Plant encoded 1-aminocyclopropane-1-carboxylic acid deaminase activity implicated in different aspects of plant development

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Proper plant development is dependent on the coordination and tight control of a wide variety of different signals. In the study of the plant hormone ethylene, control of the immediate biosynthetic precursor 1-aminocyclopropane-1-carboxylic acid (ACC) is of interest as the level of ethylene can either help or hinder plant growth during times of stress. It is known that ACC can be reversibly removed from the biosynthesis pathway through conjugation into other compounds. We recently reported that plants can also irreversibly remove ACC from ethylene production through the activity of a plant encoded ACC deaminase. Heretofore only found in bacteria, we showed that there was ACC deaminase activity in both Arabidopsis and in developing wood of poplar. Here we extend this original work and show that there is also ACC deaminase activity in tomato plants, and that this activity is regulated during tomato fruit development. Further, using an antisense construct of \textit{AtACD1} in Arabidopsis, we investigate the role of ACC deamination during salt stress. Together these studies shed light on a new level of control during ethylene production in a wide variety of plant species and during different plant developmental stages.

Hormones are a class of signaling molecules produced and sensed at very low levels; therefore control of their biosynthesis is crucial for proper plant development. The plant hormone ethylene has been studied for over a century and can positively impact plant development, such as in the initiation of fruit ripening, but ethylene accumulation can also induce widespread damage during stress responses. Ethylene is produced in two steps from the S-adenosylmethionine (SAM) that is derived from the Yang cycle. In the first committed step, SAM is converted into 1-aminocyclopropane-1-carboxylic acid (ACC) via the action of ACC SYNTHASEs (ACSs). ACC is then converted into ethylene by ACC OXIDASEs (ACOs), a particular adaptation of flowering plants. Once ACC is produced, there are few proven pathways that can divert it from conversion into ethylene. ACC can be conjugated into malonyl-1-aminocyclopropane-1-carboxylic acid (MACC) through the activity of ACC malonyl transferase or to 1-(γ-L-glutamyl-amino)cyclopropane-1-carboxylic acid (GACC) via γ-glutamyltranspeptidase. In bacteria, another pathway exists that can break down ACC obtained from plants through an irreversible deamination process. Through heterologous expression of bacterial ACC DEAMINASE (ACDs) in plants it has been possible to engineer plants that have reduced production of ethylene by affecting the native pools of ACC. Until recently no ACC deaminase pathway has ever been proven in plants, although a number of different plant genomes encode genes which bear sequence homology to bacterial ACDs. Should these genes code for active ACDs, this would provide an additional level of control for ethylene production beyond the activity of ACSs and ACOs. Recently we reported that Arabidopsis and Populus have inherent ACC deaminase activity, and we showed that this activity in Arabidopsis is due, in part, to the product of \textit{ACDDEAMINASE}...
controlled by ethylene receptor turnover. Ethylene production during ripening in tomato is essential during climacteric fruit development. We report here some of our preliminary findings in the areas of tomato fruit ripening and salt stress in Arabidopsis.

As precise control of ethylene levels is essential during climacteric fruit development, in parallel with our reported studies we also studied ACC deaminase activity in developing tomato fruit. Ethylene production during ripening in tomato is controlled by ethylene receptor turnover and conjugation of ACC by MACC and GACC. We found that tomatoes also have inherent ACD activity, and that this activity varies over ripening of the fruit (Fig. 1; solid line). During the immature green stage in tomato development ACC deaminase activity was low. This activity increased significantly during the 'late breaker' stage, just prior to the orange/red stage of development, and then decreased during later stages of tomato ripening. Also shown in this figure are the predicted levels of ethylene during fruit development. It is interesting to note that the highest amount of ACC deaminase activity coincides with the drop in ethylene levels soon after the breaker stage (Fig. 1; dashed line; based on Brady"). Our data would suggest that, in addition to ethylene receptor turnover and GACC and MACC activity, ACC deaminase activity may also help control ethylene levels. It has already been shown that constitutive expression of a bacterial ACC deaminase in tomato can delay the rate of tomato fruit ripening by reduction of ethylene production. Although ACD activity is evident during ripening in tomato, the gene responsible has not been identified. Recently a tomato gene with sequence similarity to bacterial ACC deaminases was tested for ACD activity. It was found that, despite the close sequence similarity, this gene (accession number EU639448) did not have ACD activity. Therefore, additional work must be done to isolate the gene responsible for the ACD activity we demonstrate in tomato fruit.

Our discovery of a plant encoded ACC deaminase in Arabidopsis allows us, for the first time, to downregulate ACC deaminase activity and investigate how this affects plant development. Previously, we showed that downregulation of AtACD1 using antisense resulted in up to a 30% reduction in ACD activity and up to a 2.5-fold increase in the evolution of ethylene. We showed that this difference in ACD activity was sufficient to alter hypocotyl elongation during Arabidopsis germination on different concentrations of ACC. It was unknown, however, if this difference was sufficient to affect other areas of development, such as stress response, in Arabidopsis. The expression of bacterial ACC deaminases in plants are known to increase plant resistance to a number of stressors due to decreased ethylene evolution. Based on microarray data, it is known that AtACD1 expression is upregulated 150% during salt stress and functionally it has been demonstrated that ACC production is increased in salt stressed roots and overexpression of bacterial ACDs in canola increases salt tolerance. It was unknown, however, if a reduction in native ACD activity would result in reduced vigour of plants grown on increasing concentrations of sodium chloride. We observed that there was no significant difference in rosette size, leaf production or percent dry weight between wildtype and three independent Arabidopsis lines expressing the AtACD1 antisense construct when grown on MS media without salt (Fig. 2A–C). As the concentration of salt increased in the growth media it was found that the antisense lines also did not differ from wildtype in their growth. The lack of a definitive phenotype under salt stress may mean that the level of reduced ACD activity achieved in the AtACD1 antisense lines was not sufficient to quantifiably affect the development of Arabidopsis. Additionally, as ethylene is not the only factor that affects a plant’s survival during times of salt stress, it is also possible that the plants were able to compensate for increased ethylene production in the AtACD1 antisense lines to promote normal plant development. This finding highlights the complex nature of the different signals involved in a plant’s response to salt stress and the need for a better understanding of the role of plant ACDs and how the plant may compensate for altered ACD activity.

In the known framework of ethylene synthesis our work has shown that plants do have the ability to reduce ethylene synthesis by irreversibly deaminating ACC through the action of a native ACC

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**Figure 1.** Tomato fruits exhibit ACC deaminase activity during ripening. A plot of ACC Deaminase activity (Solid Line) with known levels of ethylene production during ripening (Dashed Line; Brady") superimposed over pictures of the corresponding stage of tomato development. *Indicates significant increase in activity ("nmol mg^-1 hr^-1"). ACC deaminase activity analysis was performed on total tomato fruit protein as per Penrose and Glick (2003).
deaminase. Further to our first study, we show here that there is inherent ACC deaminase activity in tomatoes and that this activity varies during tomato ripening in a manner consistent with a factor that is involved in the regulation of ethylene levels. We also show here that transgenic Arabidopsis lines with a mild reduction in ACD1 activity do not have an obvious affect on mediation of salt stress. This finding, however, does not preclude a role for ACD1 in mediating other aspects of plant development or in affecting plant development during other types of plant stress (i.e., drought). Therefore, there still remain many questions to answer concerning the role of plant encoded ACC deaminases and many exciting avenues of ethylene regulation to pursue. The identification and exploitation of tomato, poplar and other plant ACC deaminases could be used to alter fruit ripening, wood production and stress tolerance—all aspects of plant development that are economically and scientifically important.

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Figure 2. Growth and development of Arabidopsis wildtype and three Antisense AtACD1 lines on increasing concentrations of salt. Stratified wildtype Arabidopsis (Col-0) and three independent transgenic lines expressing an antisense construct of AtACD1 (A1, A2, A3) were sown on 0 mM NaCl (Dark Grey Bars), 100 mM NaCl (White Bars), 125 mM NaCl (Black Bars) and 150 mM NaCl (Light Grey Bars) and allowed to germinate and grow for 2 weeks under long-day conditions (16 h light/8 h dark) at a light intensity of 130 to 190 μE m⁻² s⁻¹ at the rosette level at 21°C in Econair AC-60 growth chambers. Plants were analyzed for rosette diameter (A), leaf production (B) and percent dry weight (C). Error bars are ± SE.
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