1, GA_3, GA_8, GA_9 and GA_{20} were detected in extracts of both cultivars, H2 (Table 1) and 246 (Table 2). GA_4 was only detected in the 2- and 4-days post-pollination extracts of cv. 246 flowers. The timing of gibberellin accumulation was consistent between cultivars (Figure 1), but the total concentrations of extractable gibberellins were very low (max. 6.31 pmol g^{-1} fresh weight) throughout the first 70 d after anthesis (Tables 1 and 2).

DISCUSSION

Macadamia fruits contained several gibberellins (viz. GA_1, GA_3, GA_4 and GA_9) that have also been detected at low concentrations (max. 11.48 pmol g^{-1} fresh weight) in apical buds or lateral buds of macadamia cv. 344 (Fletcher and Mader, 2007). The gibberellin levels in macadamia fruits (< 7 pmol g^{-1} fresh weight) were much

Table 2. Gibberellin concentrations (pmol g^{-1} fresh weight) in macadamia cv. 246 fruits.

	Time post-anthesis (d)									
	2	**4**	**7**	**14**	**21**	**30**	**36**	**42**	**56**	**70**
GA_1	nd	0.37	nd	0.83	1.09	0.40	0.55	0.55	0.46	0.40
GA_3	nd	nd	nd	0.87	0.55	1.24	nd	nd	1.71	2.57
GA_4	0.36	0.51	nd	nd	nd	nd	nd	nd	nd	nd
GA_8	0.47	0.74	nd	nd	0.80	nd	nd	nd	nd	nd
GA_9	0.44	nd	0.47	nd	1.11	nd	nd	nd	nd	0.32
GA_{20}	1.11	0.99	nd	0.75	1.84	nd	0.45	0.48	1.33	0.87
Total	2.38	2.61	0.47	2.45	5.39	1.64	1.00	1.03	3.50	4.16

'nd' = Not detected.

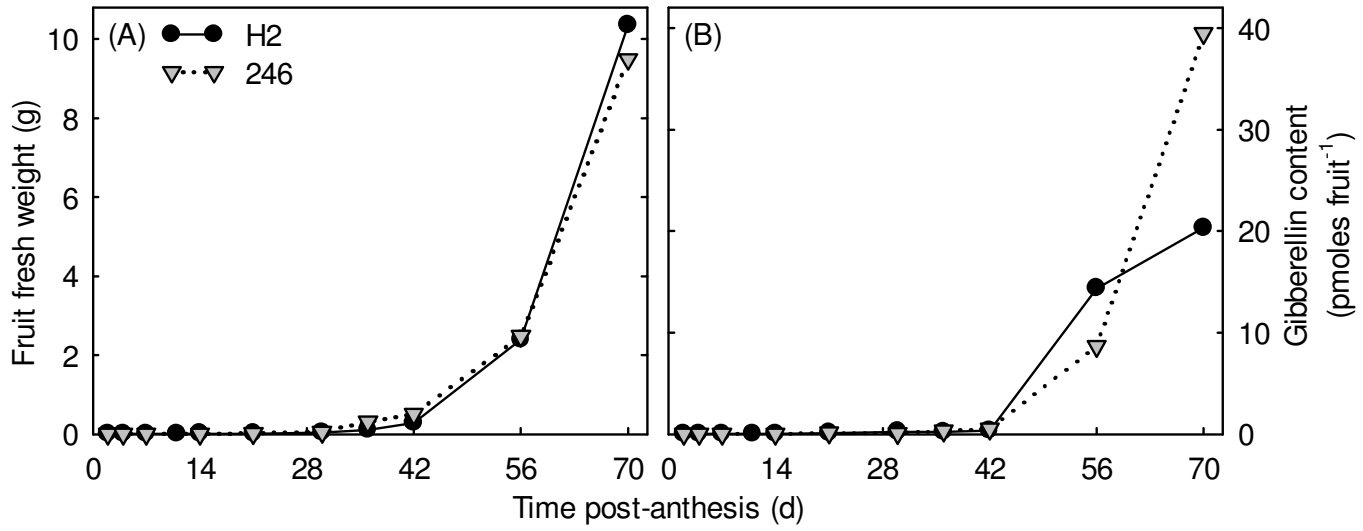

Figure 1. Fresh weight of individual fruit (A) and total contents of extractable gibberellins in the fruit (B) of macadamia cultivars, H2 and 246.

lower than the levels detected, for example, in early developing fruits of tomato and pear (~ 250 and 662 pmol g^{-1} fresh weight, respectively) and in almost-ripe fruits of Christmas rose (~ 483 pmol g^{-1} fresh weight) (Serrani et al., 2007; Zhang et al., 2007; Ayele et al., 2010). Macadamia buds do, however, contain higher levels of GA_7 (Fletcher and Mader, 2007). GA_4, GA_7 and GA_{20} are most likely converted from GA_9, and the other gibberellins are then produced directly (GA_1) or indirectly (GA_3 and GA_8) from GA_{20} (Sponsel, 1995; Ward et al., 2010).

The current results show that macadamia flowers and fruits contain very low gibberellin levels during the periods of fertilization, endosperm development and early embryo development. Macadamia pollen tubes reach the ovary within 7 days of pollination (Trueman and Turnbull, 1994a; Wallace et al., 1996) and endosperm develops rapidly for several weeks before cell walls are laid down between 35 and 77 days post-anthesis (Hartung and Storey, 1939; Sedgley, 1981; Strohschen, 1986). Embryo development is more gradual, but the first zygotic division occurs approximately 35 days post-anthesis and the endosperm is completely replaced by cotyledons by 140 d post-anthesis (Hartung and Storey, 1939; Sedgley, 1981; Strohschen, 1986). The very low gibberellin concentrations during endosperm and early embryo development of macadamia contrast with many other species, where gibberellin levels are high during early fruit development (Serrani et al., 2007; Zhang et al., 2007; Ozga et al., 2009; Ward et al., 2010).

The very low gibberellin levels in macadamia flowers and immature fruits explain, in part, the inability of paclobutrazol applications to influence fruit retention during the early phases of flower and fruit drop (Trueman, 2010a). Paclobutrazol inhibits the conversion of *ent*-kaurene to *ent*-kaurenol early in the gibberellin synthesis pathway and, thus, reduces endogenous gibberellin levels (Sponsel, 1995). Immature macadamia fruits contain very low gibberellin levels and are, therefore,

unlikely to respond to gibberellin-synthesis inhibitors. Fruit drop is also unaffected by GA_3 application (Trueman, 2010a), which tends to indicate that the processes involved in macadamia fruit retention are not sensitive to gibberellin concentrations.

Abscission of immature macadamia fruits occurs in three phases around 2, 6 and 10 weeks post-anthesis (Trueman and Turnbull, 1994b). The first phase results from fertilization failure in some flowers, but the second and third phases are strongly related to available carbohydrate levels, possibly representing an adjustment of crop load prior to the major period of biomass accumulation (Trueman and Turnbull, 1994a, b; Trueman and Wallace, 1999; McFadyen et al., 2011). Future attempts to regulate macadamia yield through the use of plant growth regulators could focus on treatments that manipulate resource supply and source-sink relationships during the final stages of immature fruit drop around 10 weeks post-anthesis.

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REFERENCES

Ayele BT, Magnus V, Mihaljević S, Prebeg T, Čož-Rakovac R, Ozga JA, Reinecke DM, Mander LN, Kamiya Y, Yamaguchi S, Salopek-Sondi B (2010). Endogenous gibberellin profile during Christmas rose (*Helleborus niger* L.) flower and fruit development. J. Plant Growth Regul., 29: 194-209.

de Jong M, Mariani C, Vriezen WM (2009). The role of auxin and gibberellin in tomato fruit set. J. Exp. Bot., 60: 1523-1532.

Fletcher AT, Mader JC (2007). Hormone profiling by LC-QToF-MS/MS in dormant *Macadamia integrifolia*: Correlations with abnormal vertical growth. J. Plant Growth Regul., 26: 351-361.

Hartung ME, Storey WB (1939). The development of the fruit of *Macadamia ternifolia*. J. Agric. Res., 59: 397-406.

McFadyen LM, Robertson D, Sedgley M, Kristiansen P, Olesen T (2011). Post-pruning shoot growth increases fruit abscission and reduces stem carbohydrates and yield in macadamia. Ann. Bot., 107: 993-1011.

Olesen T, Huett D, Smith G (2011) The production of flowers, fruit and leafy shoots in pruned macadamia trees. Funct. Plant Biol., 38: 327-336.

Ozga J, Reinecke DM, Ayele BT, Ngo P, Nadeau C, Wickramarathna AD (2009). Developmental and hormonal regulation of gibberellin biosynthesis and catabolism in pea fruit. Plant Physiol., 150: 448-462.

Sedgley M (1981). Early development of the *Macadamia* ovary. Aust. J. Bot., 29: 185-193.

Serrani JC, Sanjuán R, Ruiz-Rivero O, Fos M, García-Martínez JL (2007). Gibberellin regulation of fruit set and growth in tomato. Plant Physiol., 145: 246-257.

Sponsel VM (1995). The biosynthesis and metabolism of gibberellins in higher plants. In: Davies PJ (ed.) Plant Hormones. Dordrecht: Kluwer Academic Publishers, pp. 66-97.

Strohschen B (1986). Contributions to the biology of useful plants. 4. Anatomical studies of fruit development and fruit classification of the macadamia nut (*Macadamia integrifolia* Maiden and Betche). Angew. Bot., 60: 239-247.

Trueman SJ (2010a). Benzyladenine delays immature fruit abscission but does not affect final fruit set or kernel size of *Macadamia*. Afr. J. Agric. Res., 5: 1523-1530.

Trueman SJ (2010b). Endogenous cytokinin levels during early fruit development of macadamia. Afr. J. Agric. Res., 5: 3402-3407.

Trueman SJ, McConchie CA, Turnbull CGN (2002). Ethephon promotion of crop abscission for unshaken and mechanically shaken macadamia. Aust. J. Exp. Agric., 42: 1001-1008.

Trueman SJ, Richards S, McConchie CA, Turnbull CGN (2000). Relationships between kernel oil content, fruit removal force and abscission in macadamia. Aust. J. Exp. Agric., 40: 859-866.

Trueman SJ, Turnbull CGN (1994a). Effects of cross-pollination and flower removal on fruit set of macadamia. Ann. Bot., 73: 23-32.

Trueman SJ, Turnbull CGN (1994). Fruit set, abscission and dry matter accumulation on girdled branches of macadamia. Ann. Bot., 74: 667-674.

Trueman SJ, Wallace HM (1999). Pollination and resource constraints on fruit set and fruit size of *Persoonia rigida* (Proteaceae). Ann. Bot., 83: 145-155.

Wallace HM, Vithanage V, Exley EM (1996). The effect of supplementary pollination on nut set of *Macadamia* (Proteaceae). Ann. Bot. 78: 765-773.

Walton DA, Wallace HM (2008). Postharvest dropping of macadamia nut-in-shell causes damage to kernel. Postharvest Biol. Technol., 49: 140-146.

Walton DA, Wallace HM (2009). Delayed harvest reduces quality of raw and roasted macadamia kernels. J. Sci. Food Agric., 89: 221-226.

Ward DA, MacMillan J, Gong F, Phillips AL, Hedden P (2010). Gibberellin 3-oxidases in developing embryos of the southern wild cucumber, *Marah macrocarpus*. Phytochemistry, 71: 2010-2018.

Wilkie JD, Sedgley M, Olesen T (2008). Regulation of floral initiation in horticultural trees. J. Exp. Bot., 59: 3215-3228.

Williams RR (1980). Control of premature fruit drop in Macadamia integrifolia: effects of naphthalene acetic acid application, cincturing, and shoot tip removal. Aust. J. Exp. Agric. Anim. Husb., 20: 740-742.

Zhang C, Tanabe K, Tani H, Nakajima H, Mori M, Sakuno E (2007). Biologically active gibberellins and abscisic acid in fruit of two late-maturing Japanese pear cultivars with contrasting fruit size. J. Am. Soc. Hortic. Sci., 132: 452-458.