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RESEARCH PAPER

Alq mutation increases fruit set rate and allows the maintenance of fruit yield under moderate saline conditions

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Abstract

***Arlequin* (*Alq*) is a gain-of-function mutant whose most relevant feature is that sepals are able to become fruit-like organs due to the ectopic expression of the *ALQ-TAGL1* gene. The role of this gene in tomato fruit ripening was previously demonstrated. To discover new functional roles for *ALQ-TAGL1*, and most particularly its involvement in the fruit set process, a detailed characterization of *Alq* yield-related traits was performed. Under standard conditions, the *Alq* mutant showed a much higher fruit set rate than the wild type. A significant percentage of *Alq* fruits were seedless. The results showed that pollination-independent fruit set in *Alq* is due to early transition from flower to fruit. Analysis of endogenous hormones in *Alq* suggests that increased content of cytokinins and decreased level of abscisic acid may account for precocious fruit set. Comparative expression analysis showed relevant changes of several genes involved in cell division, gibberellin metabolism, and the auxin signalling pathway. Since pollination-independent fruit set may be a very useful strategy for maintaining fruit production under adverse conditions, fruit set and yield in *Alq* plants under moderate salinity were assessed. Interestingly, *Alq* mutant plants showed a high yield under saline conditions, similar to that of *Alq* and the wild type under unstressed conditions.**

Keywords: ABA, *ALQ-TAGL1* gene, *Arlequin*, cytokinins, fruit setting, salt tolerance.

Introduction

Fruit set is the most critical phase of fruit production and is of vital importance in agriculture (Mariotti *et al.*, 2011). The transition of the ovary into a developing fruit with the capacity to become a mature organ takes place once the flower reaches the anthesis stage and is dependent on successful completion of pollination and fertilization (Gillaspy *et al.*, 1993). The earliest phase of fruit set is characterized by a period of intense mitotic activity and involves the generation of positive signals that drive the re-activation of cell cycle-related genes (Azzi *et al.*, 2015). MADS-box genes could function as integrators of the ovary development signal triggered by fertilization (Chevalier, 2007) since they are induced immediately after anthesis in the ovary walls (Busi *et al.*, 2003).

This process is also regulated to a large extent by hormonal signals, especially auxins and gibberellins (GAs), and the response genes to both hormones are positively regulated after

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pollination (Vriezen *et al.*, 2008; de Jong *et al.* 2009*a*; Carrera *et al.*, 2012). In fact, application of either auxin or GA to tomato ovaries can trigger fruit set without the need for pollination and fertilization, although the effect of these hormones on cell division and cell expansion seems to be different (de Jong *et al.* 2009*a*). The application of auxin produces more cell layers in the pericarp, whereas the application of GA results in greater cell elongation, as well as reduced cell division activity in the pericarp (Serrani *et al.*, 2007). Also, in auxin-induced fruit setting, the locular cavities are filled with jelly and the ovules do not degenerate, but form pseudoembryos. In contrast, in GA-induced fruit, the ovules degenerate and the locular tissue barely develops (de Jong *et al.*, 2009*a*). It has been hypothesized that pseudoembryos can stimulate early fruit growth by producing hormones and may be able to act as a substitute for seeds in controlling fruit growth (Kataoka *et al.*, 2003; Goetz *et al.*, 2007; de Jong *et al.*, 2009*a*).

Studies using genetic approaches in tomato and Arabidopsis have revealed that the auxin signalling pathway is instrumental in the onset of fruit development (de Jong *et al.* 2009*a*; Ruan *et al.*, 2012). In tomato, the *SlARF7* gene, a member of the tomato auxin response factor (ARF) gene family, acts as a negative regulator of fruit set and as a transcriptional activator of auxin response-attenuating genes (de Jong *et al.*, 2009*b*). Down-regulation of the *SlARF7* gene resulted in parthenocarpic fruit development and up-regulation of the indole-3-acetic acid (IAA)-amido synthetase gene *GH3.6*. The characteristics of parthenocarpic RNAi *SlARF7* fruits suggest that the *SlARF7* gene might be involved in the crosstalk between auxin and GA (de Jong *et al.*, 2009*b*). Likewise, down-regulation of *SlARF5* resulted in seedless fruit development by regulating both the auxin and GA signaling pathways (Liu *et al.*, 2018). Also the *SlIAA9* gene, a member of the Aux/IAA family of tomato transcription factors, acts as a transcriptional repressor of auxin signalling (Wang *et al.*, 2005). Silencing of *SlIAA9* resulted in uncoupling fruit set from pollination and fertilization, and promoted fruit initiation in tomato (Wang *et al.*, 2005). The *SlARF8* gene plays a relevant role in regulating tomato fruit set, by stimulating expression of early auxin-responsive genes, initiating fruit growth and development (Goetz *et al.*, 2007). It has been proposed that *SlIAA9* and *SlARF8* genes belong to the same signalling cascade, forming a regulatory complex that prevents fruit set prior to fertilization. Pollination and fertilization promote *SlIAA9* degradation, and the *SlARF8* gene is released, resulting in the transcription of the auxin response genes (Goetz *et al.*, 2007; de Jong *et al.*, 2009a). Mutations or transgenic manipulation of these *ARF* and *Aux/IAA* genes led to the development of seedless tomato fruits (Wang *et al.*, 2005; Goetz *et al.*, 2007; de Jong *et al.*, 2009*b*; Mazzucato *et al.*, 2015; Ueta *et al.*, 2017). It has also been shown that, besides auxin and GA, ethylene participates in the tomato fruit set process by suppressing GA metabolism (Shinozaki *et al.*, 2015) and promoting ovary senescence (Shinozaki *et al.*, 2018).

Arlequin (*Alq*) is a tomato semi-dominant mutant whose most relevant feature is that the sepals become fruits. In homozygous plants, sepal-derived fruits acquire all the characteristics of a sink and mature as normal fruits (i.e. those derived from the ovary). In addition, the fruits of *Alq* show much better quality parameters than those of wild-type (WT) plants and, after ripening, they do not detach because the pedicel abscission zone (AZ) is inhibited (Pineda *et al.*, 2010). It has been demonstrated that *Alq* mutation promotes a gain-of-function phenotype caused by the enhanced expression of the *ALQ-TAGL1* gene (Giménez *et al.*, 2010). Spatial and temporal expression patterns of *ALQ-TAGL1* in WT tomato flowers indicated that its expression begins at early stages of flower development, although the highest transcript accumulation is detected at anthesis (Giménez *et al.*, 2010). The *ALQ-TAGL1* gene has an essential role as a positive regulator of fruit ripening (Itkin *et al.*, 2009; Vrebalov *et al.*, 2009; Giménez *et al.*, 2010), but also has additional effects on early development of the carpel, coinciding with the cell proliferation stage of carpel development following anthesis (Giovannoni *et al.*, 2017). Overexpression of *ALQ-TAGL1* results in homeotic alterations affecting floral organ identity that are similar to but stronger than those observed in *Alq* mutant plants (Itkin *et al.*, 2009; Vrebalov *et al.*, 2009; Giménez *et al.*, 2010). It has been reported that fruits from 35S:*TAGL1* plants and transgenic tomato plants overexpressing the *PpPLENA* gene, the peach orthologue of the *ALQ-TAGL1* gene, fail to produce seeds (Tadiello *et al.*, 2009; Vrebalov *et al.*, 2009).

Plants developing seedless fruits usually display a higher fruit set rate under standard growth conditions. For instance, tomato transgenic lines for *DefH9-RI-iaaM*, a gene that increases auxin biosynthesis in ovules, yielded a higher number of fruits than the WT under standard growth conditions (Rotino *et al.*, 2005). This is also the case for *procera* (*pro*), a parthenocarpic tomato mutant that carries a point mutation in the *SlDELLA* gene (Bassel *et al.*, 2008; Jasinski *et al.*, 2008). Compared with the WT, the *pro* mutant is able to develop a higher number of fruits per plant despite the fact that it develops fewer flowers (Carrera *et al.*, 2012). Seedlessness may also improve fruit quality through increases in total soluble solids of the fruit (Ficcadenti *et al.*, 1999; Carmi *et al.*, 2003; Carrera *et al.*, 2012; Mazzucato *et al.*, 2015; Klap *et al.*, 2017).

The pollination-dependent tomato fruit set is highly sensitive to both biotic and abiotic stresses, often leading to fruit abortion (Ruan *et al.*, 2012). There is some evidence suggesting that pollination-independent fruit set could be useful under adverse environmental conditions (e.g. low or high temperatures), where pollination and fertilization are reduced (Carmi *et al.*, 2003; Shabtai *et al.*, 2007; Klap *et al.*, 2017).

In the tomato, fruit set rate is one of the parameters of yield most affected by saline stress. Apart from the direct effects, salinity affects both pollen quantity and viability, resulting in decreased pollination-dependent tomato fruit set (Cuartero and Fernández-Muñoz, 1999; Ghanem *et al.*, 2009). In this work, we performed a detailed assessment of fruit set in the *Alq* mutant. This allowed us to demonstrate that the *Alq* mutant exhibits an increased fruit set rate with respect to the WT. *Alq* mutation promotes fruit set prior to anthesis and modifies the expression pattern of genes involved in cell division, GA metabolism, and the auxin signalling pathway. Importantly, the increased fruit set rate in *Alq* plants allows the maintenance of yield under moderately saline conditions.

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Materials and methods

Plant material

The *Arlequin* tomato mutant (*Alq*) and its genetic background have been described by Pineda *et al.* (2010). Tomato *TAGL1*-silenced (RNAi) and overexpressing (OE) lines were described by Giménez *et al.* (2010). Flower emasculation was carried out 2 d before anthesis in order to avoid accidental self-pollination. Reciprocal backcrosses by hand pollination on previously emasculated flowers were carried out with pollen collected from flowers in the anthesis stage.

Pollen viability

In vitro pollen viability assays were performed by means of staining pollen grains from 10 WT and *Alq* flowers with 0.5% 2,3,5-triphenil tetrazolium chloride (TTC) (w/v) in 0.5 M sucrose for 2 h at 50 °C in a humid box in darkness and then visualized with a Leica MZZ16F light microscope (Leica Microsystems, Wetzlar, Germany). At least 200 pollen grains were scored, taking into account their colour intensity and external morphology.

Ovary growth analysis

In order to analyse the ovary growth, flowers at three floral stages (i.e. 5 d and 2 d prior to anthesis and at the anthesis stage) were collected and the three outer whorls were removed. Ovaries were viewed and measured on a stereomicroscope (MZFLIII; Leica). At least 10 flowers from two independent trials were sampled and checked for each developmental stage.

Histology techniques

Tissue sections of ovaries were fixed in FAE [50% (v/v) ethanol, 5% (v/v) formaldehyde, 10% (v/v) acetic acid] and stored in 70% (v/v) ethanol. Subsequently, tissues were dehydrated in 100% (v/v) ethanol and embedded in paraffin (Paraplast Plus) blocks using plastic containers. Sections of material (8 µm thick) were cut with a Leica RM2025 microtome. The sections were stained with 0.05% (w/v) toluidine blue for 2–5 min and rinsed with water. The samples were observed with a Leica MZZ16F light microscope (Leica Microsystems). The number of cell layers and cell size were measured according to the method described by Serrani *et al.* (2007). At least four independent sections were made from independent ovaries at the anthesis stage.

Characterization of the reproductive development of WT, Alq, ALQ-TAGL1 RNAi, and ALQ-TAGL1 OE plants

Experiments were conducted in a controlled environment greenhouse, under the following conditions: long-day photoperiod (16 h of natural light supplemented with Osram lamps Powerstar HQI-BT, 400 W), temperature fixed at 24 °C during the day and 18 °C at night, and automatic fertirrigation. Plants were irrigated daily with Hoagland's nutrient solution (Hoagland and Arnon, 1950) in 6 litre pots containing coconut fibre. Under moderate salt stress conditions, NaCl was added to the irrigation solution to reach 50 mM concentration. The salt treatment was started at the sixth leaf stage and was maintained throughout the experiment. To assess fruit setting, both flowers and fruits from the first seven inflorescences of each plant were scored. To determine yield per plant, all the fruits from the first seven inflorescences were harvested and weighed. The seed number per fruit was also scored. At least 10 plants of each genotype were used in each greenhouse trial.

Quantification of plant hormones

Aliquots (~100 mg FW) of frozen ovaries were extracted with 80% methanol–1% acetic acid containing internal standards and mixed by shaking for 1 h at 4 °C. The extract was kept at –20 °C overnight and then centrifuged, and the supernatant was dried in a vacuum evaporator. The dry residue was dissolved in 1% acetic acid and passed through an Oasis HLB (reverse phase) column as described in Seo *et al.* (2011). For GAs, IAA, and abscisic acid (ABA), the dried eluate was dissolved in 5% acetonitrile–1% acetic acid, and the hormones were separated using an autosampler and reverse-phase UHPLC (2.6 µm Accucore RP-MS column, 50 mm length×2.1 mm id; ThermoFisher Scientific) with a 5–50% acetonitrile gradient containing 0.05% acetic acid, at 400 µl min^{-1} over 14 min. For cytokinins (CKs), the extracts were additionally passed through an Oasis MCX (cationic exchange) and eluted with 60% methanol–5% NH_4OH to obtain the basic fraction containing CKs. The final eluate was dried and dissolved in 5% acetonitrile–1% acetic acid, and CKs were separated with a 5–50% acetonitrile gradient over 7 min. The hormones were analysed with a Q-Exactive mass spectrometer (Orbitrap detector; ThermoFisher Scientific) by targeted selected ion monitoring (SIM). The concentrations of hormones in the extracts were determined using embedded calibration curves and the Xcalibur 2.2 SP1 build 48 and TraceFinder programs. The internal standards for quantification of each of the different plant hormones were the deuterium-labelled hormones.

Gene expression analyses

Total RNA was extracted from ovaries from WT and *Alq* plants at three stages of reproductive development: pre-anthesis (AD–2), anthesis (AD), and post-anthesis (AD+5). RNA preparation and gene expression studies were performed from three biological replicates according to procedures described by Giménez *et al.* (2010). Briefly, RNA integrity was first determined by agarose denaturing gel electrophoresis (Supplementary Fig. S3 at *JXB* online) and then the RNA concentration was quantified at 260 nm using a NanoDrop 2000 (ThermoFisher Scientific) spectrophotometer. Subsequently, first-strand cDNA was synthesized from 500 ng of total RNA using the First Strand cDNA Synthesis Kit (ThermoFisher Scientific) and an equimolar mixture of random hexamer and oligo$(dT)_{18}$ primers. Quantitative real-time PCRs (qRT-PCRs) were performed using gene-specific primers and the SYBR Green PCR Master Mix kit (Applied Biosystems) on the 7300 Real-Time PCR System (Applied Biosystems). Relative transcript levels were calculated using *UBIQUITIN3* controls, and the quantification of gene expression were performed using the ΔΔCt calculation method (Winer *et al.*, 1999). The sequences of the primers used in the expression analyses are listed in Supplementary Table S5.

Results

Arlequin *mutant plants have an increased fruit set rate*

Previously, we reported that the *Alq* mutant develops succulent sepals whose morphological features resemble tomato fruit pericarp (Pineda *et al.*, 2010). Indeed, at the mature stage, transformed sepals from *Alq* homozygous plants show the characteristic red colour of tomato fruits and are able to accumulate glucose, fructose, lycopene, and carotenoids at similar levels to those of normal tomato fruits. Interestingly, the conversion of sepals into fruit-like organs is not always synchronized with ovary-derived fruit development and this process can even occur in the absence of fruit setting (Pineda *et al.*, 2010). The fact that the ectopic expression of the *ALQ-TAGL1* gene promotes the homeotic conversion of sepals into fruit-like organs suggests that this gene could play a relevant role in the ovary-derived fruit setting process.

In order to check this hypothesis, an in-depth characterization of the reproductive development of WT and *Alq* plants was carried out. As shown in Table 1, fruit set was significantly higher in the *Alq* mutant. This difference was mainly due to an increased number of fruits produced in the *Alq* mutant, since the number of flowers per inflorescence was similar to that of

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Table 1. *Fruit production in WT (SLDG2) and* Alq *plants grown under standard greenhouse conditions*

Parameters		WT	*Alq*
Flowers per inflorescence		9.90±0.95 a	10.06±0.39 a
Ovary-derived fruits	Fruits per inflorescence	4.23±0.65 b	9.23±0.40 a
	Fruit setting rate (%)	42.73±5.10 b	93.11±1.41 a
	Fruit weight (g)	22.75±1.32 a	14.16±0.34 b
	Yield per plant (g)	672.68±68.98 b	869.65±27.70 a
Sepal-derived fruits	Fruits per inflorescence	ND	9.80±0.43
	Fruit setting rate (%)	ND	97.74±0.84
	Fruit weight (g)	ND	5.43±0.10
	Yield per plant (g)	ND	340.81±13.89
Ovary- and sepal-derived fruits	Fruits per inflorescence	4.23±0.65 b	9.80±0.43 a
	Fruit setting rate (%)	42.73±5.10 b	97.74±0.84 a
	Fruit weight (g)	22.75±1.32 a	19.60±0.40 a
	Yield per plant (g)	672.68±68.98 b	1210.65±27.70 a

Different parameters such as the number of flowers per inflorescence, number of fruits per inflorescence, fruit setting rate, fruit weight, and yield per plant were assessed, considering only ovary-derived fruits, only sepal-derived fruits, or both ovary- and sepal-derived fruit The values are given as the means ±SD of 10 individual plants per line. Means within each column followed by different letters are significantly different in accordance with the Student's *t*-test ($P<0.05$). ND: not detected.

the WT. In fact, considering only the ovary-derived fruits, the fruit set rate was twice as high in *Alq* (93%) than in the WT (43%). As mentioned above, mature red sepals without ovary fruit setting were also observed in *Alq* tomato plants. Thus, considering both ovary- and sepal-derived fruits, the fruit set rate increased up to 97% in the *Alq* mutant (Table 1).

As expected, the highest number of fruits per inflorescence in *Alq* had an effect on the fruit weight, which was significantly lower than that of WT plants (Table 1). Despite that, compared with the WT, the *Alq* mutant showed a significantly higher yield. In this sense, yield in *Alq* plants was 30% higher when only ovary-derived fruits were considered and almost 80% higher when the sepal-derived fruits were also taken into account (Table 1).

The facultative parthenocarpy of Alq *is not related to pollen viability loss*

The aforementioned agronomic tests indicated that *Alq* mutant plants produce a large number of fruits with very few seeds. On average, we were able to collect ~1150±200 seeds from a WT plant, whereas no more than 210±35 seeds were obtained from an *Alq* plant. It was also observed that *Alq* plants develop fruits of different sizes, some being very small while others reach a caliber similar to that of WT fruits.

To clarify the reason why *Alq* plants produce so few seeds, we first determined the proportion of fruits with and without seeds in WT and *Alq* plants. Most of the *Alq* fruits were seedless (73%) while only a small proportion of WT fruits (3%) did not contain seeds (Supplementary Table S1). Notably, the characteristics of the seedless fruits were different in both cases (Fig. 1). The WT seedless fruits had a very uniform weight, ranging from 5 g to 7 g, while those of the *Alq* mutant were very variable in weight, ranging from 3 g to 30 g. In addition, the few WT seedless fruits were the so-called 'small tiny nut fruitlets' without locular jelly (Fig. 1B), whereas those of *Alq* contained locular jelly and seed-like structures which were smaller than the normal seeds induced by fertilization (Fig. 1C, E).

With the aim of elucidating whether the lack of viability in *Alq* pollen could be the cause of the seedless fruit development, pollen viability was analysed in WT and *Alq* flowers through *in vitro* and *in vivo* assays. The results of *in vitro* analyses indicated that pollen viability was not affected in *Alq* plants as pollen grains displayed size, morphology, and staining similar to WT pollen (Supplementary Fig. S1). *In vivo* analyses were performed through self-pollination by hand and reciprocal backcrosses between WT and *Alq* plants. No differences were observed in the weight and number of seeds of WT and *Alq* fruits obtained by self-fertilization (Supplementary Table S2). Similarly, both backcrosses yielded fruits of similar size, and the average seed number using WT pollen was not significantly different from the average seed number with *Alq* pollen (Supplementary Table S2). Overall, the results showed that seedless fruit development in *Alq* plants is not related to the absence of pollen viability as, when successful pollination and fertilization of the ovules occur, *Alq* fruits are able to reach the same weight and develop the same number of seeds as WT fruits.

Alq *ovaries exhibit precocious fruit set*

In normal development, fruit set is dependent on successful completion of pollination and fertilization, and the starting point of this process is the flower anthesis stage (Gillaspy *et al.*, 1993). A few days before anthesis, the ovary enters into an 'arrest' state and, when fertilization is successfully completed, the ovary resumes growth (Klap *et al.*, 2017).

The results mentioned above indicated that the process of fruit setting in the *Alq* mutant is not pollination dependent and that its facultative parthenocarpy is not related to the lack of pollen viability. To assess possible early fruit development in *Alq* plants, we examined the ovary size in three flower stages: 5 d and 2 d prior to anthesis, and the anthesis stage. There were no significant differences in size between WT and *Alq* ovaries at either 5 d or 2 d before anthesis (Fig. 2A). However, the

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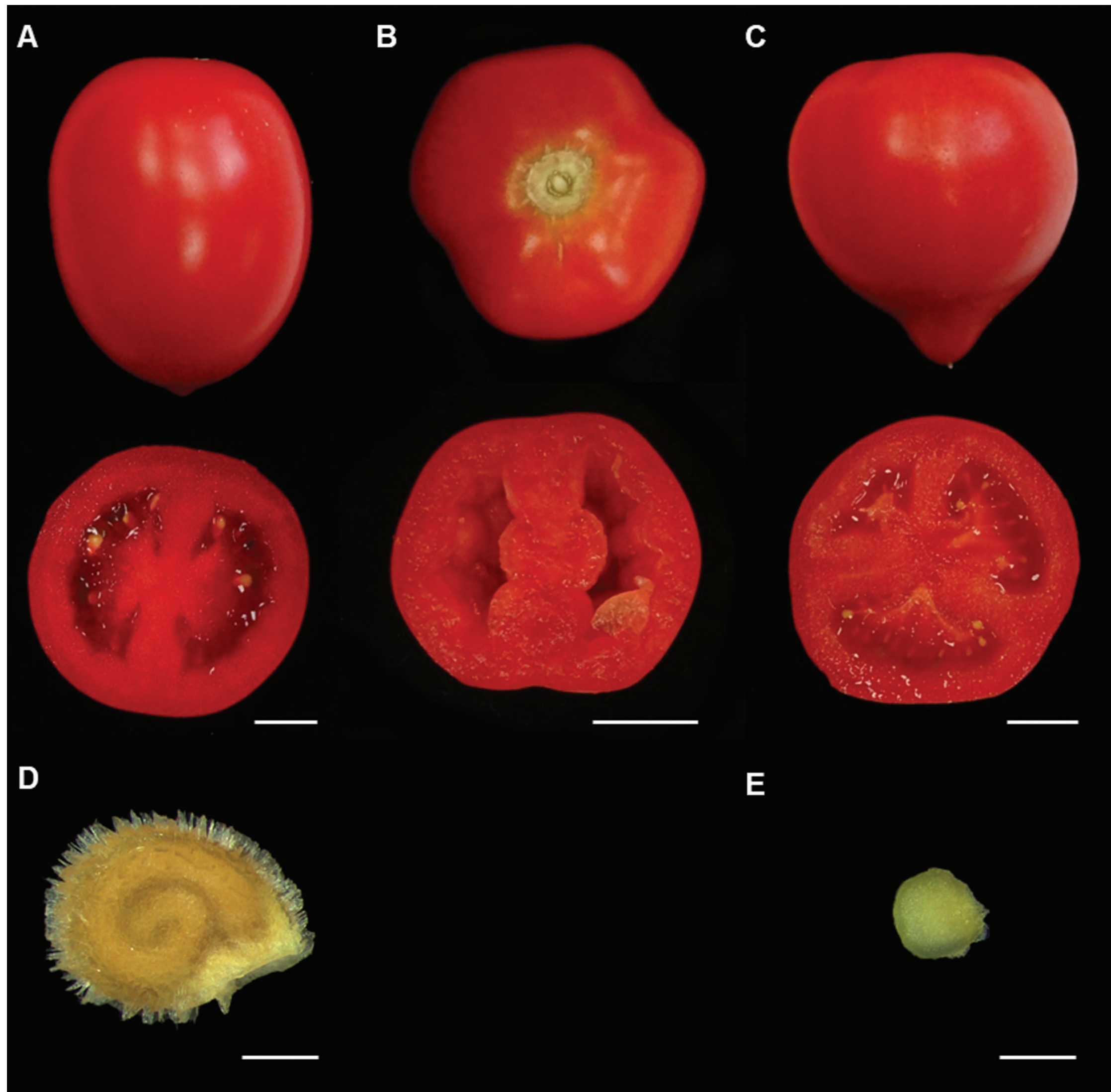

Fig. 1. Characteristics of mature fruits and seeds from WT and *Alq* plants. (A) WT seeded fruits. (B) WT seedless fruits. (C) *Alq* seedless fruits. (D) Seed from a WT fruit. (E) Seed-like structure from an *Alq* fruit. Scale bars: 1 cm in (A–C) and 1 mm in (D) and (E).

results showed a significant increase in the average size of *Alq* ovaries at anthesis (2.37±0.05 mm) with respect to the WT (2.01±0.04 mm), which means an increase of ~18% in the ovary size. To gain more insight into this feature, cross-sections of WT and *Alq* ovaries at anthesis were examined by light microscopy. We observed that the pericarp thickness of *Alq* ovaries was higher than that of the WT (Fig. 2B, C). Interestingly, the increase of pericarp thickness was associated with a significantly higher number of cell layers (Fig. 2C). WT and *Alq* pericarp cells were similar in size (Fig. 2C), suggesting that the larger size of the ovary in *Alq* flowers at the anthesis stage resulted from greater cell division rather than greater cell expansion.

To fully determine whether *Alq* flowers are actually able to develop into fruits in a pollination-independent way, flowers were emasculated at the pre-anthesis stage and tested for both ovary- and sepal-derived fruit setting. All the WT emasculated flowers aborted, whereas >45% of the *Alq* emasculated flowers were able to set ovary-derived fruits. In addition, nearly 80% of the emasculated flowers of *Alq* plants set sepal-derived fruits (Supplementary Table S3; Supplementary Fig. S2). Taken as a whole, the results indicate that the *Alq* mutant is able to set fruits in a pollination-independent way and that its facultative parthenocarpy is due to an early transition from flower to fruit.

Changes in endogenous hormone levels may account for early fruit set in the Alq *mutant*

Endogenous hormone analysis in the *Alq* mutant showed a higher level of GA_1 at the anthesis stage combined with a lower level of ABA at both 5 d and 2 d before anthesis (Fig. 3A, C). Furthermore, the *Alq* mutant showed a significantly higher level of different CKs at most of the floral stages analysed. Indeed, dihydrozeatin (DHZ) concentration significantly increased at both 5 d and 2 d before anthesis; the same happened with isopentenyladenine (iP) at 5 d before anthesis, and *trans*-zeatin (tZ) significantly increased at all floral stages analysed (Fig. 3E–G). These hormonal changes, especially the higher CK concentration at floral stages prior to anthesis, may account for the early transition from flower to fruit in the *Alq* mutant as well as for its ability to develop seedless fruits.

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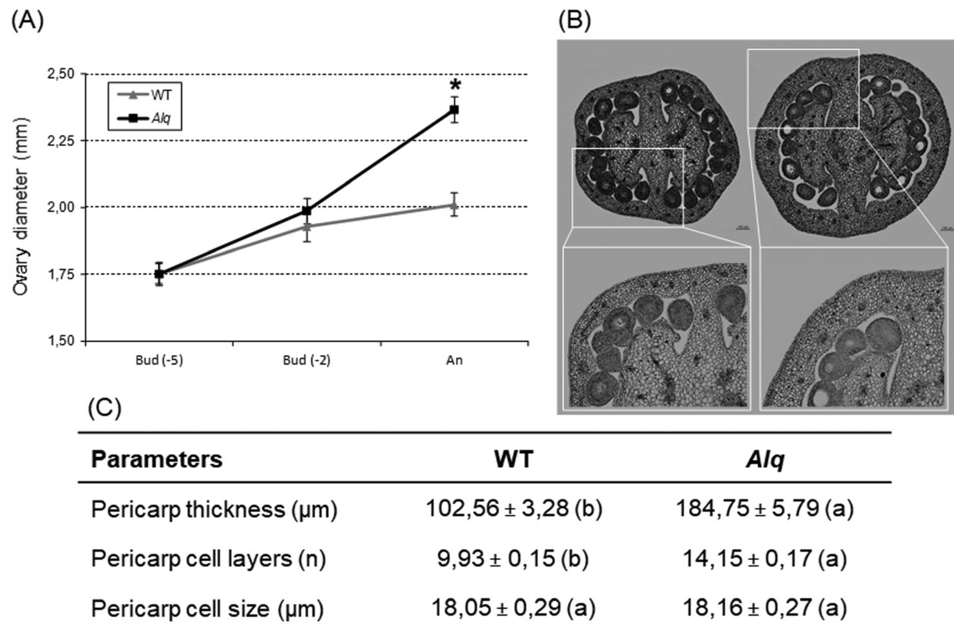

Parameters	WT	*Alq*
Pericarp thickness (μm)	102,56 ± 3,28 (b)	184,75 ± 5,79 (a)
Pericarp cell layers (n)	9,93 ± 0,15 (b)	14,15 ± 0,17 (a)
Pericarp cell size (μm)	18,05 ± 0,29 (a)	18,16 ± 0,27 (a)

Fig. 2. Precocious fruit setting in *Alq* ovaries. (A) Comparison of WT and *Alq* ovary diameter in three stages of floral development: 5 d before anthesis [Bud (-5)], 2 d before anthesis [Bud (-2)], and anthesis day (An). (B) Cross-sections of ovary pericarp in WT (left) and *Alq* (right) flowers at the anthesis stage. (C) Thickness, cell layers, and cell size in pericarp of WT and *Alq* ovaries at the anthesis stage. The values are given as the mean ±SE. In (A), error bars represent ±SE of two independent trials; for each trial, $n \geq 10$. The statistical significance of mean differences was analysed using Fisher's test ($P \leq 0.05$). Asterisks or different letters indicate statistical significance ($P \leq 0.05$).

The Alq *mutation modifies the expression pattern of tomato genes involved in different processes related to fruit set*

Comparative expression analyses were carried out in order to analyse genetic interactions of the *Alq* mutation with tomato genes involved in fruit set and reproductive development. Flowers collected at three developmental stages (2 d before anthesis, at anthesis, and 5 d post-anthesis) were chosen for comparative analysis between the pericarp tissue of WT and *Alq* plants.

The transcript levels of some genes involved in GA biosynthesis (*SlGA20ox1*, *SlGA20ox2*, *SlGA20ox3*, *SlGA3ox1*, and *SlGA3ox2*) and GA inactivation (*SlGA2ox1* and *SlGA2ox2*) were analysed. Although significant differences in the expression levels of most of the *SlGA20ox* genes were not detected, the expression of the *SlGA20ox1* and *SlGA20ox3* genes was significantly down-regulated in the *Alq* ovary at post-anthesis and the anthesis stage, respectively (Supplementary Fig. S4C, E). However, transcript levels of both *SlGA3ox1* and *SlGA3ox2* were significantly increased at the pre-anthesis stage (Supplementary Fig. S4A, B). In the case of the GA2-oxidases, no significant changes in transcript levels were observed in the *SlGA2ox1* gene, whereas the *SlGA2ox2* gene was up-regulated at pre-anthesis and anthesis stages in *Alq* ovaries (Supplementary Fig. S4F, G).

The expression of some auxin signalling genes (*SlIAA9*, *SlARF5*, *SlARF7*, and *SlARF8*) known to be involved in the control of fruit initiation was also analysed. The transcript levels of the *IAA9* gene were similar in WT and *Alq* ovaries (Supplementary Fig. S5A). Concerning the *SlARF5* gene, no significant changes in transcript levels were observed at either pre-anthesis or anthesis stages, but significant differences in the expression levels were detected at the post-anthesis stage (Supplementary Fig. S5B). Transcript levels of *SlARF7* and *SlARF8* shared similar patterns and showed a significant up-regulation in *Alq* ovaries, at both pre-anthesis and anthesis stages (Supplementary Fig. S5C, D).

The transcript levels of some genes involved in ethylene biosynthesis (*SlACO1*, *SlACO2*, *SlACO3*, *SlACS1A*, and *SlACS6*) and senescence (*SlSAG12* and *SlNAP*) were also analysed. Significant differences in the expression levels of these genes were not detected at pre-anthesis and anthesis stages, except in the case of the *SlACS1A* gene that was significantly up-regulated at the anthesis stage (Supplementary Fig. S6D). However, transcript levels of *SlACO1*, *SlACO2*, *SlACO3*, *SlACS1A*, *SlACS6*, *SlSAG12*, and *SlNAP* genes were significantly increased at the post-anthesis stage (Supplementary Fig. S6A–G).

As the fruit set process requires an intense cell division activity, transcript levels of some genes involved in the cell cycle (*CycA1*, *CycD3*, and *CDKA1*) and cell division control (*SlWUS*) were also analysed. In *Alq* ovaries, the *CycA1* gene was down-regulated at the post-anthesis stage, and the same occurred with the *CycD3* gene at both anthesis and post-anthesis stages (Fig. 4A, B). Importantly, the *CDKA1* gene was significantly up-regulated at both anthesis and post-anthesis in *Alq* ovaries (Fig. 4C). In addition, a significant up-regulation of the *SlWUS* gene was detected in *Alq* ovaries at all the floral stages analysed (Fig. 4D). The results of cell cycle gene expression levels are consistent with the changes detected in the content of endogenous hormones, especially CKs, and the early ovary to fruit transition.

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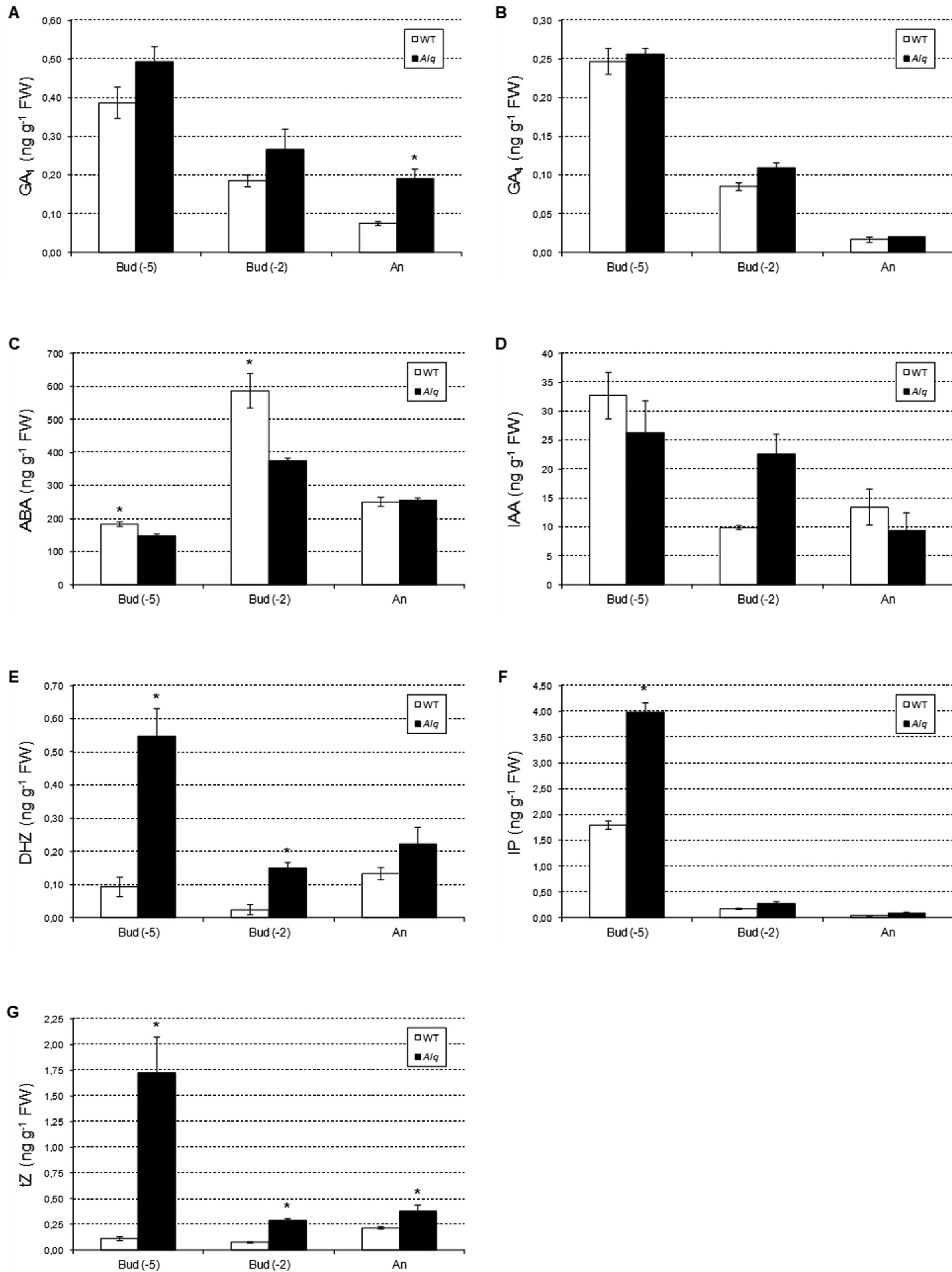

Fig. 3. Endogenous level of different hormones in ovaries from WT and *Alq* plants. The analysis was performed on entire ovaries at 5 days before anthesis [Bud (-5)], 2 d before anthesis [Bud (-2)], and anthesis day (An). (A, B) GA_1 and GA_4: gibberellins GA_1 and GA_4, (C) ABA: abscisic acid, (D) IAA: indole-3-acetic acid, (E) DHZ: dihydrozeatin, (F) IP: isopentenyladenine; and (G) tZ: *trans*-zeatin. The results show the averages and standard errors of three biological replicates (aliquots of ~100 mg each). Asterisks indicate significant differences (Student's *t*-test, $P \leq 0.05$).

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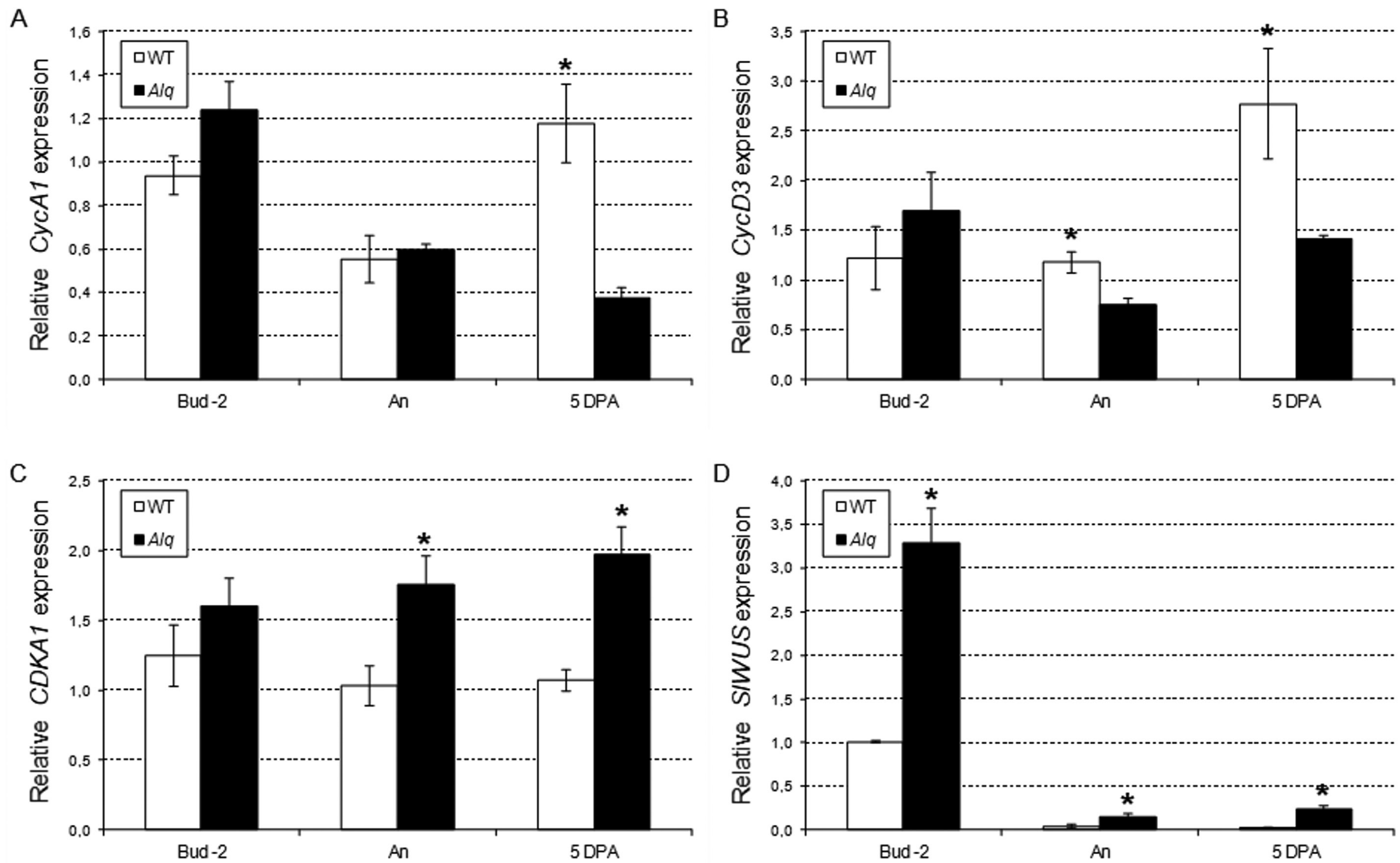

Fig. 4. Expression analyses of tomato genes involved in cell cycle (A–C) and cell division control (D). *CycA1* (A), *CycD3* (B), *CDKA1* (C), and *SlWUS* (D) gene expression in WT and *Alq* ovaries in three stages of floral development: 2 d before anthesis [Bud (-2)], anthesis day (An), and 5 d post-anthesis (5 DPA). The results show the averages and standard errors of three independent biological experiments and three technical replicates. Asterisks denote significant differences (Student's *t*-test, $P<0.05$).

Alq *plants are able to maintain fruit yield under moderate salinity conditions*

Fruit set and yield of WT and *Alq* plants were assessed under both control and moderate salt stress conditions. Plants were grown in a greenhouse without and with salt stress (50 mM NaCl) applied from the sixth leaf stage and maintained throughout the experiment.

In WT plants, the numbers of flowers and fruits per inflorescence were significantly lower in salt stress with respect to control conditions. In contrast, in *Alq* plants, no differences were found in both parameters when comparing control and stress conditions (Table 2). Notably, in both control and salt stress conditions, the number of fruits per inflorescence was significantly higher in *Alq* than in WT plants (Table 2; Supplementary Fig. S7). Likewise, fruit set rate was significantly higher in *Alq* plants, reaching 75% in control conditions and 84% under moderate salt stress. In fact, the two-way ANOVA indicated that the only relevant factor for fruit set was the line. As observed in previous experiments carried out under normal conditions, the average fruit weight was lower in *Alq* than in the WT. However, under stress conditions, the reduction in fruit weight was greater in the WT (38%) than in *Alq* (29%) (Supplementary Table S4). Indeed, as shown in Fig. 5A, almost half of the fruits produced by WT plants in control conditions had a weight ≥50 g, the other half ranged from 20 g to 50 g, and a small proportion weighed <20 g. In salinity conditions only 3% of the fruits produced by WT plants had a weight ≥50 g, almost 75% ranged from 20 g to 50 g, and >20% weighed <20 g. Thus, significant differences were found in all the weight ranges in the case of WT plants (Fig. 5A). In contrast, no significant differences were found when comparing these weight ranges in *Alq* fruits (Fig. 5B, C).

In the WT, fruit yield per plant under salt stress was significantly lower (599.4 g) than in control conditions (1097.6 g) (Table 2), representing a yield decline of >45% (Supplementary Table S4). In contrast, *Alq* plants were able to maintain fruit yield under moderate salinity conditions. Thus, when only ovary-derived fruits were taken into account, fruit yield per plant in *Alq* plants reached 928.7 g in control conditions and 834.9 under salt stress, which represents a yield decline of just 10%. When both ovary- and sepal-derived fruits were taken into account, fruit yield increased up to 1170.6 g in control conditions and 1051.8 under salt stress (Table 2). From an agronomic point of view, it is noteworthy that in both cases fruit yield in *Alq* plants was not significantly reduced by salinity.

Fruit setting decreases in ALQ-TAGL1-*down-regulated plants and increases in plants overexpressing* ALQ-TAGL1

To further validate the effect of the *ALQ-TAGL1* gene on fruit set, we evaluated yield parameters under both control and moderate salt conditions in plants in which *ALQ-TAGL1*

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Table 2. *Fruit production traits in WT (SLDG2) and* Alq *plants grown under standard and moderate salt stress conditions*

Parameters		Without salt stress		With moderate salt stress (50 mM NaCl)		Two-way ANOVA
		WT	*Alq*	WT	*Alq*	
Flowers per inflorescence		11.09±0.27 a	9.25±0.39 b	8.26±0.21 c	9.30±0.28 b	L ns S** L×S**
Ovary-derived fruit	Fruits per inflorescence	3.08±0.43 b	7.05±0.46 a	2.01±0.23 c	7.88±0.43 a	L*** S ns L×S*
	Fruit setting (%)	27.45±3.72 b	75.11±2.66 a	25.29±2.91 b	84.33±3.45 a	L*** S ns L×S ns
	Fruit weight (g)	46.72±1.10 a	22.89±3.90 b	29.03±1.38 b	16.22±1.23 c	L*** S*** L×S*
	Yield per plant (g)	1097.56±118.98 a	928.67±78.95 a	599.40±74.62 b	834.95±99.96 a,b	L ns S** L×S*
Ovary- and sepal-derived fruit	Fruits per inflorescence	3.08±0.43 b	8.58±0.29 a	2.01±0.23 c	9.28±0.29 a	L*** S ns L×S*
	Fruit setting (%)	27.45±3.72 b	93.20±3.32 a	25.29±2.91 b	99.77±0.23 a	L*** S ns L×S ns
	Fruit weight (g)	46.72±1.10 a	27.34±4.18 b	29.03±1.38 b	19.63±1.41 c	L*** S*** L×S*
	Yield per plant (g)	1097.56±118.98 a	1170.59±83.22 a	599.40±74.62 b	1051.82±112.10 a	L* S** L×S ns

Different parameters such as the number of fruits per inflorescence, fruit setting, fruit weight, and yield per plant were assessed, considering only ovary-derived fruits or both ovary- and sepal-derived fruit The values are given as the means ±SD of 10 individual plants per line and condition. Means within each column followed by different letters are significantly different in accordance with the Fisher post-hoc test ($P<0.05$). For each parameter, the two-way ANOVA was obtained to test the effect of line (L), salinity (S), and their interaction (L×S). Asterisks denote significant differences at *$P<0.05$; **$P<0.01$; and ***$P<0.0001$ probability levels; ns=not significant.

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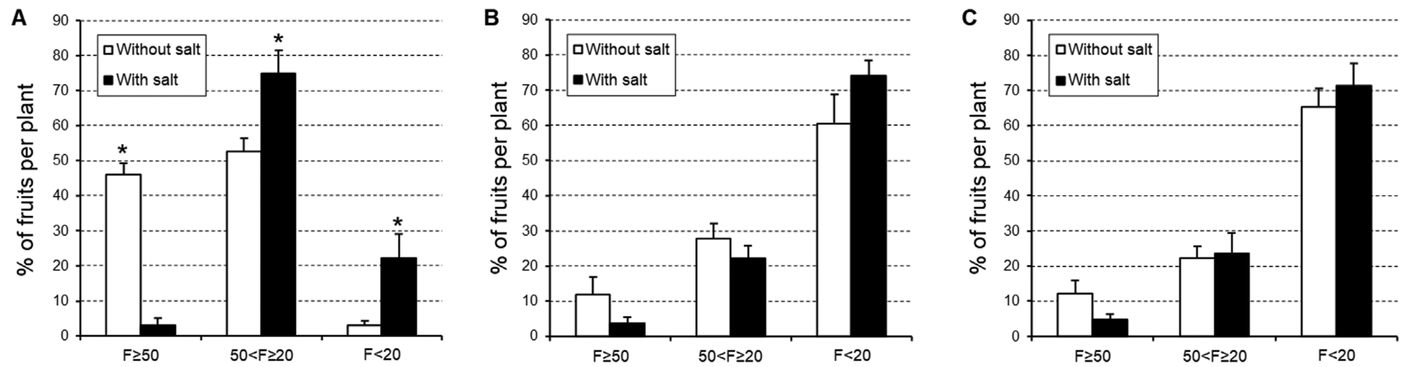

Fig. 5. Percentage of fruit per weight interval in both non-salt and salt conditions: (A) WT, (B) *Alq* (ovary-derived fruits), and (C) *Alq* (ovary- and sepal-derived fruits). Each graph illustrates the percentage of fruits whose weights are ≥50 g (F≥50), with weights between 20 g and 50 g (50<F≥20), and with weights <20 g (F<20). A total of 118 and 125 WT fruits, 220 and 255 *Alq* fruits (ovary-derived fruits), and 281 and 320 *Alq* fruits (ovary- and sepal-derived fruits) from non-salt and salt conditions, respectively, were used. Asterisks indicate significant differences (Student's *t*-test, *P*≤0.05).

was silenced or overexpressed. In both WT and *ALQ-TAGL1*-RNAi plants, fruit yield per plant under salt stress was significantly lower than in control conditions, representing a yield decline of 55% and 69%, respectively. However, no significant differences were found in *ALQ-TAGL1*-OE plants when comparing fruit yield under both conditions (Table 3). With respect to fruit set, a significant decline was observed under control conditions in the *ALQ-TAGL1*-RNAi plants, despite the fact that the number of flowers per inflorescence was lower than in WT plants. Importantly, set rate was significantly higher in *ALQ-TAGL1*-OE plants under both control conditions and moderate salt stress (Table 3). Consistent with these results, the two-way ANOVA indicated that the only relevant factor for fruit set was the line.

Discussion

Arlequin (*Alq*) is an insertion mutant whose sepals have the outstanding ability to turn, morphologically, structurally, and metabolically, into fruit-like organs which acquire all the characteristics of a sink and ripen as in ovary-derived fruits (Pineda *et al.*, 2010). Molecular characterization of the tagged gene demonstrated that *Alq* is a gain-of-function mutant and that its characteristic phenotype is due to the ectopic expression of the *ALQ-TAGL1* gene (Giménez *et al.*, 2010). The essential role of this gene as a positive regulator of fruit ripening has been shown (Itkin *et al.*, 2009;Vrebalov *et al.*, 2009; Giménez *et al.*, 2010). Several lines of evidence suggested that this gene may also play an important role in the fruit setting process. The highest accumulation of transcripts occurs 1–2 d before anthesis, at the anthesis stage (Giménez *et al.*, 2010), or immediately after in the ovary (Busi *et al.*, 2003). It has also been stated that the *ALQ-TAGL1* gene plays a role in the cell proliferation stage following anthesis (Giovannoni *et al.*, 2017). Furthermore, the fact that the ectopic expression of *ALQ-TAGL1* in the *Alq* mutant causes a homeotic conversion of sepals into functionally equivalent structures to carpels points in the same direction. Therefore, the *ALQ-TAGL1* gene could emerge as a new player in the fruit set process.

We present here compelling evidence in this respect, since the in-depth characterization of the reproductive development of *Alq* revealed that the up-regulation of the *ALQ-TAGL1* gene, caused by the T-DNA insert, doubles the fruit set rate (93% in *Alq* versus 43% in the WT, Table 1). The enhanced expression of the *ALQ-TAGL1* gene in *Alq* not only increases the fruit set rate but also promotes the ability to set fruits in a pollination-independent way (Fig. 1C; Supplementary Table S1). Notably, it has been reported that the overexpression of the *PpPLENA* gene, the peach orthologue of *ALQ-TAGL1*, also promotes pollination-independent fruit set in transgenic tomato plants (Tadiello *et al.*, 2009). Unlike that which occurs in other mutants with facultative parthenocarpy (Mazzucato *et al.*, 1998; Rojas-Gracia *et al.*, 2017), *in vitro* and *in vivo* tests have shown that the ability of *Alq* to set fruits in the absence of pollination is not related to the lack of pollen viability (Supplementary Fig. S1; Supplementary Table S2). Instead, the parthenocarpic fruit development in *Alq* is due to precocious fruit set prior to anthesis (Fig. 2). Most of the *Alq* seedless fruits bore seed-like structures, usually called pseudoembryos or aborted seeds (Fig. 1E). These pseudoembryos are typically present in auxin-induced fruits (Asahira *et al.*, 1967; Kataoka *et al.*, 2003), but have also been found in parthenocarpic tomato fruits treated with synthetic CKs (Matsuo *et al.*, 2012). Pseudoembryos in seedless fruits have also been observed in transgenic tomatoes expressing an aberrant form of *AtARF8*, an ARF expressed in the ovule (Goetz *et al.*, 2007). Similarly, in the ovules of WT flowers at stage 9 (according to Brukhin *et al.*, 2003), high levels of *ALQ-TAGL1* transcripts have been found (Vrebalov *et al.*, 2009; Giménez *et al.*, 2010). Therefore, it cannot be ruled out that enhanced expression of *ALQ-TAGL1*, through the stimulation of *SlARF8* gene expression, takes part in the development of the pseudoembryos observed in *Alq* fruits.

Our results indicated that the enhanced expression of the *ALQ-TAGL1* gene in *Alq* increases the expression of some ethylene-related genes at the post-anthesis stage (Supplementary Fig. S6A–E), consistent with the fact that the *ALQ-TAGL1* gene acts upstream of ethylene-related genes (Itkin *et al.*, 2009; Vrebalov *et al.*, 2009; Giménez *et al.*, 2010). Ethylene is involved in ovule senescence in unpollinated ovaries (Shinozaki *et al.*, 2018). The higher expression levels of both *SlSAG12* and *SlNAP* genes observed in *Alq* ovaries at the post-anthesis

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stage (Supplementary Fig. S6F, G) could be associated with the senescence of the ovules, since the *Alq* mutation promotes pollination-independent fruit set.

Besides GAs and auxins, CKs and ABA are generally believed to be involved in fruit growth and development (Mariotti *et al.*, 2011). The levels of auxin, GAs, and CKs increase during the fruit setting phase, while those of ABA decrease (Mariotti *et al.*, 2011; McAtee *et al.*, 2013; Kumar *et al.*, 2014). The exogenous application of auxin and GAs can trigger pollination-independent fruit set in tomato plants (Martí *et al.*, 2007; Serrani *et al.*, 2007, Matsuo *et al.*, 2012). Similarly, exogenous CK treatment has been reported to induce parthenocarpy in the tomato (Ding *et al.*, 2013) and it has been proposed that its endogenous level is directly correlated with fruit growth, especially in the stimulation of cell division (Kumar *et al.*, 2014). Marsch-Martínez *et al.* (2012) demonstrated that *SHATTERPROOF* (*SHP*) MADS-box transcription factor genes, the Arabidopsis orthologues of the *ALQ-TAGL1* gene, are required for CK accumulation during gynoecium and fruit development. More importantly, the concentrations of different CKs (DHZ, iP, and tZ) significantly increased before anthesis, coinciding with the time when fruit set in *Alq* occurs (Fig. 3E–G). Our results suggest that the increase of endogenous CKs promoted by the up-regulation of the *ALQ-TAGL1* gene is one of the factors that determine early fruit set in *Alq*, possibly through the activation of core cell cycle genes during the early stages of fruit development. On the other hand, the role of ABA in the tomato fruit set process is still unclear. Nitsch *et al.* (2009) showed that ABA levels are relatively high in mature ovaries and down-regulated after pollination, which suggests that ABA inhibits ovary growth until fruit set. However, it was not possible to induce fruit set by way of the application of an ABA biosynthesis inhibitor (fluridone) nor to inhibit fruit set by ABA application to pollinated ovaries. For this reason, these authors suggested that ABA is an additional player in the regulation of tomato fruit set, together with other hormones. Our results provide evidence in favour of this hypothesis. The analysis of endogenous hormones in *Alq* ovaries not only showed a dramatic increase in CK content but also a significantly lower level of ABA at both 5 d and 2 d before anthesis (Fig. 3C). This could indicate that it is not just the concentration of either CKs or ABA, but rather the CK and ABA crosstalk, that is the key event which induces early fruit set in the *Alq* mutant.

Our results also showed that *Alq* ovaries had higher pericarp thickness mainly associated with an increase in cell layers at anthesis (Fig. 2B, C). Tomato fruit development is characterized by an intense activity of cell divisions after the fruit set. As mentioned above, the endogenous level of CK is directly correlated with the fruit growth, especially in the stimulation of cell division (Kumar *et al.*, 2014). Furthermore, the active cell division induced upon flower fertilization is consistent with the up-regulation of CDKs (Joubès *et al.*, 1999). Comparative expression analyses in the *Alq* mutant revealed important changes in transcript levels of several genes involved in cell division, GA metabolism, and the auxin signalling pathway (Fig. 4; Supplementary Fig. S4; Supplementary Fig. S5). In tomato ovaries, *CDKA1* transcripts accumulate after anthesis

Table 3. *Fruit production traits from WT (Moneymaker),* ALQ-TAGL1 *RNAi, and* ALQ-TAGL1 *OE plants grown under standard and moderate salt stress conditions*

Line	Salt	Flowers per inflorescence	Fruits per inflorescence	Fruit setting	Fruit weight (g)	Yield per plant (g)
WT	0 mM	12.36±1.35 a	4.93±0.50 c	43.13±2.82 b	50.32±1.10 a	1487.59±146.31 a
	50 mM	8.74±0.36 b,c	2.26±0.13 d	25.87±1.38 c	33.86±1.29 b	662.65±68.31 b
ALQ-RNAi	0 mM	9.94±0.55 b,c	2.22±0.16 d	26.09±2.12 c	43.26±2.37 a	713.62±63.66 b
	50 mM	11.05±0.74 a,b,c	3.19±0.45 d	30.63±3.27 b,c	30.96±9.83 b	218.23±58.08 c
ALQ-OE	0 mM	11.43±0.14 a,b	10.29±0.59 a	93.09±2.86 a	17.68±1.87 c	743.17±90.93 b
	50 mM	9.10±0.13 c	7.62±1.17 b	81.83±14.17 a	15.33±3.42 c	400.49±75.05 b,c
Two-way ANOVA		L ns	L***	L***	L***	L***
		S **	S***	S ns	S***	S***
		L×S**	L×S***	L×S ns	L×S ns	L×S ns

The values are given as the means ±SD of 10 individual plants per line and condition. Means within each row followed by different letters are significantly different according to Fisher's post-hoc test ($P<0.05$). The lower row shows data obtained by two-way ANOVA, to test the effect of line (L), salinity (S). and their interaction (L×S). Asterisks denote significant differences at *$P<0.05$; *$P<0.01$; and ***$P<0.0001$ probability levels; ns=not significant

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(Joubès *et al.*, 1999). The significantly higher expression levels of the *CDKA1* gene observed in *Alq* ovaries at the anthesis stage could be associated with the early ovary growth (Fig. 4C). This precocious ovary growth in the *Alq* mutant could also be related to the up-regulation of genes encoding enzymes of GA biosynthesis (*SlGA3ox1* and *SlGA3ox2*) before the anthesis stage (Supplementary Fig. S4A, B). Moreover, *Alq* mutation influences the expression pattern of some auxin signalling pathway components that have been shown to be instrumental in the control of tomato fruit initiation. *SlARF8*, a member of the tomato *ARF* gene family, can stimulate expression of early auxin-responsive genes, initiating fruit growth and development (Goetz *et al.*, 2007). In fact, tomato transgenic plants down-regulated in the expression of the *SlIAA9* gene exhibited precocious fruit set and showed up-regulation of *SlARF8* at the bud stage (Wang *et al.*, 2005, 2009). Interestingly, the *SlARF8* gene showed strong up-regulation in *Alq* ovaries at pre-anthesis and anthesis stages (Supplementary Fig. S5D), although no significant changes were detected in the expression of the *SlIAA9* gene (Supplementary Fig. S5A). *SlARF5*, another member of the tomato *ARF* gene family, is expressed at high levels in unpollinated ovaries but maintains low expression levels in pollinated ovaries (Liu *et al.*, 2018). The higher expression levels of the *SlARF5* gene observed in *Alq* ovaries post-anthesis could be associated with the pollination-independent ovary growth (Supplementary Fig. S5B). Also, the *SlWUS* gene, which is a member of the plant-specific WUS homeobox (WOX) transcription factor family (Muños *et al.*, 2011) and is involved in promotion of cell division (Azzi, *et al.*, 2015), was significantly up-regulated from the pre-anthesis stage (Fig. 4D). Apart from its important role in the ripening process (Itkin *et al.*, 2009;Vrebalov *et al.*, 2009; Giménez *et al.*, 2010), it has been documented that the *ALQ-TAGL1* gene is involved in the regulation of fruit development through cell division control (Azzi *et al.*, 2015). Therefore, the higher expression of *ALQ-TAGL1* in the *Alq* mutant could promote pollination-independent ovary growth by altering transcript levels of genes involved in cell division and other important regulators related to fruit setting. Interestingly, our results indicated that in the *Alq* mutant, there is an increase in the expression of cell cycle genes in the ovary of flowers at the anthesis stage as well as in the early stages of fruit development (Fig. 4C). Moreover, fruit setting is significantly reduced in *ALQ-TAGL1*-silenced plants and increased in plants overexpressing *ALQ-TAGL1* (Table 3). These results suggest that the *ALQ-TAGL1* gene could act as an integrator of the ovary development signal in the fruit set process.

The ability of the *Alq* mutation to increase the fruit set rate could be of potential interest under unfavourable stress conditions. In this study, a comparative assessment of the fruit set and yield under both standard and moderate salt stress conditions was carried out in WT and *Alq* plants (Table 2). According to Cuartero and Muñoz (1999), at relatively low salinity electrical conductivities (e.g. 2.5 dS m^{-1}), the yield reduction is mainly caused by a decrease in the fruit average weight. Interestingly, we have observed that salt stress had a more severe impact on fruit weight of the WT than of *Alq*. In addition, WT plants cultivated under salt conditions significantly reduced the percentage of larger fruits and significantly increased the percentage of smaller ones. In contrast, in *Alq* plants the percentage of larger and smaller fruits was similar under standard and salt conditions (Fig. 5). Under non-salt conditions, most of the *Alq* flowers set fruit, while only one out of four flowers of WT plants did. Notably, under salt conditions, *Alq* plants developed a similar number of fruits to that in normal conditions, whereas in WT plants the number of fruits per inflorescence was significantly reduced (Table 2).

The higher fruit set observed in *Alq* plants could be favoured by the inhibition of the fruit AZ (Pineda *et al.*, 2010). According to Cuartero and Muñoz (1999), the inhibition of the fruit AZ could avoid the 'drop of developing fruit' often observed under salinity. In addition to the role played in fruit setting, auxin operates as a brake of fruit abscission (reviewed by Roberts *et al.*, 2002). Interestingly, one possible role for CKs in fruit development is to modulate auxin biosynthesis and/or polar auxin transport to prevent flower abscission (Matsuo *et al.*, 2012). Some MADS-box genes such as *JOINTLESS* and *MACROCALYX* (*MC*) regulate pedicel AZ development and activate the auxin-mediated pathway to prevent flower abscission (Mao *et al.*, 2000; Nakano *et al.*, 2012). It is known that *JOINTLESS* and *MC* interact with *ALQ-TAGL1* (Leseberg *et al.*, 2008; Giménez *et al.*, 2016). In addition, ectopic expression of the *ALQ-TAGL1* gene promotes down-regulation of *JOINTLESS* in *Alq* sepals (Pineda *et al.*, 2010). Then, the increase in endogenous CKs in *Alq* plants could play, in concert with auxins, an important role in the inhibition of the pedicel AZ associated with the interaction between the *Alq* mutation and both *JOINTLESS* and *MC* genes.

The maintenance of agricultural productivity under unfavourable stress conditions is an important goal in order to meet growing food demand through sustainable agriculture (Cuartero *et al.*, 2010; Pineda *et al.*, 2012). Recently, Klap *et al.* (2017) reported that *SlAGL6* loss of function results in fertilization-independent fruit set, improving fruit production under heat stress conditions that usually affect pollen viability and the fertilization process. Here, by evaluating long-term production, we show that *Alq* plants are able to maintain fruit yield under moderate salinity conditions based on a higher fruit set rate. Notably, when only the ovary-derived fruits are taken into account, *Alq* plants set 59% more fruits and gave 39% higher yield than the WT under saline conditions. Taking into account both sepal- and ovary-derived fruits, *Alq* plants set 74% more fruits and had 75% higher yield than the WT in stress conditions.

In summary, our results reveal that increased expression of the *ALQ-TAGL1* gene in the *Alq* mutant promotes early fruit set (i.e. prior to anthesis) which could be due to the increase in the content of endogenous CKs and the decrease in the level of ABA in pre-anthesis. In addition, the *Alq* mutant undergoes a much higher fruit set rate than the WT. Importantly, the ability of the *Alq* mutation to increase the fruit set rate allows for the maintenance of fruit yield under moderate salinity conditions.

Supplementary data

Supplementary data are available at *JXB* online.

Fig. S1. *In vitro* assays of pollen viability in WT and *Alq* plants.

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Fig. S2. Red fruits developed from emasculated flowers of the *Alq* mutant.

Fig. S3. Total RNA quality assessment using denaturing agarose/formaldehyde gel electrophoresis.

Fig. S4. Expression analyses of GA biosynthesis genes and GA inactivation genes.

Fig. S5. Expression analyses of auxin response transcription factors.

Fig. S6. Expression analyses of ethylene- and senescence-related genes.

Fig. S7. Fruits produced in inflorescences of WT and *Alq* plants under control and salt stress conditions.

Table S1. Level of facultative parthenocarpy in WT and *Alq* plants.

Table S2. Weight and seed number of fruits obtained from self-pollination by hand or reciprocal backcrosses between WT and *Alq* flowers.

Table S3. Ovary- and calyx-derived fruit set from flowers emasculated at the pre-anthesis stage of WT and *Alq* plants.

Table S4. Relative comparison of reproductive development in the salt-treated plants (with respect to untreated plants) in the WT and *Alq*.

Table S5. Primers used for real-time quantitative PCR assays.

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