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Effect of 1-Aminocyclopropane-1-Carboxylic Acid on Maize Kernel Development In Vitro

Jonathan M. Hanft,[1] Andrew J. Reed,[2] Robert J. Jones,[1] and James S. McLaren[2]

[1]Department of Agronomy and Plant Genetics, University of Minnesota, St. Paul, Minnesota 55108; and [2]Monsanto Agricultural Products Co., St. Louis, Missouri, USA

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Abstract. Pollination stimulates ethylene production in maize ears, and the application of ethephon during the pollination period can cause kernel abortion. The objective of this study was to determine if kernel abortion could be induced in vitro by the ethylene precursor 1-aminocyclopropane-1-carboxylic acid (ACC). Adding ACC to the culture medium resulted in the evolution of ethylene which caused abortion and reduced mature kernel mass. The effect of ethylene on kernel abortion and dry matter accumulation was partially negated by the addition of the ethylene-binding site inhibitor, 2,5-norbornadiene (NBD). The effect of ethylene on kernel abortion was greatest during the early stage of kernel development and was intensified by an increase in media sucrose concentration. These data suggest that ethylene could regulate kernel abortion in maize.

The role of ethylene in flower senescence and fruit ripening is well documented for horticultural crops (Abeles 1973, Halevy et al. 1984, Hoffman and Yang 1980, Nichols 1977, Nunez-Elisea and Davenport 1986, Stead and Moore 1983, Woodson et al. 1985). As a promotor of ovule growth, ethylene may be involved in directing assimilates to the developing ovules (Hirai 1982, Nichols 1976, Nichols and Ho 1975, Veen and Kwakkenbos 1984). In soybean, Urwiller and Stutte (1986) found that ethephon [(2-chloroethyl) phosphonic acid] applied at the beginning of flowering increased flower and pod abscission, decreased the number of three-seeded pods, and decreased pod dry weight. Research on the association of ethylene with maize seed development, however, is lacking.

Preliminary studies showed that ethylene evolution occurred at pollination and the application of ethephon increased kernel abortion (Dill et al. 1987). Maize kernel abortion can substantially reduce the number of harvestable kernels and occurs when plants are stressed during flowering (Westgate and Boyer 1986, Reed et al. 1988) or when kernels compete for assimilates in in vitro kernel culture (Hanft and Jones 1986). Characterizing the regulation of physiological events associated with kernel development is prerequisite to understanding the mechanisms that control the ability of the seed to survive and achieve its potential mass. The eventual genetic or hormonal manipulation of these mechanisms may be important in increasing the production efficiency of maize. Our goal was to determine if the plant hormone ethylene played a role in the regulation of seed growth and development in maize.

The effects of exogenous hormones on kernel growth and development can be studied in isolation from effects on the mother plant using in vitro kernel culture (Gengenbach 1977). Treatment of plant tissues with exogenous 1-aminocyclopropane-1-carboxylic acid (ACC) often results in ethylene synthesis via an ethylene-forming enzyme (Yang and Hoffman 1984). ACC added to the culture medium was used as a convenient method to study the effects of ethylene on maize kernel growth and development.

The objectives of this research were to (1) devise a convenient system for exposing maize kernels to ethylene; (2) describe the effects of ethylene on kernel abortion and final kernel mass; (3) identify the stage of development when kernels are most sensi-

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tive to ethylene exposure; and (4) determine if there is an interaction between assimilate supply and ethylene effects on kernel growth.

Materials and Methods

For all experiments, maize kernels were cultured in vitro according to the procedure described by Gengenbach (1977) and Jones et al. (1981). Ovules with their associated cob tissue were excised from the ears of field-grown or greenhouse-grown plants 3 days after pollination. Each cob block initially contained six ovules. Five of the ovules were removed leaving one to develop in culture. This ovule to cob ratio results in mature kernel masses closest to those achieved in vivo (70–90%). Five of these cob pieces were placed in each petri dish on agar-supported medium, petri dishes were sealed with parafilm, and the kernels cultured for 35 days. The standard culture medium contained 15% sucrose (wt/vol), 20 amino acids, macro- and micronutrients, and vitamins (Gengenbach 1977).

Ethylene Generation from ACC In Vitro

ACC was filter sterilized and added to the petri plates just before culturing. Kernels were cultured either on standard medium, medium containing 1 mM ACC (experiment 1), or on medium containing 10 mM ACC (experiment 2). Kernels were cultured in glass petri dishes fitted with a stoppered port to facilitate measurement of ethylene evolution. Each dish was sealed with parafilm to provide an airtight culture atmosphere. Samples of the culture atmosphere were withdrawn with a gas-tight syringe at intervals from 2 h to 29 days in culture. The needle was flame sterilized before penetrating the stopper to maintain a sterile environment. Atmosphere ethylene concentrations in the petri dishes were determined using a gas chromatograph fitted with a Poropak R column and a flame ionization detector. In both experiments, each media treatment had three replicates.

Effect of ACC on Kernel Abortion and Kernel Mass

Six replicates of the same three media treatments (0,1, and 10 mM ACC) were established. The number of aborted kernels was determined after 2 weeks in culture, and the mature mass of nonaborted kernels was measured after 35 days in culture.

Effect of 2,5 Norbornadiene (NBD) on Kernel Abortion and Kernel Mass

NBD was added to media with or without 10 mM ACC. The medium concentration of NBD in these two treatments was 0.8% vol/vol (Sisler et al. 1985). As with the ACC, the NBD was filter sterilized before it was added to the petri plates. In addition, kernels were cultured on standard medium and medium containing 10 mM ACC without NBD. Each treatment had five replicates. Kernel abortion and mature mass were determined as previously described.

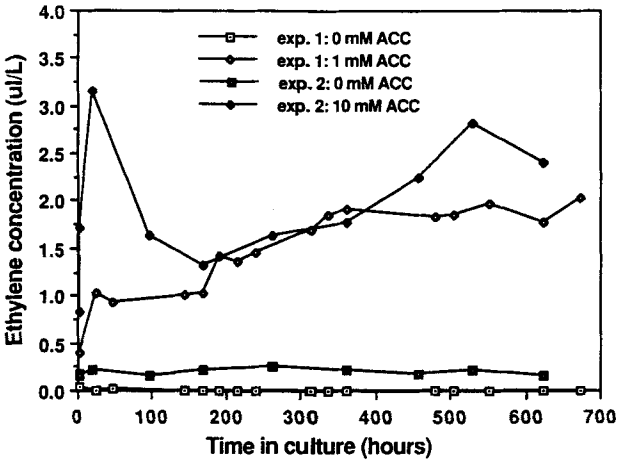


Fig. 1. Ethylene evolution from maize cob pieces cultured on medium containing ACC. In the first experiment (exp. 1), cob pieces were cultured on standard medium (□), and medium containing 1 mM ACC (◇). In the second experiment (exp. 2) cob pieces were cultured on standard medium (■) and medium containing 10 mM ACC (◆). Data points represent the mean of three petri plates each containing five cob pieces and their associated ovules.

Effect of ACC/Stage of Kernel Development

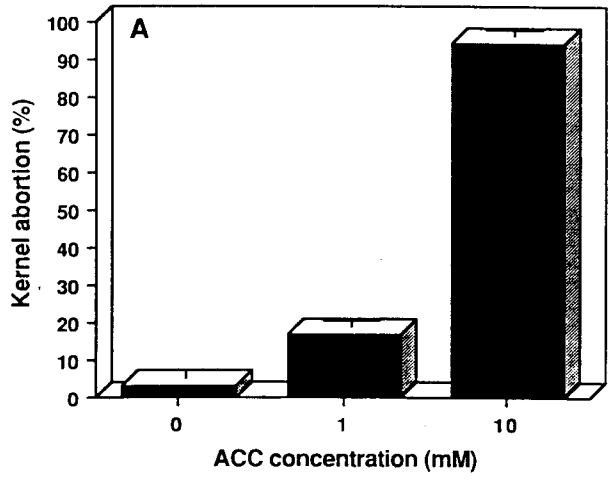
Kernels were either cultured continuously on 1 mM ACC medium beginning 3 days after pollination, or were transferred to ACC medium 10 days after pollination. To separate any transfer effects from those actually caused by the addition of ACC to the medium, a control treatment which included transferring kernels to the standard medium lacking ACC was included.

Effect of ACC/Assimilate Supply

Kernels were cultured on media containing sucrose concentrations ranging from 2–15% (58, 117, 234, and 438 mM) with or without 10 mM ACC. Kernel abortion and final mass were compared for all media sucrose and ACC combinations.

Results

The addition of ACC to the culture medium resulted in ethylene evolution from the cob tissue (Fig. 1). The presence of 1 mM ACC caused an initial rapid rise in ethylene to 1.0 $\mu l\ L^{-1}$ during the first 24 h in culture followed by a more gradual increase until 14 days in culture (336 h). Addition of 10 mM ACC to the culture medium resulted in an initial burst of ethylene reaching a maximum concentration of 3.2 $\mu l\ L^{-1}$ after 24 h in culture, and then ethylene increased from 150 h onward. Because petri dishes were sealed, ethylene concentrations reflect a balance between ethylene production and metabolism. The maximum ethylene concentrations in the cul-



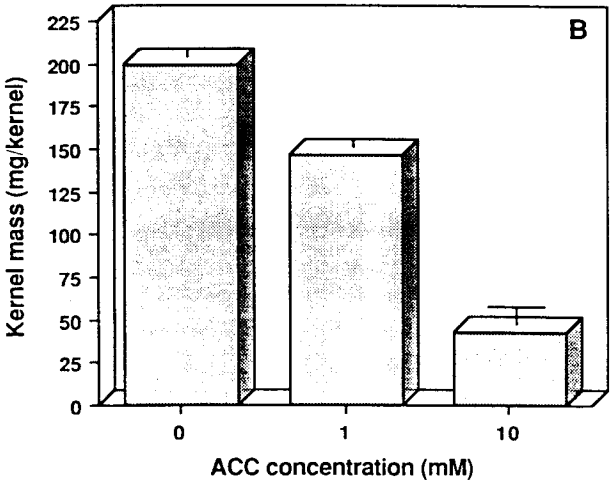
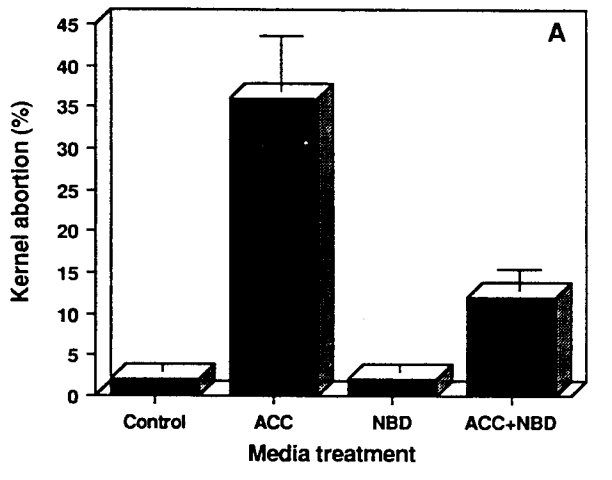


Fig. 2. Effect of ACC on kernel abortion (A) and mature kernel mass (B). Kernel abortion is the percentage of the total number of ovules that aborted by 2 weeks in culture.



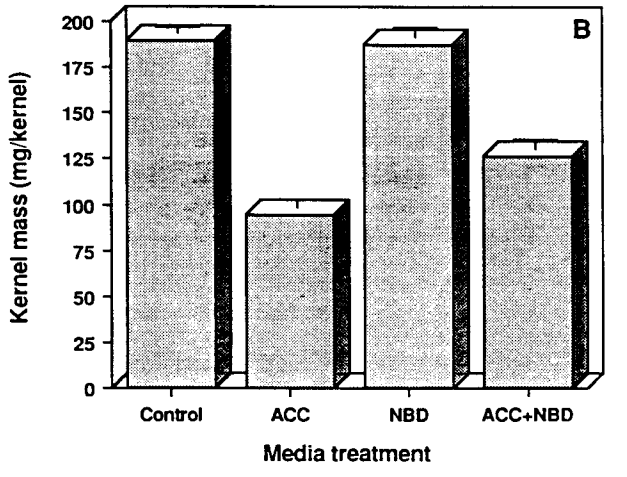


Fig. 3. Inhibition of ACC induced kernel abortion (A) and reduction in dry mass (B) by NBD. Kernels were cultured on standard medium (Control), medium containing 10 mM ACC, medium containing 0.8% vol/vol NBD, and medium containing ACC + NBD.

ture atmosphere were 2.2 and 3.2 $\mu l\ L^{-1}$ for 1 and 10 mM ACC media treatments, respectively. Wounding of the cob tissue during the culturing process probably caused the low levels ($<0.38\ \mu l\ L^{-1}$) of ethylene that evolved from cob pieces of ovules cultured on the standard medium without ACC.

Figure 2 shows the effects of ethylene on kernel growth in vitro. The presence of 1 or 10 mM ACC in the culture medium significantly increased the frequency of kernel abortion (Fig. 2A). Ninety-four percent of the kernels cultured on 10 mM ACC medium aborted, whereas 1 mM ACC caused 17% abortion. ACC also significantly reduced the mature mass of surviving kernels (Fig. 2B). The addition of 1 and 10 mM ACC in the culture medium reduced kernel mass by 27 and 79%, respectively.

The addition of the ethylene-binding site inhibitor NBD to the culture medium containing ACC partially negated the effect of ACC on kernel abortion and mass (Fig. 3A and B). Thirty-six percent of the kernels cultured on 10 mM ACC aborted, whereas only 12% of those cultured on medium containing 10 mM ACC plus 0.8% NBD aborted. NBD alone had no effect on kernel abortion. In the same experiment, 10 mM ACC reduced the mature mass of kernels by 50%, whereas the addition of NBD to the medium containing ACC resulted in a reduction in mass of only 33%. NBD alone had no effect on mature kernel mass.

The effect of ACC on kernel abortion depended

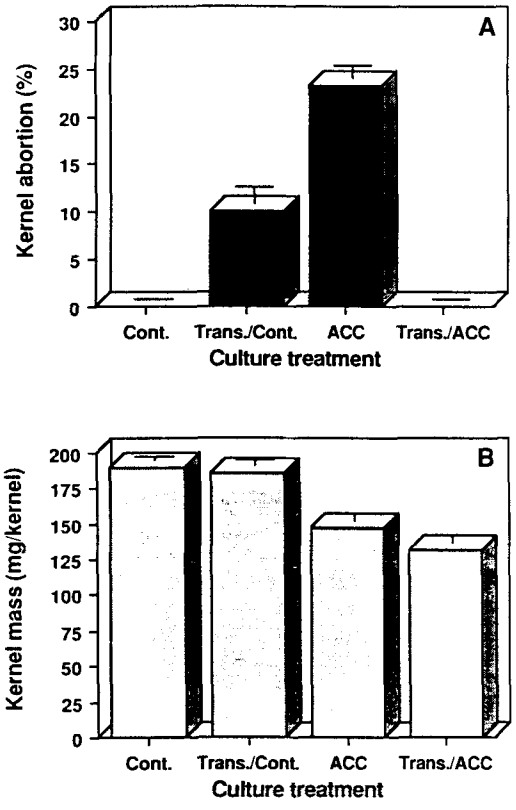


Fig. 4. Effect of ACC on kernel abortion (A) and mature dry mass (B) when supplied at two different times during grain filling. Kernels were cultured on standard medium (Cont.), on standard medium and then transferred to the same medium 10 days after pollination (Trans./Cont.), on 1 mM ACC medium (ACC), and on standard medium and then transferred to 1 mM ACC medium 10 days after pollination (Trans./ACC).

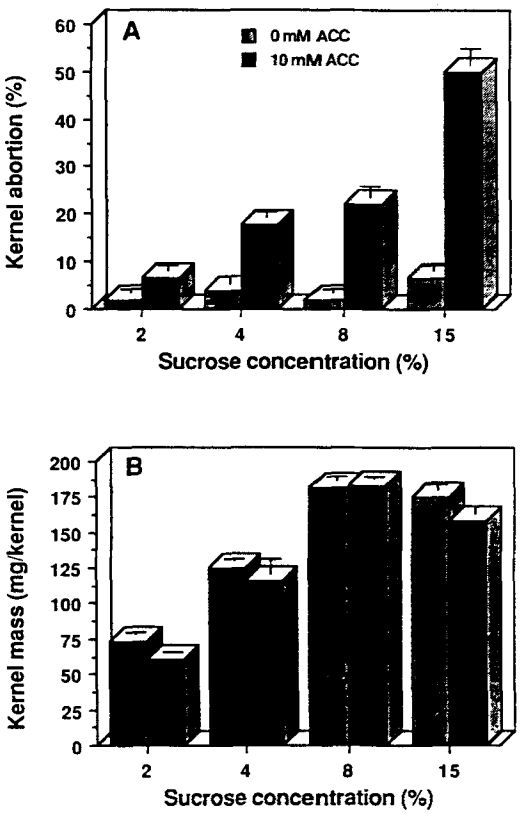


Fig. 5. Effect of media sucrose concentration on abortion (A) and mature mass (B) of maize kernels cultured in the presence or absence of ACC. Kernels were cultured on medium ranging in sucrose concentration from 2–15% wt/vol (58–438 mM).

on the stage of kernel development (Fig. 4A). Kernels cultured on 1 mM ACC beginning 3 days after pollination had an abortion frequency of 23%. None of the kernels aborted when transferred from standard medium to medium containing 1 mM ACC at 10 days after pollination. In contrast to kernel abortion, kernel mass was affected by ethylene when kernels were exposed during both periods (Fig. 4B). Kernel mass was reduced by 23 and 29%, respectively, when cultured on ACC medium continuously and after transferring 10 days after pollination.

Figure 5A shows that the effect of ACC on kernel abortion is intensified by an increase in medium sucrose concentration. As the amount of sucrose available to the developing kernels increased from 2–15% in the presence of ACC, kernel abortion increased from 7–50%. Kernel abortion did not exceed 7% for those kernels cultured in the absence of ACC. Sucrose supply had little effect on the response of mature kernel mass to ACC. The addition of ACC to the medium resulted in an 18, 8, 0, and 10% reduction in kernel mass for kernels cultured on 2, 4, 8, and 15% sucrose, respectively.

Discussion

The addition of ACC to maize kernel culture medium proved to be a convenient method for studying the effect of ethylene generated by cob explants on kernel growth and development. Apparently, the cob tissue was ACC limited, and ethylene was rapidly synthesized when ACC was included in the medium. The concentration of wound ethylene was probably too low to affect kernel development. An increase in the rate of ethylene metabolism following the initial burst in ethylene concentration in the 10 mM ACC treatment (Fig. 1) may account for the decline in ethylene concentration from 24–150 h in culture.

These experiments show that the ethylene generated by the addition of ACC to the culture medium can cause maize kernels to abort and reduce mature

mass in vitro. Since the addition of the ethylene-binding site inhibitor NBD partially offsets the increase in abortion and reduction in mass caused by the addition of ACC to the medium, we conclude that the effect on abortion and dry matter accumulation is caused directly by the ethylene evolved in culture. Ethephon applied to ears in the field at pollination also caused kernel abortion (Dill et al. 1987). This ethephon effect was also offset by the application of NBD. Volatilization of some of the NBD when it was added to the petri plates before the lids were sealed probably contributed to the failure of NBD to completely offset the effects caused by the addition of ACC to the medium.

Ethylene caused kernels to abort only during the lag phase of growth and failed to cause abortion during the linear filling period (Fig. 4A). Endosperm cell division and amyloplast initiation occur during the lag phase. These two parameters determine the capacity of the kernel to accumulate dry matter (Jones et al. 1985). Low carbohydrate supply, water stress, and high temperature (Hanft and Jones 1986, Jones et al. 1985, Westgate and Boyer 1986) also cause kernel abortion during the lag phase of growth. This developmental sensitivity seems to be an important factor in kernel abortion. Kernel abortion also occurs rapidly and is probably an active process. Thus, kernel abortion is consistent with other hormone-induced phenomena, and our data suggest that ethylene may be involved.

Since carbohydrate supply and metabolism seem to play a role in kernel abortion (Hanft and Jones 1986a, Hanft and Jones 1986b, Hanft et al. 1986), we are also interested in the interaction between assimilate supply and ethylene. In culture, ovules supplied with relatively high sucrose concentrations abort more frequently when exposed to ethylene (Fig. 5A). The relationship between plant tissue carbohydrate concentrations and ethylene production in unclear. In tobacco leaf discs, sucrose stimulates ethylene production (Philosoph-Hadas et al. 1985). Sucrose stimulates both the conversion of methionine to ACC and ACC to ethylene indicating that carbohydrates somehow enhance ACC formation and conversion of ACC to ethylene. Since we did not measure ethylene evolution from the cob blocks cultured on different sucrose concentrations, we do not know if ovules with a high supply of sugar were more sensitive to the effects of ethylene, or if more ethylene was evolved from these cob blocks.

Ethylene could function in several different ways to control the growth and development of maize kernels. At pollination, a release of ethylene could be involved in silk senescence and the formation of the constriction zone at the point of silk attachment to the ovule. Abscission of cotton bolls (Guinn 1982, Lipe and Morgan 1973) and soybean flowers and pods (Urwiler and Stutte 1986) may be controlled by ethylene. Although maize ovules do not abscise, the abortion process may be analogous to fruit abscission of other crops. Aborting maize kernels form a black layer between the developing ovule and maternal tissues (Daynard and Duncan 1969) which impedes the flow of assimilates to the developing kernel. This layer, whose development may be stimulated by ethylene, appears to have characteristics similar to an abscission zone.

Ethylene could also signal a change in the source-sink relationship between reproductive and nonreproductive tissues. In maize, tip kernels are often pollinated 4 days after those toward the middle and base of the ear and abortion occurs most frequently at the tip. Ethylene evolution from developing middle and basal kernels or from recently pollinated tip kernels could cause tip kernels to abort to ensure an adequate supply of assimilates for the earliest pollinated kernels. While the mechanisms by which ethylene may control kernel abortion are supported by the existing evidence. Further experiments are required to confirm in situ regulation of kernel development.

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