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Research Note

Occurrence of natural auxin and accumulation of calcium during early fruit development in kiwifruit

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Abstract

This note tracks for the first time the natural occurrence and metabolism of the plant hormone free indole-3-acetic acid (IAA) in young kiwifruit (*Actinidia deliciosa* var. *deliciosa*, C.F. Liang et A. R. Ferguson). Simultaneous measurements of IAA and fruit calcium (Ca) levels were examined to discover possible correlations. The IAA was detected using gas chromatography-mass spectrometry and Ca was assessed spectrophotometrically. Both determinations were carried out on fruits sampled from field-grown mature vines 6, 13, 19, 27, 35, 45, 55 and 67 days after pollination (DAP). The hormone level was greatest at 6 DAP (55 ±5 ng g^{-1} fresh weight, FW, ±SD) but declined markedly thereafter to a minimum of 5.32 ±0.5 ng g^{-1} FW by 45 DAP (about 6 weeks later). Modulation of free IAA concentration did not appear to depend on conjugation of the hormone. Results show that the IAA (ng g^{-1} FW) and Ca (mg g^{-1} FW) concentrations were closely correlated (R^2=0.92) with a linear regression slope value of 0.061. Although further research is required to identify possible causality, this relationship's possible significance in determining fruit storage quality is discussed.

Keywords: *Actinidia deliciosa*, auxin metabolism, calcium accumulation, indole-3-acetic acid.
Abbreviations: DAP, day after pollination; IAA, indole-3-acetic acid.

Introduction

Auxins are a group of plant hormones whose intricate phenomenology covers a wide range of aspects of growth and development (e.g. cell division/extension, vascular differentiation, apical dominance) (Normanly, 1997; Zhao, 2010). Indole-3-acetic acid (IAA) was the first naturally occurring auxin identified (Davies, 1995) and its synthetic analogues are used extensively to enhance crop production (Basra, 2000). In several fruit crop species including kiwifruit, it has been documented that an exogenous application of auxin influences some fruit traits (e.g. size, firmness, softening, mineral composition) (Basak, 2006; Fabbroni et al., 2007; Lorenzo et al., 2007). In particular the increased size of treated fruit has generated great interest in the use of auxin as a possible way to raise crop value. However, in some cases the application of phytohormones may be ineffective or even detrimental to production. For example, Stern et al. (2007) reported that flesh firmness at harvest (and during storage-life) of *Prunus salicina* was significantly lowered in fruit sprayed with synthetic auxin. Based on the variability of results, Basak (2006) emphasised "the ambiguous and complex effect of bioregulators on the mineral composition of apple trees" (SIC). This uncertainty is likely due to some still unclear interaction among the different classes of growth regulators (including auxin), both exogenously applied and endogenously biosynthesized (Zhao, 2010). Despite the general interest in external applications of auxin, few attempts have been made to determine the natural levels of hormones during fruit growth. The seasonal patterns of IAA content are reported in some fruit tree crops (e.g. peach, almond, citrus) (Lewis et al., 1965; Miller et al., 1987; Koukourikou-Petridou, 2003) while in the case of kiwifruit such specific information is not yet available. Therefore, this study was undertaken primarily to determine levels of endogenous IAA in a growing kiwifruit, to assess its changes during the early developmental stages in which cell division is of critical importance. Additionally, owing to the metabolic role of ester- and amide-conjugated IAA in the regulation of the free hormone level (Normanly, 1997), changes in the concentrations of these molecules were also analysed. In kiwifruit, auxins are known as anti-senescence growth regulators able to delay the ripening process through their interaction with ethylene (Bregoli et al., 2007). Ripening processes and postharvest storage life are also influenced by mineral composition, especially Ca concentration. Maintenance of relatively high Ca concentrations in fruit tissues results in a slower rate of ripening, owing to lower respiration rates, reduced ethylene production, slower softening of flesh and reduced incidence of some post-harvest disorders (Hewett et al. 1999; Ferguson et al. 2003; Thorp et al. 2003). Auxin transport seems to be necessary for Ca transport, as was concluded by Bangerth (1976) from a study on the effects of auxin transport

inhibitors in tomato and apple fruit. A further connection could be the ability of Ca to enhance the binding of auxins through the modification of the membrane binding sites at the cell scale (Ferguson, 1984). Although fruit mineral composition has been widely studied in kiwifruit also in various cultivars (Mohammadian and Koldeh, 2010), calcium accumulation as compared with auxin biosynthesis and metabolism has not been adequately explored. Hence, the second purpose of this study was to determine the amount of Ca accumulated in a kiwifruit and whether it is correlated with the prevailing concentration of endogenous IAA.

Results and discussion

IAA concentration and metabolism

The natural occurrence of IAA shows that it was at its highest concentration in very young fruits (*viz* 6 DAP), peaking at about 55 ng g^{-1} FW (Fig. 1). During the following 20 days the IAA concentration declined sharply toward values close to 11 ng g^{-1} FW, after which the hormone level was relatively stable at about 8-10 ng g^{-1} FW, except for a transient decrease to 5.32 ng g^{-1} recorded on 45 DAP (Fig. 1). The concentrations of both ester- and amide-IAA were quite stable throughout the experiment. The sole relevant oscillation of the level of these molecules occurred at the last sampling date (67 DAP) when the conjugates peaked to their greatest concentration values (about 14 and 40 ng g^{-1} FW for the amide- and ester-IAA, respectively) (Fig. 1). The level changes observed in natural free IAA are hard to compare with the literature because of the scant information on this for kiwifruit. However, the 80% decrease in IAA concentration recorded between 6 and 27 DAP (Fig. 1) is comparable with that observed in peach, almond and citrus fruits (Lewis et al., 1965; Miller et al. 1987; Koukourikou-Petridou 2003). The relatively high IAA concentration observed in young fruit is consistent with it having a stimulating effect on processes which occur intensively at this stage. For example, the pericarp cells are dividing and expanding very rapidly and the seeds (probably the primary sites for auxin synthesis) are also developing rapidly (Hopping, 1976; Davies, 1995; Ozga et al., 2002). Twenty days later, when these processes (pericarp cell division) came to an end or at least are expected to proceed more slowly (seed development) (Hopping 1976), the IAA concentration consistently reached a minimum value (at 27 DAP, Fig. 1). Such a huge decrease in IAA could be partly associated with a decline in some auxin-protecting secondary metabolites (e.g. hydroxycinnamic acids) which occurs at this stage (Montanaro et al., 2007). Apparently, the changes in the concentration of both ester- and amide-IAA did not show any clear relationships with changes in free IAA. The analysis of the conjugated forms of IAA may provide only partial information on the metabolism of the hormone. Also, along with the transport of the hormone, the contributions of biosynthesis and catabolism should be taken into account when attempting to explain changes in IAA levels. However, considering that conjugation may modulate the action of IAA by converting it into an inactive form and that some kind of conjugation reactions may commit IAA to terminal catabolism (Woodward and Bartel 2005), the conjugation activity may plausibly have contributed to keeping the concentration of free IAA at a low level after 27 DAP.

Auxin and calcium accumulation

The fruit Ca content increased exponentially during the early weeks of growth before slowing and eventually reaching an

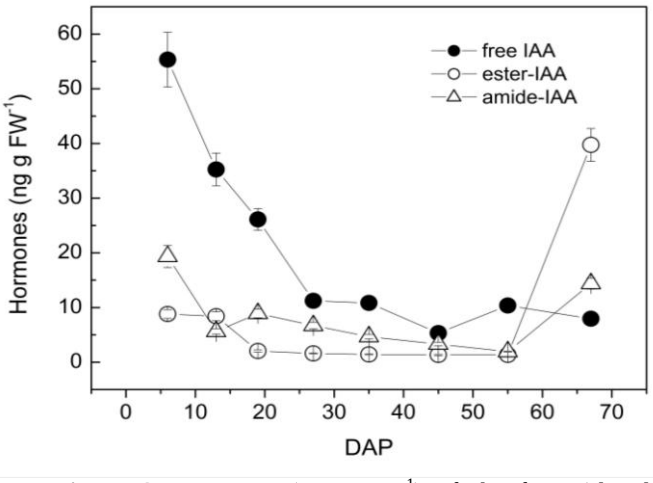

Fig 1. Concentration (ng g FW^{-1}) of the free- (closed symbols) and conjugated- (open symbols) IAA in kiwifruit during early season development. DAP = days after pollination. Each point represents the mean of three bulked samples (± SD).

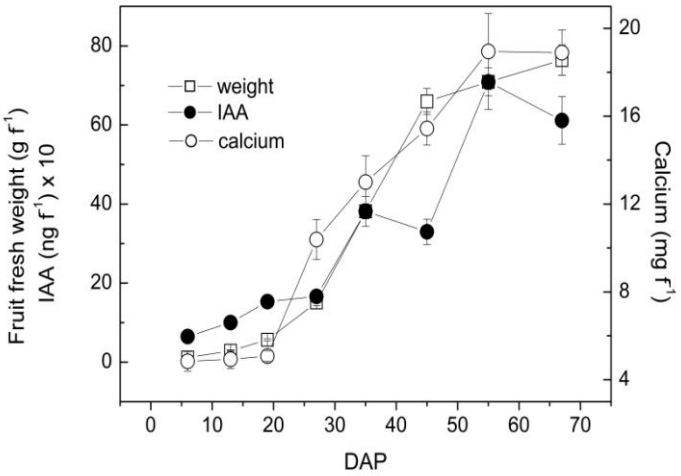

Fig 2. Fruit growth (fresh weight, g) (□), Ca content (mg $fruit^{-1}$) (○), and free IAA (ng $fruit^{-1}$) (●) measured during early season development. Bars are ±SD, DAP = days after pollination.

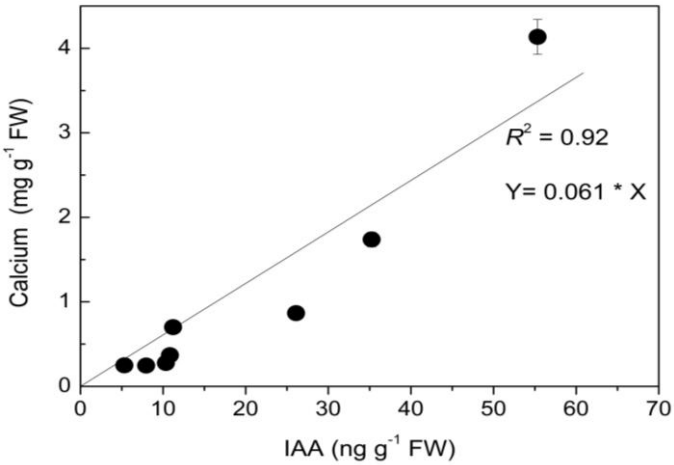

Fig 3. Correlation between free IAA and calcium concentrations observed during early season development.

asymptotic value of approximately 20 mg fruit^{-1} by 55 DAP (Fig. 2). Similarly, during the early 45 days of growth, fruit development is considerable by this time reaching about 72% of their final weight at harvest (Fig. 2). Approximately 20 days later (55 DAP), mean fruit weight attains ~85% of the harvest value confirming the importance of the early growth period for determining final fruit size (Hopping 1976). Both the pattern of fruit Ca concentration change (not shown) and Ca accumulation were substantially in agreement with earlier determinations, the latter showing the typical sigmoidal accumulation curve (Fig. 2) (Clark and Smith, 1988; Montanaro et al. 2006). The time course of the absolute content of free IAA per fruit was similar to those of Ca accumulation and of fruit fresh weight (Fig. 2). In parallel, the changes in Ca and IAA concentrations showed a tight linear relationship with a reasonably high coefficient of determination (R^2 = 0.92) (Fig. 3). Whether these correlated changes are indicative of linkage through some simple mechanism or not cannot be determined from this dataset. The slope value determined (0.061; Fig. 3) should be interpreted as the average number of units of Ca (mg g^{-1} FW) accumulated per unit of free endogenous IAA (ng g^{-1} FW) under natural conditions. How this ratio eventually changes if synthetic auxins are applied remains unclear. The ability of auxin to stimulate Ca uptake has been observed in avocado (Cutting and Bower, 1989), tomato (Banuelos et al., 1987) and apple (Stahly and Benson 1970) suggesting that increased IAA in the pericarp tissues might increase the Ca and maintain the Ca:IAA ratio rather stable. Findings on the linear correlation of IAA *vs* Ca are in line with evidence that auxin positively affects the induction and formation of vascular tissues which are the route for the import of Ca (Dražeta et al., 2004).

Materials and methods

Experimental site and fruit measurements

Trials were conducted on fruits from a commercial 'Hayward' kiwifruit orchard (*Actinidia deliciosa* [A.Chev.] C.F. Liang et A.R. Ferguson) during the 2008 growing season at Rigoli (Pisa, Italy; 43° 46' N, 10° 25' E, ~10 m a.s.l.). Vines were trained as a free Palmette (5.5 × 5.0 m planting distance) and managed organically. To minimise fruit-to-fruit variability, at bloom (May 22) about 400 fully-open flowers from the basal end of terminating fruiting shoots located in the central part of the canopy (1.5-2.5 m above the ground) were selected from 10 randomly chosen vines. Flowers were hand pollinated using pollen previously collected from male flowers of 'Tomuri' vines growing in the same orchard. Throughout the experiment, 20 uniform hand-pollinated fruits were collected from 6 shoots on 6, 13, 19, 27, 35, 45, 55 and 67 days after pollination (DAP), promptly transferred to the laboratory and weighed (fresh weight, FW). To minimise variability (Thorp et al. (2003), fruits were always sampled from the same position in the vine being the second fruit from the basal end of the shoot.

Extraction and purification of IAA

For each sampling time, 11 fruits (skin plus flesh) were cut up manually using a sharp blade and grouped into a single bulk sample. From this, three replicate samples were collected and used for the analyses. Each replicate (5-10 g FW) was processed according to the analytical protocol described in Sorce et al. (2009). Briefly, samples were homogenized, supplemented with a suitable amount of $[^{13}C]_6$ IAA as internal standard for quantitative determination of endogenous IAA and extracted three times with aqueous acetone. The aqueous phase of the pooled extracts was partitioned against diethyl ether. The organic phase was dried, re-suspended in 0.5% (v/v) acetic acid and purified by C18 SPE cartridges. The fractions collected were evaporated, re-suspended in the appropriate starting solvents and purified using HPLC. After diethyl ether partitioning, the aqueous phase was pooled with the extracted pellet and hydrolysed. The first hydrolysis separated the ester-bound IAA and the second separated the amide-bound IAA. At the end of these hydrolyses, samples were further purified by reverse phase HPLC. The HPLC fractions corresponding to the elution volume of the IAA standard were dried thoroughly, silylated and analysed by gas chromatography-mass spectrometry. Mass spectra were acquired in full scan mode. Identification and quantification of the analytes were confirmed by tandem MS. Data presented for each sample are the mean of three replicates ± SD.

Calcium analyses

On each sampling date, 9 fruits similar to those used for IAA determination were collected, the sepals and calyx residues were discarded and the remaining material (flesh plus skin) sliced and dried to constant weight (72 h at 110°C). Fruits were grouped in three bulk samples (×3 fruit each) and Ca concentrations measured on the acid digested samples (H_2SO_4 + HNO_3) using an AA-40 atomic absorption spectrophotometer (Varian, Palo Alto, CA; λ = 422.7 nm; air-acetylene flame).

Conclusion

This note tracks for the first time the natural occurrence of IAA in young kiwifruit and a strong linear correlation between IAA and Ca concentrations is reported. Although more detailed information is needed to elucidate the nature of this correlation, this is to be investigated by applications of exogenous (possibly labelled) IAA and monitoring any changes in the ratio of Ca to natural and synthetic IAA.

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