Special Research Report # 404: Postproduction

Control of Ethylene Production in Flowers

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BACKGROUND

Ethylene is one of the most important factors determining the lifespan of many flowers. In this project, we used diploid geranium flowers to investigate the control of ethylene production and its effects on the flower. Diploid geraniums have a single whorl of petals (usually 5 petals) and are highly sensitive to ethylene. Flowers treated with ethylene will abscise their petals in less than two hours. Pollination increases ethylene production in the flower and also results in petal abscission, but in this case in 3-4 hours. The biochemical pathway for ethylene is well known. Either of the last two steps in ethylene synthesis could be important for regulating ethylene production by the flower. These steps are catalyzed by the enzymes ACC synthase and ACC oxidase. We investigated the activity of these enzymes in pollinated geraniums.

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MATERIALS AND METHODS

ACC synthase was assayed by *in vivo* and *in vitro* methods. An *in vivo* assay was used for ACC oxidase. ACC levels and ACC conjugate levels were measured in various flower parts. Enzyme activities were compared with measurements of gene expression. The importance of protein synthesis was investigated by using a protein synthesis inhibitor, cycloheximide, applied through the cut stem. Some inhibitors were applied to the stigma directly to test pollination effects.

RESULTS

Pollination caused an increase in ethylene production in the pistil, which was required for petal abscission. Therefore, the pollen does not carry adequate quantity of the ethylene precursor to be responsible for petal abscission. Pollination increased ACC levels in the stigma and style by 46 fold, and ACC in the basal part of the petals by 6-fold. ACC conjugate levels also increased dramatically. Activity of ACC synthase was increased in the same tissues. ACC oxidase also increased in the stigma and style, but not in the basal part of the petals, where it was already high. One of the genes for ACC synthase increased expression after pollination, but the other, more abundant gene did not. The correlation between gene expression and activity was poor. Cycloheximide did not block pollination-induced ethylene production. This indicates that the increase in ethylene came primarily from the ACC synthase enzyme that was already present, i.e. there was no need for additional gene transcription and translation.

Since the stigma, where pollination occurs, is some distance from the abscission zone at the base of the petals, we also considered how enough ethylene reaches that abscission zone to induce petal separation. Most of the ethylene in the flower comes from the sigma and style, and very little from other parts. It is possible that ethylene diffuses inside the floral tissues from the stigma and style to the petal base. Our data indicated that ACC was unlikely to be the diffusing compound. Another possibility is that the very low levels of ethylene production in the petal base could be responsible for causing petal abscission. The identity of the signal from the stigma and style that causes the increase in ethylene at the petal base after pollination is still unknown.

CONCLUSIONS

Ethylene production increases after pollination and causes petal abscission in geranium. This increase in ethylene is not due to increased transcription of genes for ACC synthase and ACC oxidase, which occurs in carnation and orchids. Instead, the activity of pre-existing ACC synthase enzyme is increased by pollination. This activity is specifically found in the stigma, style, and petal base, and the latter is the tissue where the ethylene acts to cause petal drop.

IMPACT TO THE INDUSTRY

Many university and industry researchers are studying the manipulation of ethylene as a way to enhance the postharvest longevity of flowers. The use of genetic approaches, including blocking ethylene action and synthesis, have proven to be more complicated than anticipated. Ethylene synthesis is controlled by several enzymes, each of which is a member of a gene family (i.e. there is more than one). Studies show that only one, of several candidates, may be important for regulating ethylene in the flower. It is the opinion of this researcher that the most promising approaches for the floriculture industry are:

(1) chemical blockage of ethylene action, e.g., STS or alternatives such as EthylBloc. Such methods tend to be broadly applicable to all ethylene-sensitive flowers.

(2) If a genetic approach is to be taken, which requires a substantial investment in every species, blocking ethylene action by targeting receptors in a tissue-specific fashion is most desirable. This avoids secondary effects on other aspects of plant development for which ethylene is important.