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Full Length Research Paper

The effects of calcium on the expression of genes involved in ethylene biosynthesis and signal perception during tomato flower abscission

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Calcium regulation of the genes involved in ethylene biosynthesis and ethylene receptors in flower abscission zones (AZ) of wild-type tomato (*Lycopersicon esculentum* Mill.) was investigated in this study. Calcium treatment delayed abscission of pedicel explants. However, verapamil (VP, calcium inhibitor) treatments accelerated abscission. Results showed an increase in AZ ethylene production consistent with abscission in both control and treatment explants. Moreover, in the AZ of flower pedicels treated with VP, ethylene production, *LeACO1* and *LeETR1* expression was elevated, but *LeETR4* expression decreased. Calcium treatments delayed the onset and maximum ethylene production, maintained low *LeACO1* expression and elevated *LeETR2, 3, 4, 5* expressions. Results also found that VP and calcium had little effect on *LeACS2* expression. Our hypothesis that calcium is integral in preventing ethylene production at the point when 1-aminocyclopropane-1-carboxylic acid (ACC) is converted to ethylene was supported, but the study also revealed the regulation of expression in the ethylene receptors. The potential roles of *LeACO1*, *LeETR 3, 4, 5* and calcium in AZs during tomato pedicel explants abscission are discussed.

Key words: Abscission, ethylene receptor, calcium, tomato.

INTRODUCTION

Abscission of plant organs occurs by cell separation and plays a key role in the plant life cycle. Several studies have addressed the biochemical, molecular and genetic regulation of tomato abscission. Several studies indicate that abscission is indeed accompanied by a burst of ethylene, required to induce several processes associated with abscission, including marked changes in hydrolase, chitinase and expansins (Lashbrook et al., 1994; Volko et al., 1998; Belfield et al., 2005).

Calcium plays an important role in a wide range of physiological and biochemical processes in plants. In addition, calcium also adjusts ethylene responses in plants; for example, calcium affects “triple responses” of etiolated tomato seedlings (Zhang et al., 2002), delays fruit ripening (Ferguson, 1984), flower senescence and flower abscission (Glenn et al., 1988). Calcium, in the form of calcium pectate, binds adjacent cells and acts as a type of cementing agent within cell walls. Cementing is presumed to occur when pectic substances bind calcium ions by double salt formation. If adequate amounts of calcium are not provided during cell formation, tissues become less stable and prone to disintegration. Ca^{2+} abscission inhibition may be related, in part to the cementing effect of Ca^{2+} on cell walls through salt bridge formation between pectic components. Several lines of evidence, however, suggest that other processes are involved in abscission inhibition. For example, pulvinar tissues remain green suggesting a deferral of pulvinar senescence, presumed to be a prerequisite to the onset of ethylene responsiveness (Poovaiah and Leopold, 1973). The fact that other cations such as Mg^{2+} are markedly less effective further suggests that calcium is not simply a cementing of cell wall pectins; Tagawa and Bonner (1957) reported

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Abbreviations: AZ, Abscission zones; **VP,** verapamil; **SAM,** S-adenosylmethionine; **ACC,**1-aminocyclopropane-1-carboxylic acid; **ACS,** 1-aminocyclopropane-1-carboxylic acid synthase; **ACO,** 1-aminocyclopropane-1-carboxylic acid oxidase; **ETRs,** ethylene receptors; **SDS,** sodium deodecyl sulfate; **AR,** abscission rate; **CDPK,** calcium-dependent protein kinase; **1-MCP,** 1-methylcyclopropene.

similar effects for Ca^{2+} and Mg^{2+} in cell wall hardening. Cell wall cement degradation occurs largely during the later stages of abscission (Dela and Leopold, 1969; Morre, 1968) and Ca^{2+} inhibition is most effective when applied immediately after cutting the explant. The addition of Ca^{2+} extends stage 1, which again supports retardation of one or more senescence processes. Abscission inhibition by Ca^{2+} in the presence of added ethylene suggests that Ca^{2+} can suppress ethylene responsiveness; and ethylene biosynthesis depression by Ca^{2+} results in the suppression of normal senescence (Poovaiah and Rasmussen, 1973). Collectively, these results suggest that the effects of Ca^{2+} on abscission may be related to deferral of senescence development rather than to the influences of Ca^{2+} in cementing cell walls.

Ethylene production in plant tissues results from the metabolism of methionine. The rate-limiting steps in fruit ethylene biosynthesis include the conversion of S-adenosylmethionine (SAM) to 1-aminocyclopropane-1-carboxylic acid (ACC) via ACC synthase (ACS) and the subsequent metabolism of ACC to ethylene by ACC oxidase (ACO). ACS is the rate-limiting enzyme in the biosynthetic pathway and ethylene production is modulated mainly via regulation of ACS gene expression (Kende, 1993; Wang et al., 2002). In tomato, both steps are transcriptionally regulated and encoded by multigene families (Vriezen et al., 1999; Wagstaff et al., 2005; Annalisa et al., 2006) and two systems have been proposed to explain the regulation of ethylene during plant development (Lelievre et al., 1997). System 1 provides for the base ethylene level present in vegetative tissues and preclimateric and non-climateric fruits. System 2 is responsible for the high levels of ethylene production associated with flower senescence (Oetiker and Yang, 1995). Specific members of the *LeACS* and *LeACO* gene families are proposed to regulate the transition from system 1 to 2 ethylene production (Nakatsuka et al., 1998; Barry et al., 2000). Based on gene expression patterns and ethylene regulation, *LeACS2* and *LeACS4* are proposed to mediate system 2 ethylene production, whereas *LeACS1* and *LeACS6* function in system 1 in green fruit and vegetative tissue (Nakatsuka et al., 1998; Barry et al., 2000). Both *ACO1* and *ACO3* transcripts accumulate during leaf, fruit and flower senescence. Furthermore, data suggest that *ACO1* is wound-inducible in leaves. All three ACC oxidase genes are expressed during flower development, each showing a distinct temporal pattern of accumulation. In addition, the ACC oxidase transcripts are spatially regulated throughout flower development; *ACO1* is predominantly expressed in the petals, stigma and style; *ACO2* expression is mainly restricted to tissues associated with the anther cone; and *ACO3* transcripts have been shown to accumulate in all floral organs examined with the exception of the sepals (Barry et al., 1996).

Ethylene is detected by ethylene receptors (ETRs) and by six different genes for *LeETR1* and *LeETR2*; *NR*; and *LeETR4*, *LeETR5* and *LeETR6* have been identified in tomato (Klee, 2002; Klee and Tieman, 2002; Klee and Clark, 2004). A prediction of the negative regulation model of ethylene receptors predicts that increased levels of receptor should reduce ethylene sensitivity (Ciardi et al., 2000). Studies addressing phenotypic effects to ethylene response showed that transgenic tomato expressing the ethylene receptor gene *LeETR1* exhibited shorter internodes and delayed abscission (Whitelaw et al., 2002) and reduced expression of *LeETR1*. *LeETR1* or *LeETR2* transgenic tomato lines, *ale1* and *ale2*, displayed similar ethylene response phenotypes, however, *ale1* exhibited an increased ethylene response relative to *ale2*. The effects of ethylene, particularly accelerated abscission resulting from *LeETR1* transgenic expression, this suggests that *LeETR1* elicits a more important role in the ethylene signaling pathway among genes in the *LeETR* gene family. *NR*, *LeETR4* and *LeETR5* are induced during fruit ripening, organ senescence and abscission, and pathogen infection, and are found at lower levels in vegetative tissue (Tieman et al., 2000; Ciardi et al., 2000; Tieman and Klee, 1999). A reduction of the *LeETR4* transcript in transgenic tomato mutants resulted in marked morphological changes associated with increased ethylene responsiveness, including a strongly epinastic petiole, accelerated flower senescence and delayed fruit ripening (Tieman et al., 2000).

Flower abscission is associated with transcriptional regulation of the ACS, ACO and ethylene receptor genes. However, far less is known of the calcium inhibiting ethylene production mechanisms. This study investigated the affects of calcium treatments on two ethylene pathways during tomato flower abscission: (1) Ethylene biosynthesis pathway, particularly inhibition of ethylene production and (2) ethylene signal perception pathway.

MATERIALS AND METHODS

Plant material

Seeds of the cultivar Liaoyuanduoli (*Lycopersicon esculentum* Mill.), a popular tomato variety bearing an indeterminate inflorescence from Northeast China, were planted in September 2004 at the Shenyang Agricultural University. Plants were cultivated in a glass greenhouse (25 ± 3°C day, 15 ± 3°C night) under natural light (300 $\mu E \cdot m^{-2} \cdot s^{-1}$) in 15 cm of soil. Fertilizer was applied three times and was comprised of soluble nitrogen (N), phosphorus (P) and potassium (K) in a ratio of 1 N: 0.25 P: 1.5 K, resulting in a 1.22 kg $N \cdot m^{-2}$ application. Plants were watered twice a week. The soil organic matter, pH and relative water content were 40 $g \cdot kg^{-1}$, 6.6 and 75%, respectively.

Open flowers were required to study petiole abscission and characterized as follows: all five petals were reflexed back from the anther cone, exhibited a yellow color and the floral opening angle was approximately 90°. The flowers were excised from the inflorescence and immediately trimmed into explants under water to reduce the risk of xylem embolism and dehydration. Pedicel explants were cut into 20 mm lengths following flower removal.

Chemical treatments and abscission rate investigation

Fifty explants were inserted from the proximal end into a Petri dish

Table 1. Time to 50% abscission based on calcium and VP treatments in tomato pedicel explants sampled at anthesis without ethylene treatment.

Treatment	Time to 50% abscission (h)
Control	26 ± 2.52 b
Calcium	32 ± 1.89 a
VP	23 ± 2.71 c

Numbers followed by the same letter are not significantly different (P < 0.05) according to Duncan's multiple range test. Data in Table 1 are means ± S.E. (n = 5).

containing 0.9% agar medium. Different Ca^{2+} treatments were applied to each of the nine dishes and one dish served as the control and received no treatment (80 mmol·L^{-1} $CaCl_2$ and 1 mmol·L^{-1} VP). Treatment concentrations were selected based on our previous work (Xu et al., 2009). Dishes were subsequently placed in a container with a glass cover (40 × 25 × 20 cm^3) and the explants incubated at 25°C. The number of abscised pedicels was recorded after 8, 16, 24, 32, 40 and 48 h, following the methods of Wang et al., (2005).

The control and treatment explants were obtained at incubation periods of 0, 8, 16, 24, 32 and 40 h. Explants were cut above and below the abscission zones (AZ), including a 2 mm long segment at the joint-like position. The samples were frozen in liquid N_2 and stored at -80°C for enzyme extraction and Northern blot analysis.

Ethylene production

Treated explants were inserted into vials, placed in inverted 125 ml Erlenmeyer flasks and capped with rubber serum stoppers for 2 h. Ethylene production was estimated using gas chromatography within each flask and fresh explant weight was subsequently measured.

RNA extraction

Explants were incubated for 0, 8, 16, 24, 32 and 40 h, and subsequently left for 0, 8, 16, 24, 32, 40 and 48 h without further treatment. Following incubation, RNA was extracted from a 2 mm wide piece of tissue containing the flower AZ (zone tissue). A minimum of 1 g of frozen tissue was used to extract total RNA with plant TRIZOL reagent.

RT-PCR and partial cDNA cloning

Two micrograms of total RNA were extracted from the abscission zone of an ethylene treated tomato flower 8 and 24 h. RNA from samples treated with ethylene was used to synthesize first-strand cDNA using SuperScript II RNase H-Reverse Transcriptase (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. *LeACS2* (440 bp) partial cDNA cloning was performed using a primer pair designed based on *LeACS2* cDNA gene sequence motifs (NCBI AF001000). The template (1 µl of cDNA) was obtained from the AZ of 48 h tomato explants. The primer sequences were as follows: F-5'- GCTGATCCTGGCGATGCATT -3', R-5'- ACTCGAAATCCTGGTAA ACCT -3'. *LeACO1* (507 bp) partial cDNA cloning was performed using a primer pair designed based on *LeACO1* cDNA gene sequence motifs (NCBI u70480). The template (1 µl of cDNA) was obtained from the AZ of 48 tomato explants. The primer sequences were as follows: F-5'- AAGAAGTGCATGGAAC AAAGG -3', R-5'- GACCACTGACTTTGTCATCTT -3'. *LeETR1* (517 bp) partial cDNA cloning was performed using a primer pair designed based on *LeETR1* cDNA gene sequence motifs (NCBI u70481). The primer sequences were as follows: F-5'- ATTCTGCTGGACC TGGACTT -3', R-5'- AGAGTAACTCCATCCATTCCAA -3'. *LeETR4* (375 bp) partial cDNA cloning was performed using a primer pair designed based on *LeETR4* cDNA gene sequence motifs (NCBI u70481). The template (1 µl of cDNA) was obtained from the AZ tissue of 24 h tomato explants and the primer sequences were as follows: F-5'- CTGTGGCTGCTATTAGGATG -3', R-5'- CATCTTGCAACATGGA GAGCA -3'. The primer sequences for LeETR2 (NCBIAF043085, 313 bp) were as follows: F-5'- TTTCCCCAAGTTGTTCTG -3', R-5'- CTTCGTTACCATCCTGCT -3' while the primer sequences for LeETR3 (NCBI U38666, 349 bp) were as follows: F-5'- CGCAGCA GGAAATAGCAT -3', R-5'- CATAACCACAGCGACCGT -3'. LeETR5 (NCBI AY600439, 368 bp) had primer sequences of F-5'- TTTCCAACAGACCCTCG -3', R-5'- AGAAATCGGTTGCTCCA -3' while LeETR6 (NCBI AY079426, 574 bp) primer sequences were as follows: F-5'- CTGCTCCTCCAACATACG -3', R-5'- TCATCGG CGAGTCCTG -3'.

Northern blot analysis

Total RNA was fractionated by electrophoresis in a 1.4% (w/v) agarose gel containing formaldehyde, subsequently blotted onto nylon membranes and incubated at 65°C for 1 h in hybridization buffer (5 × sodium chloride/sodium phosphate/EDTA [SSPE; pH 7.4], 5 × Denhardt's solution, 1% [w/v] sodium deodecyl sulfate (SDS) and 10% [w/v] Dextran sulfate). Random priming was employed to generate DIG-labeled probes (DIG prime II kit, Ambion, Austin, TX) derived from tomato cDNAs encoding *LeACS2*, *LeACO1, LeETR1* and *LeETR4* partial cDNA. 18S and 28S ribosomal RNA genes were used as a loading control. Synthetic oligonucleotide probes were purified using Bio-spin P6 chromatography columns (Bio-Rad, Hercules, CA). Probes were subsequently heat denatured, added to hybridization buffer and incubated with membranes at 65°C overnight. Membranes were washed once with 2 SSPE for 20 min at room temperature, two to three times with SSPE and 1% (w/v) SDS for 15 to 30 min at 65 °C and exposed for 1 h to X-ray film or a Phosphor Imager (Bio-Rad). The densitometry analysis was performed by using Quantity One 4.1.1 software (Bio-Rad).

Statistical analyses

All experiments were repeated at least six times. The significance of differences among treatments was determined according to Duncan's multiple range test (P < 0.05).

RESULTS

The time required for full abscission of flower pedicels subjected to each treatment was recorded and the average time required for 50% of the explants to abscise was determined (Table 1). Approximately, 50% abscission was observed in the controls after 26 h, but the addition of $CaCl_2$ delayed the time to reach 50% abscission by approximately 6 h. However, verapamil (VP) treatment accelerated the time to reach 50% abscission by approximately 3 h.

The relative AR (abscission rate) for control explants peaked at approximately 39% after 32 h (Figure 1). Abscission

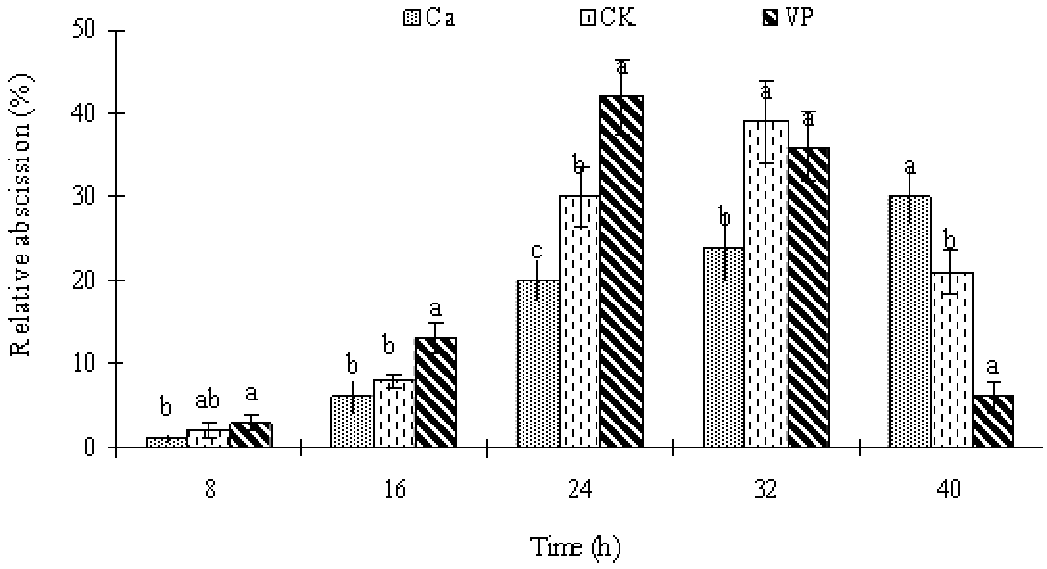


Figure 1. Effects of calcium and VP on relative abscission rates of tomato pedicel explants sampled at anthesis. Vertical bars indicate ± S.E. (n = 5).

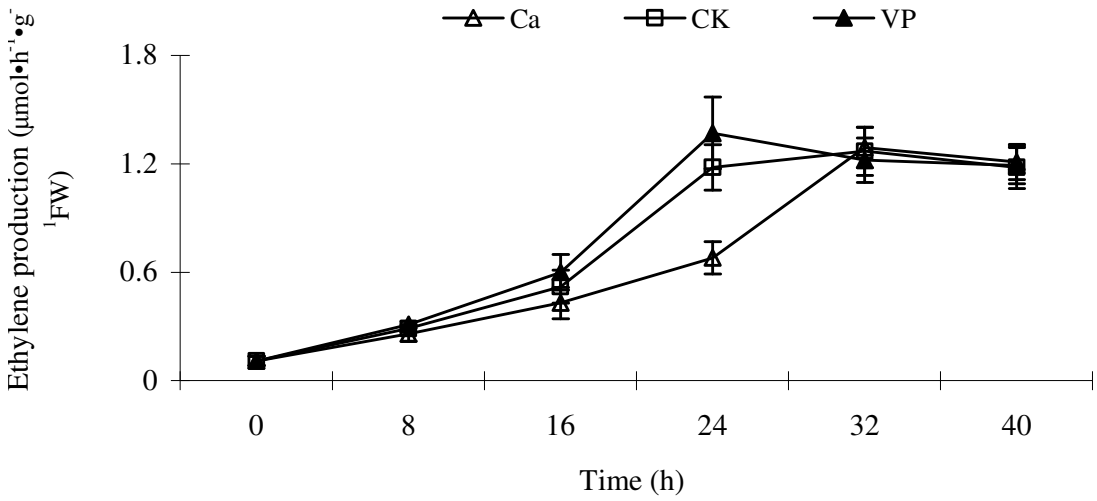


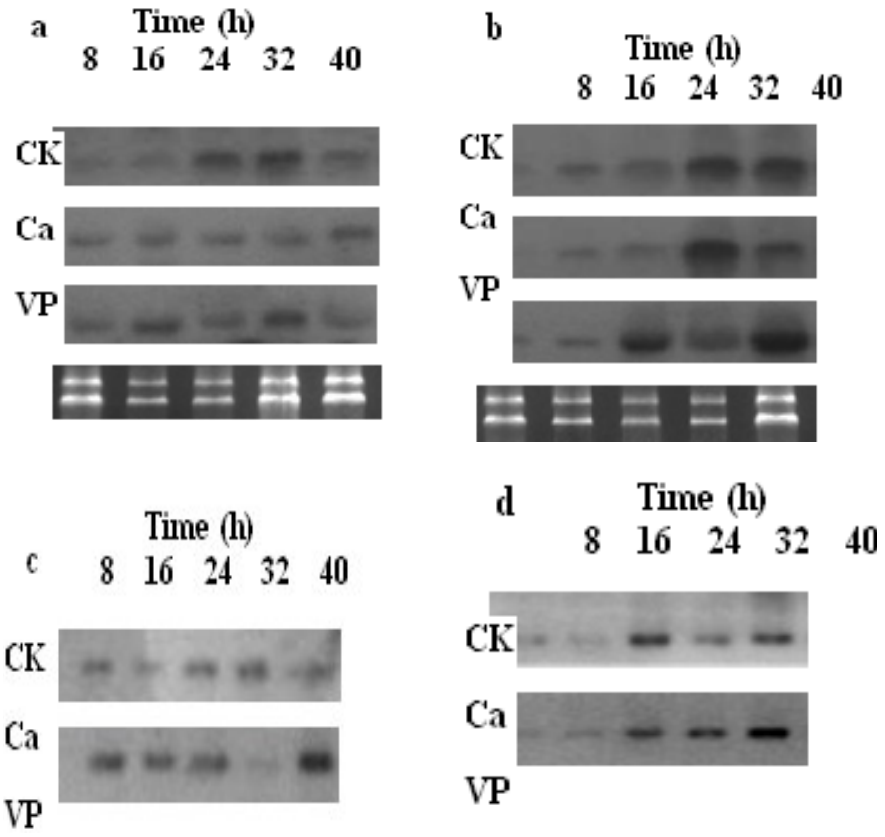
Figure 2. Effects of calcium and VP on endogenous ethylene production in AZ of tomato pedicel explants sampled at anthesis. (Δ), control (CK); (□), 80 mmol·L^{-1} calcium treatment; (▲), 5 mmol·L^{-1} VP treatment. Vertical bars indicate ± S.E. (n = 5).

rates in VP treatment explants were accelerated and reached a peak (42%) at 24 h, while Ca^{2+} treatments significantly ($P < 0.05$) delayed the relative abscission rate prior to 32 h and peaked at 40 h.

In all treatments, endogenous ethylene levels began to rise immediately after the explants were achieved. The highest ethylene concentration was obtained with VP treatment, which peaked at 24 h and then slowly declined. A similar trend was observed in control explants, with ethylene concentrations peaking at 32 h. However, the ethylene concentration was slightly lower than the VP-treated explants. Ethylene production in calcium-treated explants steadily increased over time, reaching control production levels at 32 h (Figure 2).

AZ *LeACS2* mRNA accumulation levels treated with Ca^{2+} and VP are shown in Figure 3a. Results detected *LeACS2* expression after 8 h, a peak at 24 h and a decline by 40 h in the control AZ. VP treatment showed peak *LeACS2* expression at 16 h compared to the control. Pedicel explants exposed to 80 mM Ca^{2+} demonstrated low and stable expression levels during abscission.

Figure 3b depicts *LeACO1* mRNA accumulation levels in AZ treated with calcium and VP. Control *LeACO1* expression during AZ flower abscission increased up to



Figures 3. Effects of calcium and VP on (a) *LeACS2*, (b) *LeACO1*, (c) *LeETR1* and (d) *LeETR4* mRNA expression in tomato pedicel explant AZs at anthesis.

40 h, but reached the highest level after 32 h. Relative to the control, 24 h of VP treatment resulted in a 3-fold increase in *LeACO1* expression; however, the calcium treatment exhibited little effect on *LeACO1* expression. Pedicel explants exposed to Ca^{2+} for 32 h resulted in a 2-fold increase in *LeACO1* expression, but VP expression showed a 3-fold decrease compared to the control. However, a decrease in *LeACO1* expression was detected at 40 h in pedicel explant AZs treated with Ca^{2+}, while VP had little effect.

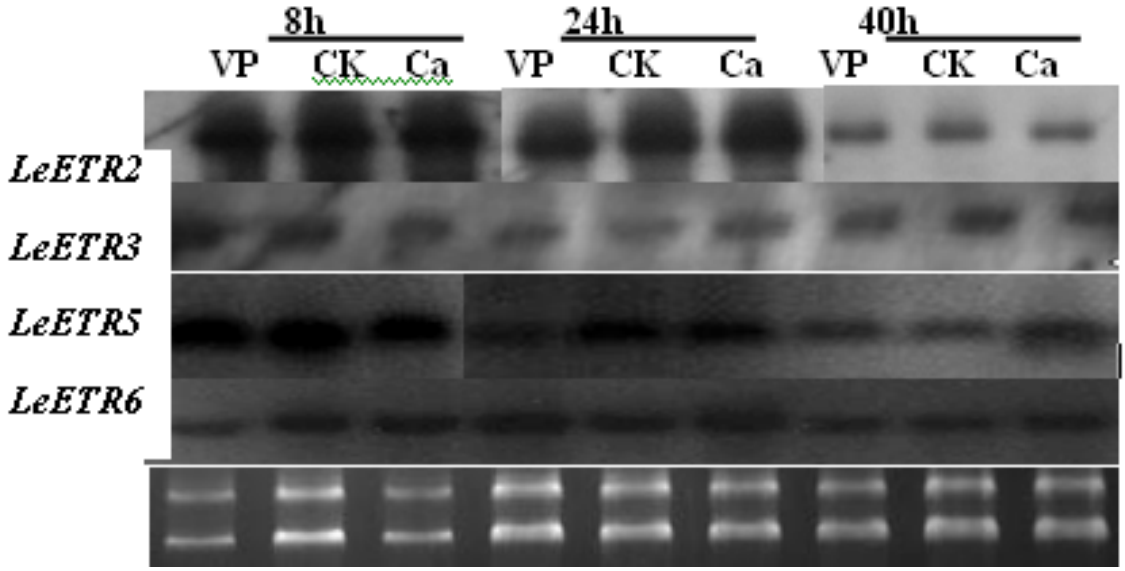
During flower abscission, a decline in *LeETR1* expression was observed after 16 h followed by an increase, which peaked at 32 h. Relative to the control, a respective 10 and 13-fold increase in *LeETR1* mRNA expression was demonstrated for VP at the 16 and 24 h treatments. *LeETR1* expression in pedicels for 16 and 24 h Ca^{2+} treatments exhibited a respective 3-fold and 2-fold increase in *LeETR1* mRNA expression levels compared to the control. However, when explants were treated for 32 h with VP or Ca^{2+}, expression levels decreased a respective 3-fold and 5-fold. Relative to the control explants, a 40 h Ca^{2+} treatment increased *LeETR1* expression 3-fold, while VP treatment inhibited *LeETR1* expression (Figure 3c).

LeETR4 expression during flower abscission indicated a peak in expression levels after 24 h, followed by a decline at 32 h and then a return to increased expression by 40 h. *LeETR4* mRNA expression in the AZ increased a respective 5 and 10-fold at the 32 and 40 h Ca^{2+} treatments compared to the control. In contrast, following a 24, 32 and 40 h VP treatment, *LeETR4* expression decreased 20, 4 and 2-fold, respectively, (Figure 3d).

The expression of *LeETR2* and *LeETR5* were decreased during abscission. In AZ of pedicels, the *LeETR2* and *LeETR5* were enhanced at 24 h Ca^{2+} treatment (2 and 1.5 fold of that time in the control, respectively), however, it only enhanced the *LeETR5* expression at 40 h. Besides, a 24 h VP treatment inhibited the *LeETR2* and *LeETR5* expressions. The expression of *LeETR3* was declined at 24 h and then increased up to the 40 h. VP treatment increased the *LeETR3* expression at earlier abscission (8 h), while Ca^{2+} treatment mainly enhanced the expression at the later abscission stages (40 h). It was shown that *LeETR6* was in a stable level through the abscission and little changed after calcium or VP treatment (Figure 4).

DISCUSSION

Dramatic increases in ethylene production have been



Figures 4. Effects of calcium and VP on *LeETR2*, *LeETR3*, *LeETR5* and *LeETR6* mRNA expression in tomato pedicel explant AZs at anthesis.

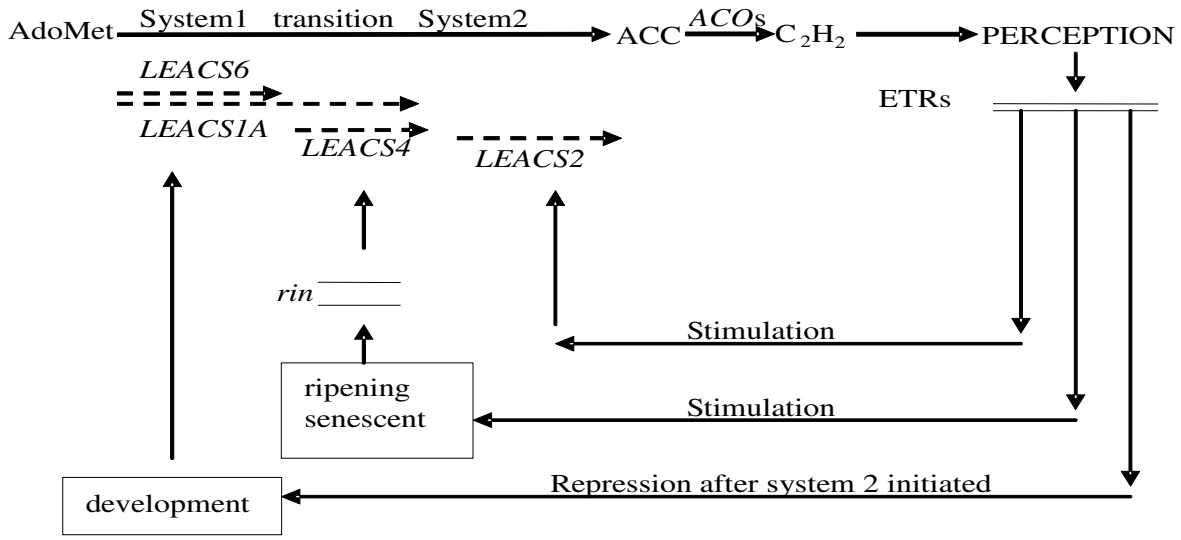
reported during a number of developmental events, including germination, leaf and flower senescence and abscission and fruit ripening (Yang and Hoffman, 1984; Mattoo and Suttle, 1991; Abeles et al., 1992). It has been proposed that *LEACS1A* and *LEACS6* are involved in the production of system 1 ethylene in green fruit (Barry et al., 2000). System 1 continues throughout fruit development until a competence to ripening is attained, whereupon a transition period is reached, during which *LEACS1A* expression increases and *LEACS4* is induced. During this transition period, system 2 ethylene synthesis (autocatalysis) is initiated and maintained by ethylene-dependent induction of *LEACS2* (Barry et al., 2000) (Figure 5).

An *ACO* gene expression pattern is highly regulated with transcripts of individual members accumulating to varying degrees at distinct developmental stages (Barry et al., 1996). In the present study, *LeACO1* expression in the AZs of tomato was found to be delayed in the calcium treated samples. The results also revealed that the calcium targeting step, which prevented ethylene production, was located at the point of ACC conversion to ethylene. Wang et al., (2005) suggested that two mechanisms can account for this process: calcium decreases relative electrolyte leakage, which prevents the accessibility of ACC to ACO; or calcium inhibits expression of *LeACO*, reducing ACO protein levels.

Calcium primarily decreased *LeACO1* expression prior to 32 h, while VP induced high expression levels at 24 h. Furthermore, as the *LeACO1* and *LeACS2* expression is both ethylene-dependent, showing the different expression patterns during calcium treatments, this indicated that *LeACO1*and *LeACS2* are located in the different ethylene signal pathway and calcium mainly adjust the initiator. Calcium resulting in increased turnover of the ACS protein is most likely responsible for ACS proteins phosphorylation but for ACS gene expression (Spanu et al., 1994). Tatsuki and Mori (2001) found that in tomato cells, *Le*ACS2 was phosphorylated by a calcium-dependent protein kinase (CDPK) from extracts of wounded tomato fruits. Support for the role of calcium and CDPK interference in regulating ACS protein stability has come from studies in pea seedlings treated with Ca^{2+} channel and calmodulin-binding inhibitors.

Patterson and Bleecker (2001) defined four stages in abscission. The lag stage in the abscission process of tomato pedicel explants was documented. However, the exact timing or period of the stage remains unknown. In this research, contrast to stable *LeACS2* expression levels, where calcium or VP exhibits only minor effects, *LeACO1* shows a substantial increase during abscission. This increase is notably consistent with the promotion of ethylene production and abscission rates. These results indicated that *LeACO1* expression levels may be a marker of the abscission stages progression, which initiated stage two.

Every ethylene receptor is expressed in different temporal and spatial patterns dependent on developmental stage and external stimuli. *LeETR1* and *LeETR2* are expressed constitutively in all tissues throughout development, *NR* is up-regulated at anthesis and both *NR* and *LeETR4* are up-regulated during ripening, senecence, abscission (Payton et al., 1996; Tieman et al., 2000) and pathogen infection (Ciardi et al., 2000). *LeETR5* is also expressed in fruit, flowers and during pathogen infection (Tieman and Klee, 1999). However, *LeETR4* uniquely exhibits `functional compensation', where its expression compensates for reductions of other receptor mRNAs. Thus, the *LeETR4*



Figures 5. Model proposing the regulation of *ACS* gene expression during the transition from system-1 to system-2 ethylene synthesis in tomato (Barry et al., 2000).

gene monitors tissue ethylene sensitivity and contributes to the maintainance of the overall `normal' levels of other ethylene receptor RNAs.

LeETR1 expression peaked earlier than *LeETR4* for VP and exhibited little influence on abscission delay; however, calcium increased *LeETR4* expression consistent with a lower abscission rate. The different *LeETR1*, *LeETR4* and *LeETR5* expression levels between Ca^{2+} and VP treatments suggested that *LeETR4* and *LeETR5* may serve a more important role in delaying abscission than *LeETR1*. The Ca^{2+} treatment only enhanced the later *LeETR3* expression while VP mainly inhibited its earlier expression indicating that *LeETR3* play important role in calcium adjust pathway during abscission. The expression of *LeET R6* which show little change means no major effect on abscission.

Calcium has been shown to play a number of roles in cell wall formation, cell elongation and middle lam separation of flower or leaf abscission. It has been demonstrated in calcium explant systems that abscission inhibition is induced by endogenous or exogenous ethylene. Furthermore, abscission is related to senescence retardation and/or enhancement of abscission delays by endogenous auxin. In other words, calcium acts in lag stage (stage I) of explant abscission, in which ethylene has only a minor effect.

1-MCP (1-methylcyclopropene), an ethylene action inhibitor, competitively binds to an ethylene receptor and delays fruit ripening and tissue senescence (Abdi et al., 1998; Blankenship and Unrath, 1998; Sisler, 1991). It has been suggested that 1-MCP remains bound to the component metal in the receptor and effectively blocks the receptor. Therefore, the formation of an active complex between ethylene and its receptor is prevented. Flower abscission in tomato is sensitive to ethylene, although the degree of sensitivity varies among different abscission stages. In tomato pedicel explants, this difference could be due to different receptor gene expression levels, rather than ethylene biosynthetic genes. Calcium adjusts the expression levels of ethylene receptors to ethylene signals, which is indicated by different ethylene responses (Raz and Fluhr, 1992; Kwak and Lee, 1997). The actions may be limited by treatment methods, concentration and period of sensitivity. However, it is assumed that 1-MCP binds permanently to ethylene receptors present at the time of treatment, and the recovery of ethylene sensitivity is due to the availability of new binding sites (Feng et al., 2004), such that a continued effect can only be achieved by repeated treatments. Results from Ca^{2+} treatments rely largely on plant sensitivity periods during a period of insensitivity, and this showed negligible calcium effects on ethylene signal action. Currently, it is not known if calcium forms complex compounds with ethylene and elicits the same effect as 1-MCP, or adjusts the ethylene receptor protein conformation. Further work is warranted to enhance our understanding of the effects of calcium on the expression of the ethylene biosynthesis gene and ethylene receptor.

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