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ORIGINAL PAPER

Indole-3-acetic acid metabolism and growth in young kiwifruit berry

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Abstract The patterns of auxin concentration and metabolism were investigated in distinct kiwifruit portions and compared with the rate of fruit growth during early developmental stages. Indole-3-acetic acid (IAA) level was higher in inner fruit tissues, particularly in younger fruit, while the hormone was barely detectable in outer tissues. Modulation of free IAA concentration did not appear to depend tightly on conjugation of the hormone. Despite the lack of a strong correlation between the levels of IAA and enzymes involved in its catabolism, in some portions of the fruit a low hormone level corresponded to a higher IAA degradation activity. An inverse correlation was also observed between hormone levels and the appearance/increase in some bands with high mobility in peroxidase gel activity assay. Phenols, compounds with a potential auxin-protecting activity, appeared to be involved mostly in photoprotection of the fruit than in the regulation of IAA levels. Beyond catabolism and conjugation, other metabolic pathways, particularly those occurring in the developing seeds, may have decisively influenced auxin levels in fruit tissues, as well as the amount of the hormone exported from the fruit. The latter, estimated by analyzing the concentration of IAA in the sap exuded from the pedicel, showed a time course which was similar to that displayed by inner fruit tissues. Furthermore, similarities were found between the pattern of IAA concentration in inner fruit tissues and fruit growth rate. The possible role of IAA in promoting growth during early fruit development is discussed.

Keywords Fruit growth · Guaiacol peroxidase · IAA degradation · Indole-3-acetic acid · Kiwifruit · Phenols

Introduction

Most of chemical, structural and functional fruit traits change as fruit develops. In berries of kiwifruit (*Actinidia* spp.), as in other fleshy fruit, the mineral composition, the vascular system and the structure of the skin greatly change during the growing season, in response to several internal and external factors (see Montanaro et al. 2014 for review). A series of physiological processes occurring during fruit growth affect fruit size at harvest, which is a key determinant of yield. Plant growth regulators strongly influence those processes, being major endogenous signals for the regulation of fruit growth (Gillaspy et al. 1993; Davies 2010). In several fleshy fruit species, auxin in combination with gibberellins and cytokinins play a major role in the regulation of fruit set, including the formation of seeds which in turn control cell division and fruit growth (Gillaspy et al. 1993; Kumar et al. 2014). Seed number is then positively correlated with fruit size and prolonged storage life, likely because it influences the accumulation of those nutrients involved in fruit structural stability (e.g., Ca) (Brookfield et al. 1996; Volz et al. 1996; Ferguson et al. 1999; Howpage et al. 2001; McPherson et al. 2001). Fruit calcium accumulation depends, among others, on factors affecting the structure and function of the vascular system and the driving force of xylem stream, which greatly change during the early fruit developmental stage (i.e., 70–80 days after pollination, DAP) (Montanaro et al.

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2014). Considering the relevance of xylem flux for both fruit water balance and nutrient import (Montanaro et al. 2015) and the involvement of auxin in certain steps of xylem formation (Sorce et al. 2013), a detailed knowledge of seasonal changes of auxin concentration and metabolism is of great importance. It has been previously described the natural occurrence of auxin in young kiwifruit (Sorce et al. 2011), however its metabolism has not yet been explored in depth. Therefore this paper aimed at analyzing the time course of indole-3-acetic acid (IAA) concentration and metabolism in kiwifruit, focusing on early fruit developmental stage, when fruit considerably enlarge and attain 80–90% of the final size (Pratt and Reid 1974). The levels of both free and conjugated hormone molecules, as well as the activity of enzymes involved in IAA catabolism (Arezki et al. 2001; Normanly et al. 2010) were determined. Based on the evidence that phenolic compounds may act as IAA-protectants (Krylov and Dunford 1996), potentially affecting IAA levels, this study also aimed at determining the concentration of phenols in order to further elucidate hormone metabolism.

The spatial distribution of minerals in fruit tissues is not uniform, with a higher concentration being found in the inner pericarp (including the seeds) than in either the outer pericarp or the pith; also the abundance of nutrients changes with the tissue considered (e.g., skin, flesh) (Faust et al. 1969; Ferguson 1980; Clark and Smith 1988, 1991). Such a tissue/position variability of nutrients has received increasing attention in relation to the incidence of some diseases (Amarante et al. 2013), however explanations for that variability are still limited. For certain phloem-immobile nutrients, such a gradient could be related to an uneven development of the xylem, in response to auxin signals. Therefore, this study was designed to analyse IAA metabolism separately in various portions of the fruit along a longitudinal (proximal, median, distal) and a transversal gradient (inner and outer). The data of auxin metabolism were compared with those of fruit growth, to search for correlative evidence between hormone physiology and fruit development.

Auxin may have a role in fruit nutrition not only through the impact on xylogenesis but also because of its involvement in the exchange of solutes outside and inside the fruit. For example, an outgoing flux of IAA from the fruit may promote the import of some cations (e.g., Ca^{2+}) (Stahly and Benson 1970; Bangerth 1976; Banuelos et al. 1987; Cutting and Bower 1989; Brown and Ho 1993). Hence, polar auxin transport may be involved in this process. Given that part of polar auxin transport occurs outside the cells, it seems reasonable to assume that the concentration of apoplastic auxin may be an estimate of the potential magnitude of that transport (Bangerth 1976). Since the apoplastic IAA concentration has not been adequately investigated in kiwifruit, this study aimed also at determining its value in the apoplastic sap artificially extruded from the fruit; this may be informative of the potential Ca-IAA exchange in kiwifruit berry.

Materials and methods

Experimental site and fruit sampling

Trials were conducted at a commercial kiwifruit orchard (*Actinidia deliciosa* [A.Chev.] C.F. Liang et A.R. Ferguson cv. Hayward) in central Italy, as previously described (Sorce et al. 2011), in the 2013 growing season. At bloom (May 18th) approx. 400 fully-open flowers from the basal end of terminal 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 and hand pollinated using pollen previously collected from male flowers of the cultivar 'Tomuri' growing in the same orchard. Approximately 30 uniform fruit were collected at 19, 27, 35, 45, 56 and 67 DAP from 6 shoots (×3 vine) at each sampling date, sealed into plastic bags to minimize their transpiration and promptly transported to the laboratory. A final fruit sampling at 139 DAP was executed only for fresh weight determination. At each sampling time, 20 fruit were weighed (fresh weight) and cut into three portions discarding the pedicel; the cutting lines were at 33 and 66% of berry length in order to separate the proximal (P), median (M) and distal (D) portions with similar thickness (Fig. 1); each portion was splitted in two sub-portions, namely 'inner' pericarp (including central columella and seeds) and 'outer' pericarp (including skin) (Fig. 1). These

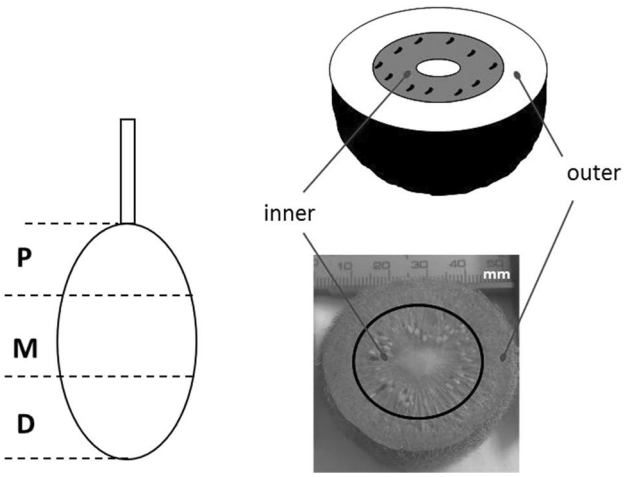

Fig. 1 Schematic representation of (*left*) the different fruit portions (*D* distal; *M* median; *P* proximal) and (*right*) tissues (*inner* and *outer*) analysed in this study

sub-portions were frozen and analyzed separately for IAA concentration, IAA catabolic enzyme activities and phenols. The remaining ~10 fruit were used for the collection of the sap as described below.

Indole-3-acetic acid analysis in fruit tissues

From each sub-portion three replications were made. Samples were chopped, dipped in cold 70% (v/v) aqueous acetone (1:5, w/v) and homogenized by mortar and pestle. Each replication (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, resuspended in the appropriate starting solvents and purified using HPLC. After diethyl ether partitioning, the aqueous phase was pooled with the extracted pellet and hydrolyzed. The first hydrolysis separated the ester-bound IAA and the second one 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 analyzed by gas chromatography-mass spectrometry. Mass spectra were acquired in full scan mode. Identification and quantification of the analyte were confirmed by tandem MS. Data presented for each sample are the mean of three replications ± SE and are expressed as ng g^{-1} FW.

Fruit sap collection and IAA analysis

The apoplastic sap was collected by a Scholander pressure chamber using a procedure similar to that reported by Albacete et al. (2008). For each sampling date ~10 fruit were used. Fruit pedicel was cut at the proximal end and inserted into a silicone tube passing through the rubber septum of the chamber. The standard pressure for all measurements was 1.3 MPa, to allow maximum flow rate of exuded sap according to Mazzeo et al. (2013). The exuding sap was collected in three sub-samples (2–5 ml each) from three different replications of 3–4 fruit each. Sap samples were stored at −20 °C until analysis. Just before IAA determination, samples were diluted to 5 ml with distilled water, adjusted to pH 2.8 with HCl and supplemented with a suitable amount of $[^{13}C]_6$IAA as internal standard. Samples were purified on C18 SPE cartridges, the volume of the fractions putatively containing IAA was reduced under low pressure, then the fractions were dried out under a nitrogen stream. Derivatization and GC–MS analysis of samples were performed as reported previously. Indole-3-acetic acid content was expressed as ng ml^{-1} sap.

Enzyme extraction and assay

Freeze dried fruit sub-portions were ground in an ice-cold mortar with liquid nitrogen and extracted in phosphate buffer (0.06 M, pH 6.1) containing PVP (1/1, w/w) and the homogenate was centrifuged (10,000×*g*, 5 min). The supernatant was recovered and used as a crude enzyme extract. Guaiacol peroxidase (POD, EC 1.11.1.7) activity was determined as described by Arezky et al. (2001) using as substrate 1% guaiacol. Enzymatic activity was determined following guaiacol oxidation by H_2O_2 (extinction coefficient 26.6 mM^{-1} cm^{-1}) at 470 nm, one unit oxidising 1.0 µmol guaiacol per min and was expressed as U g^{-1} protein. The extract indole-3-acetic acid oxidation activity was measured as described in Beffa et al. (1990). The reaction solution contained 100 µM $MnCl_2$, 50 µM *p*-coumaric acid, 15 µg IAA and 200 µl of the extract in 1 ml 6.66 mM phosphate buffer pH 6.0. After incubation at 24 °C for 40 min, 2 ml of modified Salkowski reagent (Pilet and Lavanchy 1969) were added. IAA degradation was read at 535 nm after 30 min and expressed as U g^{-1} protein, one unit being equivalent to 1 µg IAA degraded by 1 ml of extract in 40 min. Protein measurement was performed according to Bradford (1976), using BSA as standard.

Electrophoretic peroxidase separation

Electrophoresis was performed on 10% PAGE as in Milone et al. (2003) with minor modifications. Tris-HCl 1.5 M pH 8.8 was used. Equal amounts (8.4 µg) of proteins extracted from the different fruit portions were loaded onto activity gel. After running (200 V, constant current of 35 mA gel^{-1}), bands were visualized after incubation in the dark for 90 min in 1 M Na-acetate buffer pH 4.6 with 0.04% benzidine and 10 mM H_2O_2. Enzyme activity appeared as dark brown bands. Incubation was also performed for 15 min in Na-acetate buffer pH 4.6 with 1 mM guaiacol and 10 mM H_2O_2.

In situ guaiacol peroxidase determination

Sub-portions of fresh fruit, at each sampling date, were sectioned using a cryostat. Cross sections (30 µm) were immediately immersed into a solution of colourless guaiacol/H_2O_2 (5 mM H_2O_2, 5 mM guaiacol in 60 mM phosphate buffer, pH 6.1). Peroxidase activity was revealed by dark/brown colour due to the conversion of guaiacol to tetraguaiacol. After 10 min of incubation the slices were washed three times in the same buffer and mounted in glycerol for

the microscopy analysis (Lepeduš et al. 2005 with minor modifications). A blank in the absence of H_2O_2 was made.

Extraction and determination of phenols

Total phenols were measured according to Arezki et al. (2001) in freeze dried fruit sub-portions. Phenolic extracts were obtained after centrifugation of freeze dried fruit sub-portions homogenized in HCl 0.1 N and left at 20 °C for 3 h. 300 µl of extract were added to 1.5 ml H_2O + 0.1 ml Folin–Ciocalteu reagent and left so for 3 min. After addition of 400 µl Na_2CO_3 (20% w/v) and incubation at 100 °C for 1 min, the samples were cooled in ice bath and the absorbance at 750 nm was measured. Phenolic compounds content was calculated as equivalent of gallic acid (GAE mg g^{-1} FW) on the base of a standard calibration curve.

Fruit growth

Fruit length (mm) and mass (g) were measured on 20 fruits randomly chosen from the vines used for fruit sampling (see above) at approx. 1–5-day interval during the early 50 DAP, thereafter fruit length was measured at 10–15-day interval time until day 139 DAP. Fruit length and mass values were used to calculate the relative length and mass growth rate (RGR) of the fruit over the period of two consecutive observations, and the average RGR values were referred to the mid-point of that period, according to Mazzeo et al. (2013).

Statistical analyses

The analyses were performed using Past3 software. Data were reported as mean ± SD. After testing for normality (Shapiro–Wilks test) and homogeneity of variance (F test), Student *t* test or one-way ANOVA followed by *post hoc* Bonferroni multiple comparison test were used to separately examine the differences between tissues (inner and outer) or fruit portions (P, M, D), respectively, at each sampling date. Differences among means were considered significant for *p*-values ≤0.05.

Results

Free and conjugated auxin concentration

The concentration of free IAA was significantly higher in the inner pericarp than in the outer one in the three fruit portions (P, M, D) and throughout the studied period, with the sole exception of the last sampling date (67 DAP) (Fig. 2, left column). In the inner tissues, the free IAA concentration showed an overall decline, which was more pronounced in very young fruit (between 19 and 27 DAP). Notably, the initial value of IAA concentration was higher in the inner tissues of M portion (approx. 55 ng g^{-1} FW) than in those of P and D (35 and 18 ng g^{-1} FW, respectively). In the outer pericarp, free IAA exhibited a similar course across the three transverse portions of the fruit, although the concentration of the hormone peaked at the last sampling date in the P and M portions (67 DAP). Nevertheless, these peak values were relatively low, being similar to the lowest ones that had been detected in the inner tissues. Among the fruit portions analyzed, the D one showed lower hormone concentrations when compared with M and P portions. In the outer tissues of D, IAA was even constantly below the detection limit.

The time course of conjugated IAA displayed irregular patterns. Apparently, changes of both ester (Fig. 2, middle column) and amide IAA (Fig. 2, right column) followed different patterns in comparison to that of the free hormone and, in most cases, free IAA was more abundant than its conjugated forms. Remarkably, in M at the end of the experiment (67 DAP), amide IAA peaked in the inner tissues, whereas ester IAA peaked in the outer ones, attaining the greatest concentration value overall (approximately 200 ng g^{-1} FW; Fig. 2).

Apoplastic auxin

The level of free IAA detected in the exuded sap declined throughout the period of observation, starting from ~10 ng ml^{-1} at 19 DAP down to approximately 2 ng ml^{-1} at 67 DAP (Fig. 3). The decrease was more pronounced in very young fruit: between 19 and 27 DAP the concentration of free auxin underwent a 40% reduction in comparison with the initial value.

Peroxidases, IAA degradation and phenols

Peroxidases and IAA degradation activities are reported in Fig. 4a, b, respectively. As only the inner pericarp showed appreciable values for the activity of these enzymes, barely detectable in the outer pericarp, data are reported only for the inner portions of the fruit. Enzymatic activity yielded an irregular pattern. An increase in activity of peroxidases was recorded in different intermediate sampling times with maximum values, of 110 (P and M portions) and 146 U g^{-1} (D portion), reached at different sampling dates for the different fruit portions. Until 45 DAP IAA degradation activity was significantly lower in M than in P and D portions. At the last sampling date, there was a general decline of both the enzymatic activities.

Electrophoretic results relative to peroxidases in inner pericarp are shown in Fig. 5. Only results from incubation in benzidine are reported, as the brown bands obtained from

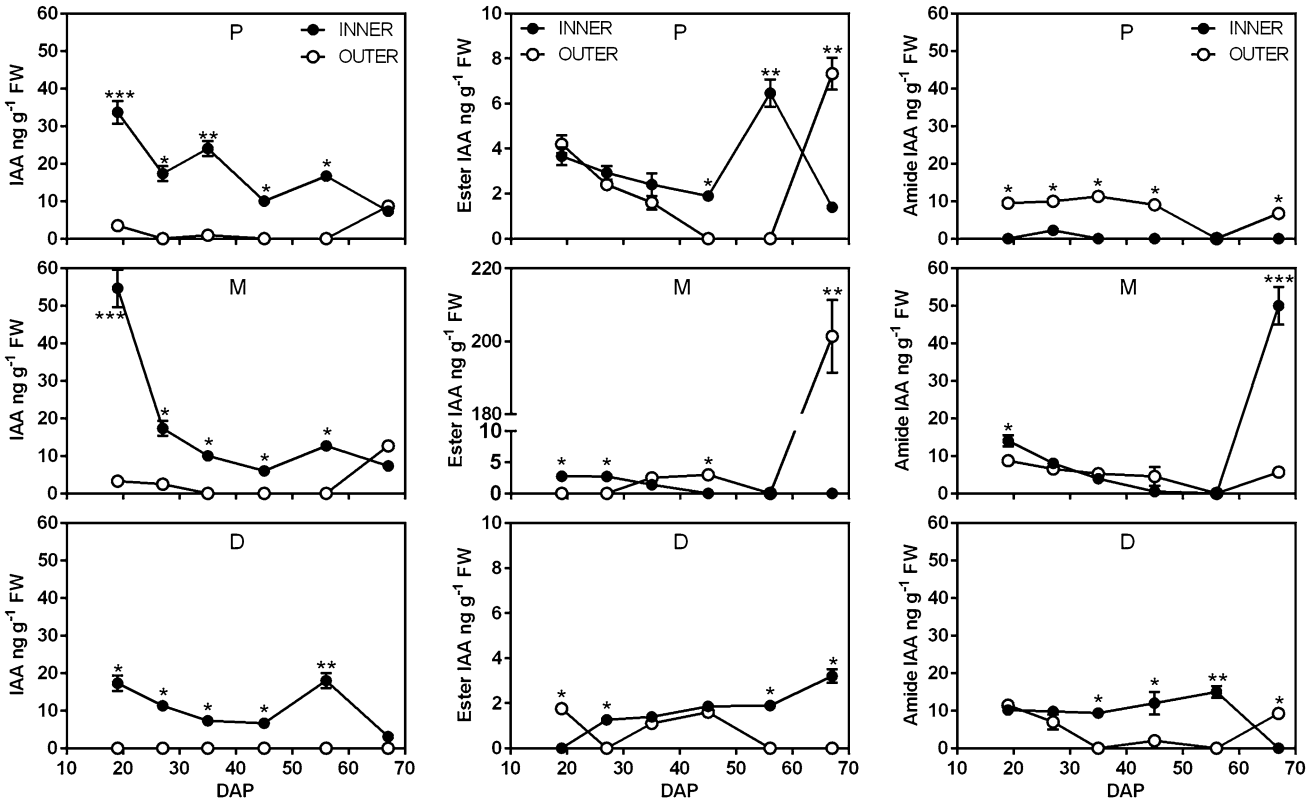

Fig. 2 Changes of free (*left*), ester conjugated (*middle*) and amide conjugated (*right*) IAA concentrations in inner and outer pericarp tissues of different fruit portions (*D* distal; *M* median; *P* proximal) during the experiment. *DAP* days after pollination. *Asterisks* indicate values that are significantly different, at each date, between inner and outer tissues (*$p \leq 0.05$; **$p \leq 0.01$; ***$p \leq 0.001$). The results represent the mean of three replications ± SD

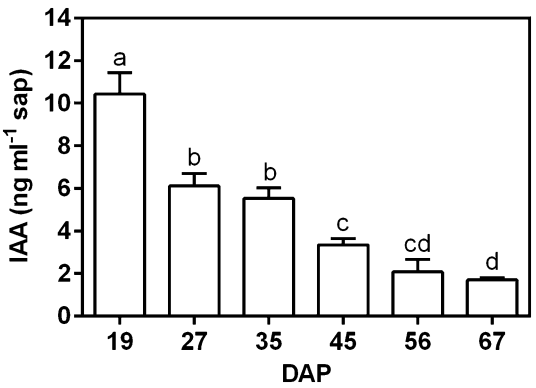

Fig. 3 Concentration of free IAA in the apoplastic sap collected from the cut pedicel of the fruit. *DAP* days after pollination. *Different letters* indicate values that are significantly different ($p \leq 0.05$). The results represent the mean of three replications ± SD

incubation in guaiacol began rapidly to fade after staining. Similar banding patterns were shown when benzidine and guaiacol were used as hydrogen donors. Staining revealed bands with lower and higher mobility, whose pattern changed depending on the portion considered. Noteworthy, the activity of bands with greater mobility increased during fruit growth in P and D portions, respectively (Fig. 5), while in the M one these bands appeared only in relatively late developmental stages, >45 DAP (Fig. 5).

Initial concentrations of phenolic compounds were significantly higher in the outer tissues than in the inner ones, being 4.2–5.8 and 2–2.4 mg g^{-1} FW, respectively (Fig. 6). By the second sampling date, the values of phenols detected in the outer tissues were still higher than in the inner ones, except for the P portion. Phenols declined by more than 50% at the end of the experiment in the outer tissues (Fig. 6). On the contrary, in the inner tissues the phenol content had different patterns, depending on the fruit portion, but it changed little with time (Fig. 6).

The in situ determination showed a comparable pattern of POD activity in fruit of different age. In particular, the activity was recorded in differentiating vascular bundles (Fig. 7b, c) and in the layer of cells delimiting the locule, i.e., the portion of inner fruit holding seeds (Fig. 7a). At an early stage of fruit development (19 DAP), the area of hypostase showed a dark staining (Fig. 7d), while at later stages (56 DAP) peroxidases were localized at the seed coat (Fig. 7e, f).

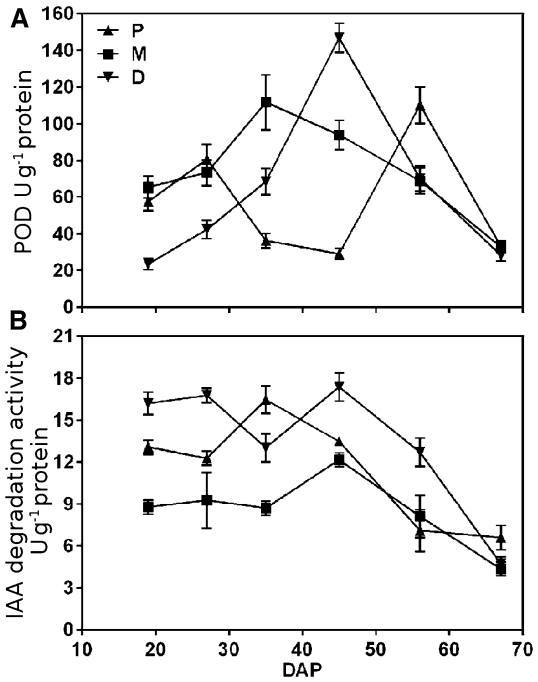

Fig. 4 Guaiacol peroxidase (POD, **A**) and IAA degradation activities (**B**) in different portions (*D* distal; *M* median; *P* proximal) of inner tissues of fruit. Enzymatic activity is reported as U g^{-1} protein. *DAP* days after pollination. *Different letters* indicate values that are significantly different ($p \leq 0.05$) at each sampling date. The results represent the mean of three replications ± SD

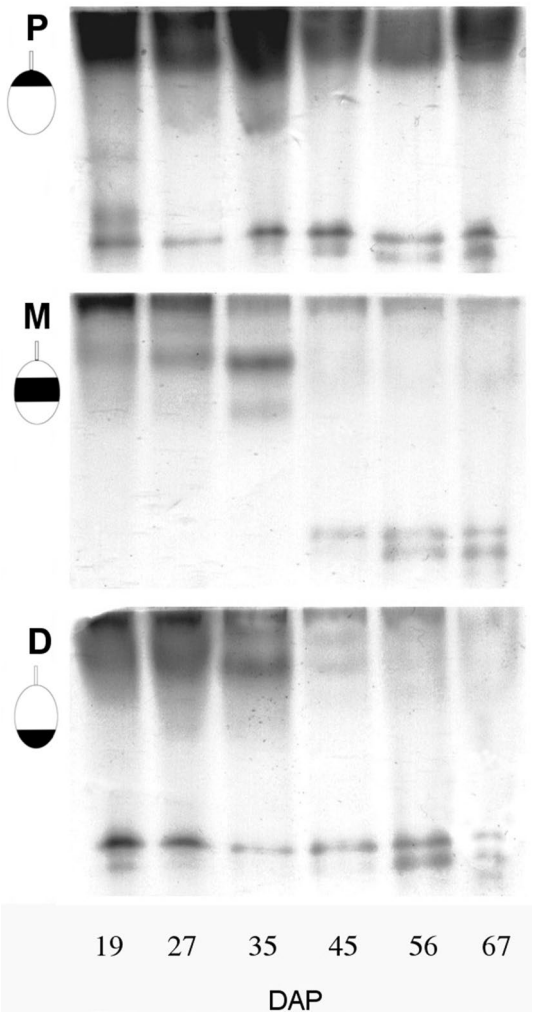

Fig. 5 Native PAGE gels of guaiacol peroxidase in different portions (*D* distal; *M* median; *P* proximal) of inner tissues of fruit during the experiment. *DAP* days after pollination

Fruit growth

Fruit growth (mass and length) showed a rapid increase in the early ~4 weeks after pollination, when the length and mass RGR were at their highest values (Fig. 8). Thereafter, fruit growth proceeded slowly and RGRs declined to attain the lowest values at ~80 DAP. At the last sampling date (67 DAP), the average fruit length was nearly 63 mm (90% of the final value), and fruit mass reached ~80 g $fruit^{-1}$ (94% of final mass).

Discussion

Free IAA showed a heterogeneous pattern in the three fruit portions. The higher auxin amount detected in inner tissues is conceivably related to the presence of the developing seeds, whose role as strong auxin source is well documented (Ozga et al. 2002; Normanly et al. 2010; Tiwari et al. 2013). Consistent with this data, the low level of auxin observed in the outer pericarp might be attributable to the lack of this hormone source and possibly to a scarce, if any, lateral translocation of IAA (either free, or conjugated) toward the peripheral region of the pericarp. This could be due to the limited development of radially-oriented vascular tissues in kiwifruit (Ferguson 1984). A similar radial gradient was observed in tomato fruit, where IAA was more abundant in the core region than in the peripheral one (Kojima 2005; Pattison and Català 2012), as well as in apple fruit (Devoghalaere et al. 2012). The time course of free IAA in inner tissues (particularly in the M portion) resembles that of the whole berry (Sorce et al. 2011), suggesting that the core of the fruit has a dominant influence on the concentration of auxin in the whole organ.

Similar declining patterns of IAA were reported for a closely related kiwifruit species (*Actinidia chinensis*, Li et al. 2015), mango (Cutting et al. 1986) and strawberry (Chen et al. 2016). Fruit growth was fast during the initial stage (early 4 weeks), when cell division typically occurs (Hopping 1976). By 67 DAP the mean fruit length had attained approx. 90% of the average length of the mature fruit with rates of the relative mass and length growth

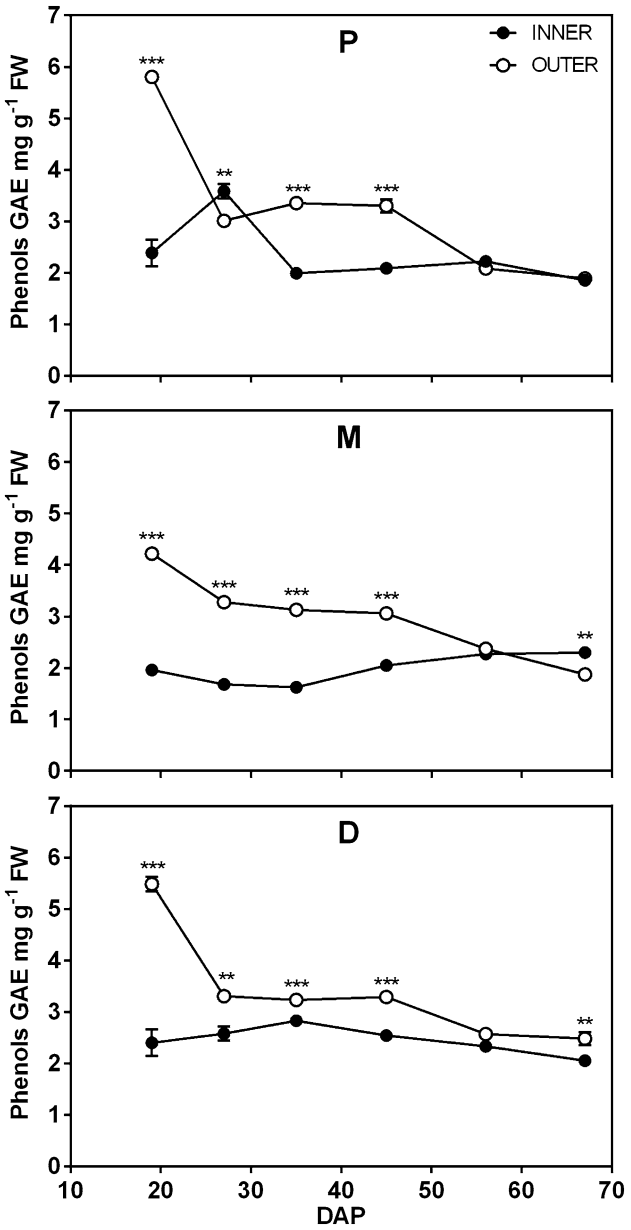

Fig. 6 Phenol concentrations in inner and outer pericarp tissues of different fruit portions (*D* distal; *M* median; *P* proximal), during the experiment. Concentration is reported as mg of gallic acid equivalent (GAE) g^{-1} FW. *DAP* days after pollination. *Asterisks* indicate values that are significantly different, at each date, between inner and outer tissues (*$p \leq 0.05$; **$p \leq 0.01$; ***$p \leq 0.001$). The results represent the mean of three replications ± SD

characteristic of kiwifruit (Morandi et al. 2010; Mazzeo et al. 2013). The time course of fruit elongation and fresh mass increase were markedly similar to that of free IAA concentration in the inner fruit tissues. There are several ways by which auxin may enhance the sink strength of the fruit: by increasing cell number, cell size and by regulating cell differentiation (including xylem differentiation; Varga and Bruinsma 1976; Brenner and Cheikh 1995). As a consequence, a role may be envisaged for IAA in the stimulation of kiwifruit growth during this early developmental stage.

Auxin has been associated, along with other factors, to Ca movement and distribution (Bangerth 1976; Banuelos et al. 1987; Cutting and Bower 1989; McLaughlin and Wimmer 1999; Vanneste and Friml 2013; Hocking et al. 2016). The free IAA of the fruit apoplast may be involved in a mutual relationship between basipetal auxin transport and acropetal Ca movement, as reported for tomato, apple and avocado (Stahly and Benson 1970; Bangerth 1976; Banuelos et al. 1987; Cutting and Bower 1989). This mechanism could gain particular importance for Ca nutrition of fruit growing under conditions that limit fruit transpiration (e.g., low *VPD*) (Montanaro et al. 2010, 2015). The time course of the apoplastic IAA appears to be related to that of the inner pericarp, which should be expected if the seeds are to be considered a major auxin source. Free IAA of the fruit sap may be partly exported from the fruit (Kojima 2005; Pattison et al. 2014) and present data suggest that the changes of free IAA concentration detected in inner fruit tissues (putatively arising from the hormonal metabolism of the developing seeds) may have affected the amount of IAA potentially exported from the fruit, as previously shown to occur in tomato (Pattison and Català 2012).

The concentration of free IAA in the tissues depends on the differential regulation of biosynthesis, conjugation, catabolism and transport (Woodward and Bartel 2005; Ljung 2013; Pattison et al. 2014). Based on the present results, it is difficult to envisage a role for conjugation in the modulation of free auxin during fruit growth, thus confirming our previous observations in kiwifruit (Sorce et al. 2011). Nevertheless, at 67 DAP peaks of ester IAA (in P and M portions) and amide IAA (in M) were detected in outer and inner tissues, respectively. Notably, the peak of ester IAA in the outer M portion seems to be the origin of the peak of ester IAA that was detected in the whole fruit with similar age in a previous investigation (Sorce et al. 2011). Given that the concentration of free auxin must decline at the end of the growing stage, to allow the fruit to proceed to the ripening process (Bregoli et al. 2007), a possible explanation for the aforementioned peaks of conjugates may be the enhancement of IAA metabolism. This would fulfil the need for a further level of control on the concentration of the hormone, to fine tune fruit development.

Enzymatic catabolism is generally considered to be a major regulator of the free auxin pool (Normanly et al. 2010). In fact, the presence of POD and IAA degradation activity was detected only in inner tissues, where the content of IAA was generally higher in comparison with the outer regions of the fruit, underlining the possible involvement of these enzymes in the control of fruit auxin content

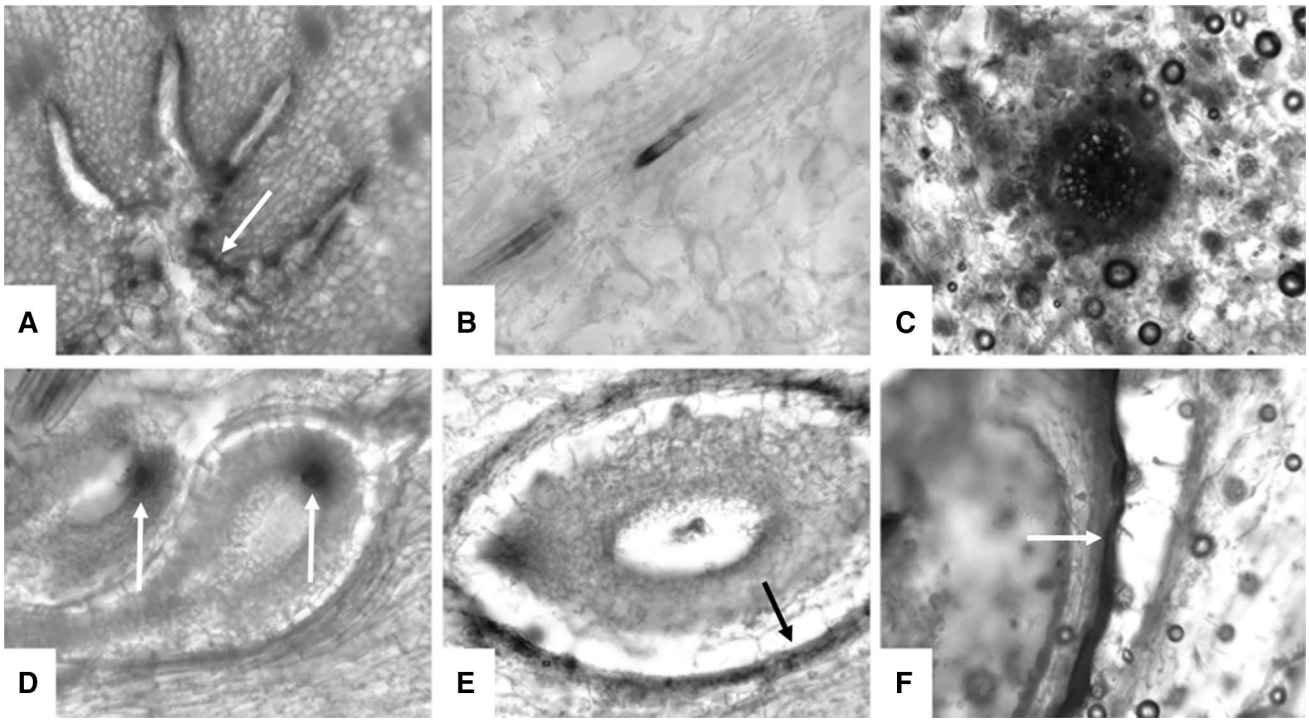

Fig. 7 Cross sections of inner pericarp tissues of different fruit portions for in situ determination of guaiacol peroxidase activity. **A** Cells delimitating the locule (19 DAP, proximal portion), **B, C** vascular bundles (19 and 56 DAP respectively, middle portion), **D** hypostase (19 DAP, middle portion), **E** seed and **F** particular of seed coat (56 DAP, middle portion). *DAP* days after pollination. *Bars* indicate 200 µm. *White* or *black arrows* in **A, D, E** and **F** highlight POD activity staining

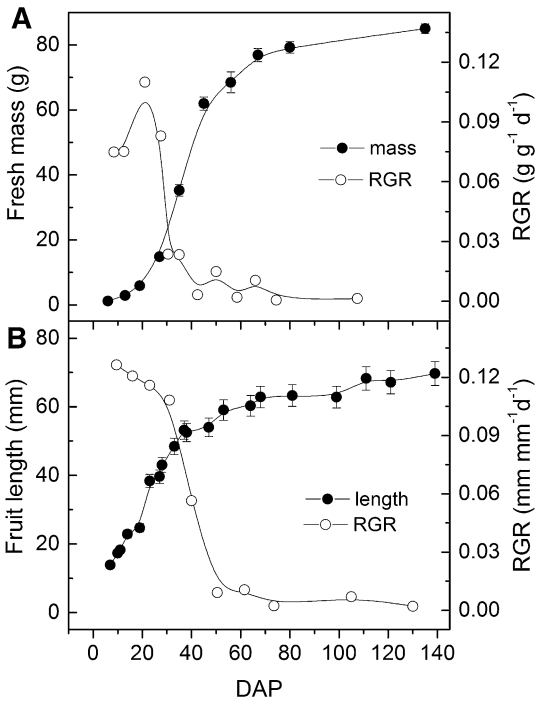

Fig. 8 Seasonal trend of **A** fresh weight and **B** length and their relative growth rates (RGR) measured in fruit during the experiment. Each value represents the mean ± SD of 20 fruits. *Lines* are illustrative only

(Thomas et al. 1982; Purgatto et al. 2002). There was not a tight correspondence between the trend of enzymatic activities and IAA concentration during the growth of kiwifruit. It has recently been found that other enzymes play a key role in the regulation of auxin levels: in *Arabidopsis* and in rice a dioxygenase for auxin oxidation (DAO) has recently been identified as main regulator of the catabolism of IAA (Zhao et al. 2013; Mellor et al. 2016; Porco et al. 2016; Zhang et al. 2016) with POD playing only a minor role in IAA homeostasis. This could at least in part explain the lack of correlation between enzymatic activities and IAA content. The study about the presence and the expression of the gene *DAO* (Mellor et al. 2016; Porco et al. 2016) in kiwi could give a more actual picture of auxin metabolism in this species. Remarkably, the distal portion of the fruit showed a low IAA content and an overall higher IAA degradation activity. Despite the lack of a clear relation between POD activity and IAA content, some more information on their possible interaction could be inferred from the in-gel POD activity assay. An inverse relation could be found between the activity of POD bands with higher mobility and the levels of free IAA. For example, middle fruit portions showed higher free IAA content in younger fruit when these activity bands were absent, while later in the season, when the concentration of free IAA was lower,

such bands were detected. Consequently, it could be suggested that these enzymatic forms may give a contribution to the modulation of the concentration of free IAA. Further studies about metabolites of IAA are however necessary to confirm a role of peroxidases in the regulation of auxin levels. Furthermore, the study of peroxidase in situ may hint at other roles that this enzyme could play in kiwifruit growth. The localization of POD activity in correspondence of the vascular bundles was probably linked with the role of this enzyme in the process of lignin biosynthesis (Lee et al. 2007), a fundamental step of xylem formation. A similar role could explain the presence, at early developmental stages, of peroxidases in correspondence of the seed hypostase, which is constituted by cells with lignified walls (Marzinek and Mourão 2003), and also in seed teguments during the late stages of growth. The POD activity detected in correspondence of the seed locules could contribute both to the regulation of the concentration of the IAA produced by the seeds and to the protection of these organs from reactive oxygen species, that are key components of seed biology (Bailly 2004).

Phenolic compounds are important secondary metabolites (Kefeli et al. 2003), involved in various physiological processes of fruit growth and development (Cheniany et al. 2010) and are also known as protective agents against peroxidase-induced IAA degradation (Krylov and Dunford 1996; Montanaro et al. 2007). These compounds are defensive factors under biotic and abiotic stress conditions (Solar et al. 2006), playing an important role in protection from UV radiation (Lattanzio et al. 2006; Huyskens-Keil et al. 2007) and, accordingly, their content was shown to increase in kiwifruit under high irradiance during early maturation (Montanaro et al. 2007). This important putative shielding action was in agreement with the higher concentration of phenols recorded around 19–27 DAP in the outer tissues, where the level of irradiance could have stimulated the biosynthesis of these photoprotecting molecules. Because late in the season (~40 DAP) the fruit develops a suberized periderm (Montanaro et al. 2014) that could prevent an excessive penetration of sunlight, the need for photoprotective molecules declines. The eventual action of phenols as IAA-antioxidants could have been played only in the inner portions of fruit, where POD activity was recorded.

In conclusion, auxin might operate positively in regulating the development of kiwifruit berries: besides the direct stimulation of tissue growth, IAA would induce the differentiation of new xylem vessels, thus putting forward this growth regulator as a key factor in the control of early development. The concentration of the free hormone is regulated by several mechanisms, among which biosynthesis (probably in the developing seeds) and enzymatic catabolism could also play a role, although their importance may change, depending on the fruit tissue and the developmental stage. Phenols seem to be at least partially involved in the regulation of IAA levels in the inner part of the fruit, while they could play a significant role in photoprotection in the outer tissues.

The present work may contribute to foster our understanding of the physiological implications of auxin changes for fruit development in kiwifruit and represents a starting point for further investigations on this matter.

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