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*Original Article*

# Endogenous hormonal status in Pummelo fruitlets cultivar Thong Dee: relationship with pre-harvest fruit drop

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**Abstract**

Relationship between endogenous hormonal status with pre-harvest fruit drop in pummelo fruitlets cultivar Thong Dee was studied during January 2013-August 2013. The results indicated that the concentration of IAA in normal and dropped fruitlets tended to decrease gradually during fruit development and the concentration of $GA_3$ in normal and dropped fruitlets increased continually throughout the study. However, the concentration of ABA in normal and dropped fruitlets decreased during fruit development. The concentration of IAA and $GA_3$ in normal fruitlets were significantly higher than those in dropped fruitlets during fruit development, whereas the concentration of ABA in normal fruitlets were significantly lower than that in dropped fruitlets. Consideration of plant growth promoters (IAA and $GA_3$) and plant growth inhibitor (ABA) ratio showed that IAA/ABA ratio in normal fruitlets at 6 week, 8 week and 10 week after fruit set were 7.54, 7.99 and 9.42, respectively, which were significantly higher than those in dropped fruitlets (1.81, 3.21 and 3.77, respectively). $GA_3$/ABA ratio in normal fruitlets at 6 week, 8 week and 10 week after fruit set were 4.54, 5.64 and 25.26 respectively which were significantly higher than those in dropped fruitlets (0.65, 3.10 and 6.71, respectively). Moreover, peel and pulp of normal fruitlets had significantly higher IAA concentration (2.63 and 3.85 $mgL^{-1}$) than dropped fruitlets (2.07 and 1.45 $mgL^{-1}$). Peel and pulp of normal fruitlets had also significantly higher $GA_3$ concentration (2.52 and 4.20 $mgL^{-1}$), whereas peel of normal fruitlets had significantly lower ABA concentration (0.39 $mgL^{-1}$) than dropped fruitlets (5.28 $mgL^{-1}$) but ABA concentration in pulp could not be detected either in normal or in dropped fruitlets. For the IAA/ABA and $GA_3$/ABA ratio measurement, it was found that the peel of normal fruitlets had significantly higher IAA/ABA ratio (6.74) than dropped fruitlets (0.39), whereas the peel of normal fruitlets had significantly higher $GA_3$/ABA ratio (6.46) than dropped fruitlets (0.38). Therefore, it was concluded that dropped pummelo fruitlets had the lower auxin and gibberellin concentration than those in normal pummelo fruitlets but had higher ABA concentration than that in normal pummelo fruitlets. For the plant growth promoter and plant growth inhibitor ratio, the normal pummelo fruitlets had higher IAA/ABA and $GA_3$/ABA ratios than those in the dropped pummelo fruitlets. Balance of plant hormones is involved in pre-harvest fruit drop in pummelo cultivar Thong Dee.

**Keywords:** endogenous hormonal status, pummelo, pre-harvest fruit drop, Thong Dee

## 1. Introduction

Fruit set is defined as the transition of a quiescent ovary to a rapidly growing young fruit, which is an important process in the sexual reproduction of flowering plants. Fruit set depends on the successful completion of pollination and fertilization (Gillaspy *et al.,* 1993). After fruit set, fruit growth and development increase until harvesting. However, pre-harvest fruit drop can occur during fruit growth and development. Many factors play important role in fruit drop of plants. Imbalance plant hormone is one of those factors resulting in pre-harvest fruit drop.

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In citrus, after petal fall, two types of fruitlets can be identified in citrus and other fruit crops as (1) fast-growing, reaching maturity, (2) slow-growing, early senescing (Abbott, 1986; Zacarias *et al*., 1995). Early senescence of slow-growing fruits is proposed as an efficient mechanism for nutrient optimization through the recycling of nutrients from senescing organs and relocation to developing ones (Valpuesta *et al*., 1993). Endogenous hormones and their balance play a modulating of nutrients to the developing organs (Browning, 1986). Gibberellin application can reduce early fruit abscission in parthenocarpic citrus varieties (Fornes *et al*., 1992). On the other hand, abscisic acid (ABA) is known to counteract the physiological effects of gibberellins in fruit growth. Increasing levels of ABA in developing citrus fruits are related to the early fruit abscission in citrus (Talon *et al*., 1990). ABA has been found to be involved in the senescence of slow-growing fruits under temperate environmental conditions (Zacarias *et al*., 1995). However, balance of plant hormone between plant growth promoter (auxin, gibberellic acid and cytokinin) and plant growth inhibitor (ethylene and ABA) has been taken into consideration about fruit drop in fruit crops. Moreover, Ruiz *et al.* (2001) reported that plant hormone concentration has effect on fruit drop in citrus. ABA and jasmonic acid promote fruit drop in citrus and dropped citrus fruits have less plant growth promoter: plant growth inhibitor ratio than those in normal citrus fruits. Sagee and Erner (1991) and Hofman (1988) found that, in sweet orange cv. Valencia and Shamouti, leafy inflorescence–borne fruitlet had faster fruit development than leafless inflorescence-borne fruitlet which trend to drop in the early stage of fruit growth. However, analyzing endogenous hormones found no significant difference in GA-like activity between leafy inflorescence–borne fruitlet and leafless inflorescence-borne fruitlet. ABA concentrations were significantly higher in leafless inflorescence-borne fruitlet than in the leafy inflorescence–borne fruitlet. These results in the ratio of $GA_3$ to ABA in fruitlets be always higher in leafy inflorescence–borne fruitlet than in leafless inflorescence-borne fruitlet. These results were discussed as a reason for better fruit set in leafy inflorescence–borne fruitlet than leafless inflorescence–borne fruitlet.

In pummelo, no information is available concerning the role of endogenous growth promoters and growth inhibitors in the regulation of early fruit abscission at 6-10 weeks after fruit set. Therefore, the goal of this study was to provide evidence for the hypothesis that fruitlet abscission in pummelo is a function of the plant growth promoter and growth inhibitor ratio.

## 2. Material and Methods

### 2.1 Plant material

Pummelo cultivar Thong Dee in a commercial orchard grown in Nakhon Pathom province was used for the study. Fifty pummelo inflorescences per tree (five trees with the same age and the same size used for the study) were selected at full bloom stage for getting the same fruit age for fruit set evaluation. After fruit set, twenty fruitlets per tree were selected totally 100 fruits used for this study. The research was conducted during January 2013-August 2013.

### 2.2 Plant hormone evaluation during fruit development

Five pummelo fruitlets per tree were collected at 6 week, 8 week and 10 week after fruit set were taken for plant hormone analysis (auxin, gibberellic acid and abscisic acid) using Kelen *et al.* (2004)'s method due to the suitable fruit size and weight for sample preparation in plant hormone analysis.

### 2.3 Plant hormone in dropped and normal fruits

Dropped fruitlets and normal fruitlets at the same age (6 , 8 and 10 week after fruit set) were sampled for auxin, gibberellic acid and abscisic acid analysis using Kelen *et al.* (2004) 's method. Plant growth promoters (auxin, IAA and gibberellic acid, $GA_3$): plant growth inhibitor (abscisic acid, ABA) ratio was measured. At four months after fruit set, four normal fruitlets and four dropped fruitlets per tree were collected. Peel and pulp of those fruits were separated for IAA, $GA_3$ and ABA analysis.

### 2.4 Plant hormone analysis

#### 2.4.1 Chemicals and reagents

Analytical reagent grade chemicals were used, unless otherwise indicated. Water, with a conductivity lower than 0.05 S/cm, and acetonitrile (Merck) were of HPLC grade. Sodium hydroxide (Merck) and phosphoric acid (Merck) were used for pH adjustment. Potassium hydrogen phthalate (dried at 110°C before use, Fluka) was used as the reference value standard. Methanol, phosphate buffer components, ethyl acetate, hydrochloric acid, diethyl ether and sodium sulfate were all of analytical purity (Merck) and were used for extraction of the plant hormones. ABA, IAA and $GA_3$ were all of standard purity (Sigma) and used without further purification. The stock standard solutions (1000 ppm) of these hormones for LC studies were prepared by dissolving in the HPLC mobile phase. Working solutions were prepared by diluting the stock solutions with the same HPLC mobile phase at appropriate concentrations. Working solutions were prepared fresh on the day of use. These solutions were filtered before injections.

#### 2.4.2 Extraction

Ten grams of fresh tissue per sample was homogenized with 70% (v/v) methanol and stirred overnight at 4°C. The extract was filtered through a Whatman filter and the methanol evaporated under vacuum. The aqueous phase was adjusted to pH 8.5 with 0.1 M phosphate buffer and then partitioned with ethyl acetate 3 times. After removal of the ethyl acetate phase, the pH of the aqueous phase was adjusted to 2.5 with 1 N HCl. The solution was partitioned with diethyl ether 3 times, and then passed through andydrous sodium sulfate. After that the diethyl ether phase was evaporated under vacuum and the dried residue containing hormones was dissolved in 2.0 mL of methanol and stored in vials at 4°C

#### 2.4.3 Apparatus

The chromatographic analysis was performed on a Shimadzu Model LC. The chromatographic system consists of a Shimadzu Model LC 10 ADVP pump with an auto injector (SIL 10 AD VP) and diode array detector (SPDM 10 A DAD). This equipment has a column oven (CTO 10 AVP) and a degasser system (DGU 14 A). The column used was a Luna C18, 250 mm x 4.6 I.D. stainless steel analytical column with 5 m particle size (Phenomonex). The e.m.f. values used to evaluate the pH of the mobile phase were measured with a Mettler Toledo MA 235 pH/ion analysis apparatus using a Hanna HI 1332 Ag/AgCl combination pH electrode. All solutions were externally thermostated at 25±0.1°C. The electrode was stabilized in appropriate acetonitrile : water mixtures before the e.m.f. measurements. In this study, pH measurements in acetonitrile-water binary mixtures were performed by taking into account the operational definition of pH.

#### 2.4.4 Chromatographic procedure

Throughout this study, the mobile phases used were acetonitrile-water (26:74; 30:70 % ; v/v). In these media, 30 mM phosphoric acid was adjusted to different pH values with sodium hydroxide. The Luna C18 column was equilibrated for each mobile phase condition with a time limit of 30 min. The column temperature was maintained at constant 25±0.1°C. The separation was carried out by isocratic elution with a flow rate of 0.8 mL/min. An injection volume of 10 µL was used for each analysis. The standard solution of the individual acid was prepared in the mobile phase and chromatographed separately to determine the retention time for each acid. The signal of the compounds was monitored at 208, 265 and 280 nm for $GA_3$, ABA and IAA, respectively. Capacity factors were calculated from k = (tR-to)/to, where to was the hold-up time, and tR was the retention time of each hormone for each mobile phase. In this equation the hold-up time, to, was established for every mobile phase composition using potassium bromide solution [Merck, 0.01% (w/v) in water, λmax= 200 nm]. The retention times and capacity factors of the solutes were determined from 3 different injections. Peak identification was based on retention time and spiking of the sample.

### 2.5 Statistical analysis

All the parameters were statistically tested by the independent sample t-test method at the .05 significant levels. The data of IAA/ABA and $GA_3$/ABA were transformed by arcsine formula before statistical analyzing

## 3. Results

Plant hormone analysis showed that the concentration of IAA in normal and dropped fruitlets tended to decrease gradually throughout fruit development and the concentration of $GA_3$ in normal and dropped fruitlets increased continually throughout the study. However, the concentration of ABA in normal and dropped fruitlets decreased during fruit development (Figure 1). The results showed that the concentration of IAA and $GA_3$ in normal fruitlets were significantly higher than those in dropped fruitlets during fruit development, whereas the concentration of ABA in normal fruitlets were significantly lower than that in dropped fruitlets (Table 1). Consideration in plant growth promoters and plant growth inhibiter ratio showed that IAA/ABA ratio in normal fruitlets at 6, 8 and 10 weeks after fruit set were 7.54, 7.99 and 9.42, respectively, which were significantly higher than those in dropped fruitlets (1.81, 3.21 and 3.77, respectively) (Table 1). $GA_3$/ABA ratio in normal fruitlets at 6, 8 and 10 weeks after fruit set were 4.54, 5.64 and 25.26 respectively which were significantly higher than those in dropped fruitlets (0.65, 3.10 and 6.71, respectively) (Table 1).

At 4 month after fruit set, peel and pulp of normal and dropped fruitlets were separated and taken for IAA, $GA_3$ and ABA analysis. The result showed that peel and pulp of normal fruitlets had significantly higher IAA concentration (2.63 and 3.85 $mgL^{-1}$) than dropped fruitlets (2.07 and 1.45 $mgL^{-1}$) (Table 2). Moreover, peel and pulp of normal fruitlets had also significantly higher $GA_3$ concentration (2.52 and 4.20 $mgL^{-1}$) whereas peel of normal fruitlets had significantly lower ABA concentration (0.39 $mgL^{-1}$) than dropped fruitlets

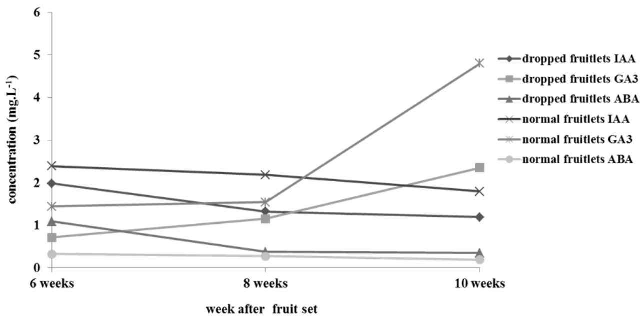


Figure 1. IAA, $GA_3$ and ABA concentration ($mgL^{-1}$) of dropped fruitlets and normal fruitlets during fruit development in pummelo cultivar Thong Dee.

Table 1. Plant hormone concentration ($mgL^{-1}$) in dropped and normal pummelo fruitlets

| Kind of fruitlet | 6 weeks after fruit set | | | | | 8 week after fruit set | | | | | 10 weeks after fruit set | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | IAA | $GA_3$ | ABA | IAA/ ABA | $GA_3$/ ABA | IAA | $GA_3$ | ABA | IAA/ ABA | $GA_3$/ ABA | IAA | $GA_3$ | ABA | IAA/ ABA | $GA_3$/ ABA |
| Dropped fruitlets | 1.98 | 0.71 | 1.09 | 1.81 (7.74) | 0.65 (4.63) | 1.19 | 1.15 | 0.37 | 3.21 (10.33) | 3.10 (10.15) | 1.32 | 2.35 | 0.35 | 3.77 (11.20) | 6.71 (15.02) |
| Normal fruitlets | 2.39 | 1.44 | 0.32 | 7.54 (15.95) | 4.54 (12.31) | 2.18 | 1.54 | 0.27 | 7.99 (16.43) | 5.64 (13.75) | 1.79 | 4.80 | 0.19 | 9.42 (17.88) | 25.26 (30.19) |
| t-test | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * |

* indicates the significant difference at p-value < .05 level analyzed by independent sample t-test. Number in the parenthesis was the transformed data which was statistically analyzed by independent sample t-test.

Table 2. Plant hormone concentration ($mgL^{-1}$) in peel and pulp of pummelo cultivar Thong Dee at 4 month after fruit set.

| Part of pummelo | Kind of fruitlet | IAA | $GA_3$ | ABA |
|---|---|---|---|---|
| Peel | Normal | 2.63 | 2.52 | 0.39 |
| | Dropped | 2.07 | 2.03 | 5.28 |
| t-test | | * | * | * |
| Pulp | Normal | 3.85 | 4.20 | nd |
| | Dropped | 1.45 | 0.84 | nd |
| t-test | | * | * | |

nd = non-detectable
* indicates the significant difference at p-value < .05 level analyzed by independent sample t-test

($5.28\ mgL^{-1}$) but ABA concentration in pulp could not be detected either in normal or in dropped fruitlets (Table 2).

For the IAA/ABA and $GA_3$/ABA ratio measurement, it was found that the peel of normal fruitlets had significantly higher IAA/ABA ratio (6.74) than dropped fruitlets (0.39), whereas the peel of normal fruitlets had significantly higher $GA_3$/ABA ratio (6.46) than dropped fruitlets (0.38) (Figure 2).

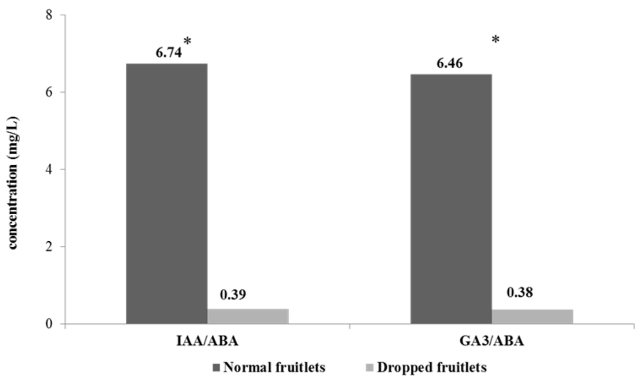


Figure 2. IAA/ABA and $GA_3$/ABA ratio in peel of normal and dropped pummelo fruitlets cultivar Thong Dee at 4 months after fruit set. * indicates the significant difference at .05 level analyzed by independent sample t-test.

## 4. Discussion

Changes in auxin, gibberellin and ABA in normal and dropped fruitlets of pummelo cultivar Thong Dee were studied. Our findings support the hypothesis that a complex hormonal signal which includes auxin, gibberellin as well as inhibiting compounds, basically, ABA are involved in the regulation of abscission processes during fruit development. It was found that IAA concentration in both normal and dropped fruitlets decreased during fruit development whereas $GA_3$ concentration increased during fruit development. It could be that IAA plays important role in cell division which takes place in the beginning stage of fruit growth. That may be in the first months after fruit set. Thus, the highest IAA concentration occurred in that period of fruit growth. As cell division completed, two and three months after fruit set, IAA concentration decreased gradually. As for $GA_3$ concentration, we found that $GA_3$ concentration continually increased during fruit development. $GA_3$ plays important role in cell elongation which takes place in fruit growth and development after cell division has completed. Therefore, it could be that, as cell division has completed after the first months of pummelo fruit development, cell elongation would play an important role in fruit development instead of cell division resulting in $GA_3$ increasing. For ABA concentration, the study found a lower concentration than IAA and $GA_3$ concentration during fruit development. It could be that 1-3 months after fruit set is the period of fruit growth and development which need plant growth promoter activity (IAA and $GA_3$) more than plant growth inhibitor activity (ABA).

From this study, normal fruitlets had higher IAA and $GA_3$ concentration than dropped fruitlets, whereas normal fruitlets had lower ABA concentration than dropped fruitlets. After petal fall, two types of fruitlets can be identified in citrus and other fruit crops: (1) fast-growing, reaching maturity, (2) slow-growing, early senescing (Abbott, 1986; Zacarias *et al*., 1995). In this study, normal fruitlets had fast-growing fruit development, whereas dropped fruitlets had slow-growing fruit development. These may result in the higher IAA and $GA_3$ concentration in normal fruitlets than in dropped fruitlets and the lower ABA concentration in normal fruitlets than in dropped fruitlets. This is according to Pozo (2011) who reported that firm fruits had a higher content of plant growth promoter ($GA_3$) than plant growth inhibitor (ABA). Koukourileou-Petridou (2003) found that June drop of almond fruits started almost simultaneously with the decrease in the concentration of extractable free and ester IAA from seeds and the amount of diffusible free IAA from persisting fruits. Also, it has been reported that increasing levels of ABA in developing citrus fruits are related to the early fruit abscission in citrus (Talon *et al*., 1990). Moreover, ABA acid has been found to be involved in the senescence of slow-growing fruits under temperate environmental conditions (Zacarias *et al*., 1995), whereas Pozo (2001) and Talon *et al*. (1997) found that ABA are involved in the regulation of abscission processes during fruit set in citrus. Moreover, Gomez-Cadenas *et al*. (2000) reported that fruitlet abscission induced by carbon shortage in citrus is regulated by ABA.

Valpuesta *et al.* (1995) reported that early senescence of slow-growing fruits is proposed as an efficient mechanism for nutrient optimization through the recycling of nutrients from senescing organs and relocation to developing ones. Endogenous hormones and their balance play a modulating role in the mobilization of nutrients to the developing organs (Browning, 1986). Therefore, endogenous hormone balance could be also involved in fruit drop of fruit crops including pummelo cultivar Thong Dee in this study. The results of this study showed that normal fruitlets had significantly higher IAA/ABA ratio and $GA_3$/ABA ratio than dropped fruitlets during the study. This result is accordance with the report of Pozo (2001), who found that content of growth promoting (G)/inhibiting substance (I) ratio (G/I ratio) of firmly attached fruitlets was higher than that of non-growing early abscising fruits during the fruit set period. Pozo (2001) also suggested that fruitlets abscission is a function of the preceding hormonal status in reproductive organs. The senescence of abscising fruitlets, expressed by a lower growth rate, could be associated with photosynthate translocation from senescing to developing fruits. This process appears to be regulated by lower G/I values. According to our results, normal fruitlets had higher IAA/ABA and $GA_3$/ABA ratios than dropped fruitlets; IAA and $GA_3$ are the plant growth promoters, whereas ABA is the plant growth inhibitor. Fornes *et al.* (1992) reported that gibberellin can reduce early fruit abscission in citrus. On the other hand, abscisic acid is known to counteract the physiological effects of gibberellins in fruit growth (Talon *et al*., 1990).

Changing of endogenous hormone concentration may be involved in a lowered photosynthate translocation to pummelo fruits resulting in ABA increasing and IAA and $GA_3$ decreasing. This endogenous hormonal status made pre-harvest fruit drop in pummelo. Moreover, the observation of pummelo dropped fruitlets showed seed abortion that may result in less auxin biosynthesis in dropped fruitlets.

## 5. Conclusions

Dropped pummelo fruitlets had the lower auxin and gibberellin concentration than normal pummelo fruitlets but had the higher ABA concentration than normal pummelo fruitlets. For the plant growth promoter and plant growth inhibitor ratio, the normal pummelo fruitlets had higher IAA/ABA and $GA_3$/ABA ratios than dropped pummelo fruitlets. Thus, balance of plant hormones is involved in pre-harvest fruit drop in pummelo cultivar Thong Dee.

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## References

Abbott, D.L. 1986. A tree physiologist's view of growth regulators. Acta Horticulturae. 179, 293-299.

Browning, G. 1986. The physiology of fruit set. In Manipulation of Fruiting, C.U. Wright, editor. Butter Wareh, pp. 195-198.

Fornes, F., Van Rensburg, J., Sanchez-Perales, M., and Guardiola, J.L. 1992. Fruit setting treatments effects on two Clementine mandarin cultivars. Proceedings of the International Society of Citriculture. 1, 489-492.

Gillaspy, G., Ben-David, H. and Gruissem, W. 1993. Fruit: a development perspective. Plant Cell. 5, 1439-1451.

Gomez-Cadenas, A., Mehouachi, J., Tadeo, F.R., Primo-Millo, E. and Talon, M. 2000. Hormonal regulation of fruitlet abscission induced by carbohydrate shortage in citrus. Planta. 210, 636-643.

Hofman, P.J. 1988. Abscisic acid and gibberellins in the fruitlets and leaves of Valencia orange in relations to fruit growth and retention. Proceedings of the International Society of Citriculture. 1, 355- 362.

Kelen, M., Demiralay, E.C., Sen, S. and G. Ozkan. 2004. Separation of abscisic acid, Indole-3-acetic acid, Gibberellic acid in 99 R (Vitis berlandieri x Vitis rupestris) and Rose Oil (*Rosa damascene* Mill) by reversed phase liquid chromatography. Turkey Journal of Chemistry. 28: 603-610.

Koukourileou-Petridou, M.A. 2003. The relation between the levels of extractable and diffusible IAA in almond fruits and their "June drop". Plant Growth Regulation. 39, 107-112.

Pozo, L.V. 2011. Endogenous hormonal status in citrus flowers and fruitlets: relationship with postbloom fruit drop. Scientia Horticulturae. 91, 251-260.

Ruiz, R., Garcia-Luis, A., Monerri, C. and Guardiola, J.L. 2001. Carbohydrate availability in relation to fruitlet abscission in citrus. Annals of Botany. 87(6), 805-812.

Sagee O. and Erner, Y. 1991. Gibberellins and Abscisic acid contents during flowering and fruit set of 'Shamouti' orange. Scientia Horticulturae. 48, 29-39.

Talon, M., Hedden, P. and Primo-Millo, E. 1990. Hormonal changes associated with fruit set and development in mandarins differing in their parthenocarpic ability. Physiologia Plantarum. 79, 400-406.

Talon, M., Tadeo, F.R., Ben-Cheikh, W., Gomez-Cadenas, A., Mehouachi, J., Pérez-Botella, J. and Primo-Millo, E. 1997. Hormonal regulation of fruit set and abscission in citrus: classical concepts and new evidence. Acta Horticulturae. 463, 450-456.

Valpuesta, V., Quesada, M. and Reid, M. 1993. Senescencia y Abscision. In Fisiologiay Bioquimica Vegetal, J. Azcon-Bieto and M. Talon, editors, McGraw-Hill Interamericana de Espana, Madrid, pp. 367-425.

Zacarias, L., Talon, M., Ben-Cheikh, W., Lafuente, M.T. and Primo-Millo, E. 1995. Abscisic acid increases in non-growing and paclobutrazol treated fruits of seedless mandarins. Physiologia Plantarum. 95, 613-619.