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Ethylene biosynthesis in relation to cyanide metabolism

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Abstract. Cyanide is a co-product of ethylene biosynthesis in higher plants via the ACC pathway. In certain physiological states, such as fruit ripening and flower senescence, and in many environmental conditions, such as flooding and chilling, ethylene biosynthesis is greatly induced. Cyanide is toxic to plants if it accumulates in plant tissues; however, during fruit ripening, the co-product cyanide is shown to be rapidly conjugated to form L-3-cyanoalanine derivatives by the enzyme L-3-cyanoalanine synthase. Recent evidence shows that cyanide, the coproduct of ethylene biosynthesis, causes phytotoxic effects on plants subjected to auxin-type herbicide treatments. It points to the possibility that under certain severe stress conditions, the induced ethylene, and thereby cyanide, may cause the death of the plants.

Keywords: Cyanide metabolism; Ethylene biosynthesis; L-cyanoalanine synthase; Cyanogenic.

Abbreviations: **ACC**, 1-aminocyclopropane-1-carboxylic acid; **AOA**, 2-aminoxyacetic acid; $\mathbf{[I]_{0.5}}$, the inhibitor concentration that inhibits 50% of the enzyme activity.

Contents

Introduction

Ethylene is a plant hormone known to regulate many important events in plant growth and development. Its effects on the triple responses in etiolated dicot seedlings and the ripening of climacteric fruits are well established. Research on the physiology and biochemistry as well as on the molecular biology of ethylene has been thriving. The knowledge derived from these studies has benefit to various agricultural systems, and the trend is accelerating. Many authors have reviewed the biology of ethylene (Abeles et al., 1992; Kende, 1993; Yang and Hoffman, 1984).

Cyanide is a harmful ion, hazardous to life, which forms a very stable complex with the active site metal (e.g., Fe and Mg) in enzymes, thereby inhibiting vital functions in cells such as respiration, carbon fixation, and nitrate reduction. Cyanogenic compounds are widespread in plant species, and are mostly stable compounds when conjugated to saccharrides forming cyanogenic glycosides. Hydrogen cyanide is released from the cyanogenic compounds during tissue disruption, infection, or food processing. The existence of cyanogenic compounds in plant species has caused cyanide poisoning in humans and animals when the compounds are ingested with foodstuffs. A cyanogenic

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starchy crop, such as cassava (*Manihot esculenta*), is a major components of human diet in some areas where cases of acute cyanide poisoning are still occasionally reported. Many review articles have been devoted to cyanogenic compounds in plant species, including their chemical structures, distributions, physiological roles, biosynthesis, and degradation. For details, please refer to the following articles: Moller and Poulton, 1993; Nahrstedt, 1988; Poulton, 1989; Seigler, 1991.

In this review, we shall focus on cyanide production as a coproduct of ethylene biosynthesis in higher plants, the fate of these sources of cyanide, and its possible physiological consequences.

Cyanide - a Coproduct of Ethylene Biosynthesis

Ethylene biosynthesis in higher plants is established through the following pathway: Methionine → S-Adenosylmethionine → 1-Aminocyclopropane-1-carboxylic acid (ACC) → C_2H_4 (Yang and Hoffman, 1984). By employing the ^{14}C tracer labeled at different positions of ACC, Peiser et al., in a 1984 paper demonstrated that during the in vivo oxidation of ACC to ethylene, the carboxyl carbons, C-1 and C-2,3 of ACC are metabolized into CO_2, L-3-cyanoalanine derivatives, and ethylene, respectively (Peiser et al., 1984; Pirrung, 1985). Although no free HCN was identified, it was asserted that C-1 of ACC is initially liberated as HCN, but is rapidly conjugated into L-3-cyanoalanine derivatives as soon as it is liberated. This notion was based on the observation that the metabolic fate of the C-1 of ACC during its conversion to ethylene was identical to that of administered HCN and that the amount of HCN-conjugates formed was equivalent to that of ethylene produced (Peiser et al., 1984). Thus, the degradation of ACC into ethylene can be represented by the following equation: ACC + 1/2 O_2 → C_2H_4 + HCN + CO_2 + H_2O. This reaction was further confirmed by Dong et al. (1992) in vitro using the purified ACC oxidase from apple. A stoichiometric production of ethylene and HCN from ACC was clearly shown during the catalytic reaction, which also required ascorbic acid, CO_2 and Fe^{++} as cofactors.

Metabolism of Cyanide in Plant

Since no free HCN was detected even in plant tissues which produced ethylene at very high rates, Peiser et al. (1984) suggested that plants must have ample capacity to metabolize the HCN originating from ACC.

In higher plants the key enzyme to detoxify HCN is L-3-cyanoalanine synthase (EC. 4.4.1.9), which catalyses the following reaction:

$$\underset{\text{(L-cysteine)}}{HS\text{-}CH_2\text{-}CH(NH_2)\text{-}COOH} + HCN \rightarrow \underset{\text{(L-3-cyanoalanine)}}{NC\text{-}CH_2\text{-}CH(NH_2)\text{-}COOH} + H_2S$$

L-3-cyanoalanine synthase is widely distributed in higher plants (Miller and Conn, 1980). L-3-cyanoalanine thus formed is further metabolized to asparagine or to γ-glutamyl-L-3-cyanoalanine (Akapyan et al., 1975; Blumenthal et al., 1963; Blumenthal, 1968; Hendrickson and Conn, 1969; Manning, 1986; Peiser et al., 1984; Pirrung, 1985). L-3-cyanoalanine synthase has been purified about 4000-fold from blue lupine seedlings by Akapyan et al. (1975); this enzyme is pyridoxal-dependent and can be inhibited by 2-aminoxyacetic acid (AOA) or 3-aminoxypropionic acid.

A Case Study - Determination of ACC Dependent Cyanide Formation in Plant Tissues

In a case study (Yip and Yang, 1988), we showed that HCN does accumulate in plant tissue which actively converts ACC to ethylene, when the conjugation of HCN to L-3-cyanoalanine is inhibited by AOA. By employing an isotope dilution method, we estimated that the steady state concentration of HCN in the tissues which actively produce ethylene was no higher than 0.2 μM.

Methodology

Measurement of ethylene. Gas chromatography using alumina column equipped with a flame ionization detector (FID) is the most common method of analysing ethylene (Yip and Yang, 1993). A short column can be easily prepared by using 40–60 mesh alumina (or Porapak) packed in a 0.5 m × 3 mm i.d., copper tube. The column must be tightly packed to ensure good performance. Before operating, it is often necessary to bake the column 1 or 2 days at around 150–180°C with a slow flow of carrier gas (nitrogen or helium) to condition it for best result. Once the column has been conditioned, it is advisable to maintain the oven temperature at about 80°C and fix the carrier gas flow rate at around 40 ml/min for operation. The flow rates of hydrogen and oxygen are usually set at 20 ml/min and 10 ml/min, respectively, to maintain the flame for the FID. Under these conditions, the retention time for ethylene should be about 0.6–0.9 min, and the sensitivity of the column is about 0.01 ppm. Normally 1 to 2 ml of gas sample can be injected.

Colorimetric determination of HCN. HCN was analyzed mainly by a modified method developed by Lambert et al. (1975). To 200 μL of trap solution (0.1 N NaOH), 100 μL 1 M acetic acid, 1 ml 0.25% succinimide/ 0.025% N-chlorosuccinimide reagent, and 200 μL 3% barbituric acid in 30% pyridine were added, and the reaction mixtures were then shaken vigorously. After 10 min, the absorbance at 580 nm was determined. This method can measure HCN down to 0.1 nmol. In a second method developed by Guilbault and Kramer (1966), cyanide was measured based on its catalytic activity to form a purple compound by reacting with ρ-nitrobenzaldehyde and o-dinitrobenzene. To 50 μL trap solution (0.5 N NaOH), 500 μL 0.1 M ρ-nitrobenzaldehyde (dissolved in cellosolve) and 500 μL 0.1 M ρ-dinitrobenzene (dissolved in cellosolve) were added. Cyanide content was estimated

by following the rate of increase in absorbance at 560 nm in a spectrophotometer.

Estimation of tissue [HCN] by isotopic dilution. Radioactive NaCN (0.8 nmol, 38 nCi) was added to the tissues, which were then homogenized with water. After centrifugation at 5000 *g* for 2 min, the supernatant was transferred into a 25 ml flask, which was then sealed with a rubber serum cap fitted with a plastic center well containing 200 µL of 0.1 N NaOH solution. The supernatant was acidified to pH 1 by the injection of H_2SO_4 solution. The flasks were kept at room temperature for 24 h to allow HCN to be distilled into the NaOH trap solution. Ten µL of the trap solution was assayed for its radioactivity by scintillation counting, and the remaining solution was employed for the HCN assay by the modified Lambert method as described above. The amount of HCN initially present in the tissue homogenate was calculated from the following equation: The amount of HCN in the tissue = (the amount of labelled NaCN added) × (C_o-C)/C, where C_o is the specific radioactivity of NaCN added to the tissue, and C is the specific radioactivity of HCN recovered in the trap. In order to improve the recovery of HCN during the homogenization, 10 mM of AOA solution was used as the homogenization medium to inhibit further metabolism of HCN, and the homogenate was kept at low temperature to minimize the loss of HCN into the gas phase. Normally about 10–20% of HCN was recovered with this procedure.

Assay of L-3-cyanoalanine synthase activity in plant tissues. Plant tissue was homogenized with 2.5 ml 0.1 M Tris buffer (pH 8.5) per g tissue. After centrifugation at 10,000 *g* for 10 min, the supernatant was employed for the enzyme assay. The enzyme assay was modified from Miller and Conn's method (Miller and Conn, 1980). The reaction was performed in a sealed 14-ml test tube containing 0.2 ml enzyme solution, 0.8 ml Tris buffer (0.1 M, pH 8.5), 25 mM L-cysteine, and 25 mM NaCN in a total volume of 1 ml. After incubation at 35°C for 30 min, the reaction was stopped by injecting 100 µL 20 mM N,N-dimethyl-ρ-phenylenediamine in 7.2 N HCl and 100 µL 30 mM $FeCl_3$ in 1.2 N HCl through the serum cap. The color developed due to the presence of H_2S was read at 650 nm, using Na_2S as the standard.

Results and Discussion

Apple slices were prepared from apple fruits which produced ethylene at a high rate (2–8 nmol/g-h). Thus, if apple tissue is not capable of detoxifying HCN and if this HCN does not diffuse out of the tissue, HCN concentration within the tissue would increase at a rate of 2 to 8 µM per h. It should be noted that the K_i value of HCN for the HCN-sensitive enzymes, such as cytochrome c oxidase, are in the 10 µM range (Theologis and Laties, 1978). Since the pKa of HCN is 9.3, the cyanide in plant tissues exists predominantly in the form of HCN, which is also volatile. As shown in Table 1, when apple slices were presoaked in the absence or presence of AOA or ACC, no HCN was trapped from the gas space of the incubation flasks. However, a significant amount of HCN was recovered when the tissues were treated with both AOA and ACC. These results are in accord with the current notion about ACC metabolism in apple. Postclimacteric apple fruit tissue is characterized by its high ACC turnover rate because apple fruit produces ethylene at a high rate (2 to 8 nmol/g-h), while maintaining a relatively low ACC level (2 to 10 nmol/g). Thus, a sustained high rate of ethylene production requires continuous ACC synthesis (Yang and Hoffman, 1984). Although AOA inhibits the conversion of HCN into L-3-cyanoalanine by inhibiting L-3-cyanoalanine synthase (Akapyan et al., 1975), HCN was not detected in the gas phase. This can be explained by

Table 1. Effect of AOA and ACC treatments on ethylene production and HCN accumulation in apple slices and mungbean hypocotyls. Data are from Yip and Yang (1988).

Treatment	C_2H_4 (nmol/g)	HCN	
		In gas phase (nmol/g)	In tissue (nmol/g)
Apple			
Control	7	ND*	0.2
AOA	0.2	ND	0.1
ACC	18	ND	0.2
AOA + ACC	14	1.6	1.7
Mungbean hypocotyl			
Control	0.1	ND	ND
AOA	0.02	ND	ND
ACC	24	0.3	1.9
AOA + ACC	17	2.4	8.1

For each treatment, eight apple slices weighing 2 g were presoaked for 2 h in 2% KCl (control) or 2% KCl containing AOA (5 mM), ACC (1 mM), or AOA (5 mM) + ACC (1 mM), as indicated. The slices were were blotted dry, and transferred to enclosed flasks fitted with center wells containing NaOH. After incubation for 2 h, the ethylene accumulated in the gas phase and the HCN trapped in the NaOH solution were assayed. HCN concentration in the tissue was estimated by the isotope dilution method. For the mungbean hypocotyls, conditions were the same as for apple slices except that 10 segments (0.9 g) each were presoaked in 5 ml 50 mM Mes buffer (pH 6.1) containing AOA (5 mM), ACC (10 mM), or AOA (5 mM) + ACC (10 mM), and were incubated for 4 h.

*Not-detectable or less than 0.1 nmol of HCN in the trap.

the fact that AOA also strongly inhibits the conversion of SAM to ACC (Yu et al., 1979), which serves as the donor of both ethylene and HCN. In contrast, when exogenous ACC was administered along with AOA, high ethylene production was maintained and the tissue became cyanogenic as indicated by the recovery of HCN from the gas phase. In the presence of exogenous ACC, ethylene production was enhanced, but the apple tissue remained non-cyanogenic (Table 1), presumably because the tissue had ample capability to detoxify the HCN produced during ethylene production.

By employing the isotope dilution method, we determined the HCN concentration in apple slices and in mungbean hypocotyls after the treatments with AOA and/or ACC. Table 1 also shows the relationship between ethylene production, HCN released into the gas phase, and HCN remaining in apple tissue following various treatments for 2 h. Control apple slices released no HCN and their internal [HCN] was estimated to be no higher than 0.2 μM, whereas ACC-plus-AOA treated slices released a significant amount of HCN and the tissue [HCN] increased to 1.7 μM. Similar results were obtained in mungbean hypocotyls. Following the treatment with 10 mM ACC, ethylene production increased 200-fold, and the amount of HCN became detectable in the gas phase as well as in the tissue. This trend became more pronounced when mungbean hypocotyls were treated simultaneously with both AOA and ACC (Table 1), where the tissue [HCN] went up to 8.1 μM. In order to verify the validity of the Lambert's method we employed, we compared the amount of HCN recovered from mungbean hypocotyls as determined by the Lambert's method and that by the method developed by Guilbault and Kramer (1966). Since the HCN values obtained from these two independent methods agreed well, we assumed that our HCN assays were valid.

We have also examined the effect of AOA and ACC on the L-3-cyanoalanine synthase activity extracted from the apple tissue. As expected, AOA significantly inhibited the enzyme activity with an $[I]_{0.5}$ of about 0.1 mM. Moreover, ACC at high concentration also exhibited an inhibitory effect on the enzyme activity; at 10 mM the inhibition was about 50%.

In order to examine the changes in the capability to detoxify HCN during the ripening process, we followed the changes in extractable L-3-cyanoalanine synthase in avocado fruit at different ripening stages. The results are shown in Table 2. In spite of the fact that ethylene increased 1000-fold at the climacteric stage, L-3-cyanoalanine synthase activity only doubled; no HCN was detected in the gas phase or in the tissue. Similar results were reported in senescing carnation flower by Manning (1986), who observed that both ACC oxidase and L-3-cyanoalanine synthase activities increased during the flower senescence; however, the increase in ethylene production was several hundred-fold, whereas the increase in L-3-cyanoalanine synthase activity was only twofold. These observations suggest that these plant tissues possess a high basal level of L-3-cyanoalanine synthase capable of detoxifying HCN generated during ethylene production from ACC.

Steady State Concentration of Cyanide in Tissues that are Actively Producing Ethylene from ACC

Since plant hormone ethylene is produced by all plants, and cyanide is a co-product of ethylene biosynthesis, it is not surprising that L-3-cyanoalanine synthase is not confined to cyanogenic plants but occurs widely throughout the plant kingdom. A major source of cyanide is from the hydrolysis of cyanogenic glycosides in those plant species which accumulate them. For those plant tissues which do not accumulate cyanogenic glycosides, but produce ethylene at a high rate, ethylene biosynthesis can be the major source of HCN (Wurtele et al., 1985). Hence, when the ethylene production rate and the capability to detoxify HCN in a tissue are known, it is possible to estimate the steady state [HCN] level within the tissue. The overall reaction is shown below:

$$\text{ACC} \xrightarrow{v_1} \text{HCN} \xrightarrow{v_2} \text{L-3-cyanoalanine}$$

where

v_1 = HCN production rate = C_2H_4 production rate, and

v_2 = the rate of HCN metabolism to L-3-cyanoalanine

Assuming that the rate of HCN metabolism follows the Michaelis-Menten kinetics, then

$$v_2 = \frac{V \times [HCN]}{K_m + [HCN]}$$

where V is the maximum rate of HCN metabolism at the saturating concentration of HCN and is therefore related to the amount of L-3-cyanoalanine synthase. The K_m for L-3-cyanoalanine synthase in vitro has been estimated to be about 0.5 mM (Akapyan et al., 1975; Hendrickson and Conn, 1969). Since this K_m value is very large in relation

Table 2. Changes in ethylene production rate, tissue cyanide content and extractable L-3-cyanoalanine synthase activity in avocado fruits at different stages of ripening. Data are from Yip and Yang (1988).

Stage of ripening	Ethylene (nmol/g-h)	HCN (nmol/g)	L-3-cyanoalanine synthase activity (nmol/mg protein-h)
Preclimacteric	0.004 ± 0.004	ND*	159 ± 14
Climacteric peak	4.25 ± 1.08	ND	282 ± 27
Postclimacteric	1.75 ± 0.21	ND	139 ± 5

*Not detectable, or less than 0.1 nmol HCN/g.

Table 3. The steady state HCN levels in unripe and ripe apples. Data are from Yip and Yang (1988).

Stage	C_2H_4 (v_1) nmol/g-h	Cyanoalanine synthase (V) nmol/g-h	v_1/V	[HCN] calculated (μM)	[HCN] observed (μM)
Unripe	0.01	630	1:63000	0.01	<0.1
Ripe	3.3	1650	1:500	1.0	0.2

The values of [HCN] calculated = 500 μM X v_1/V; see the text for detail. The values of [HCN] observed represent the determined HCN concentrations in the tissue; the methods employed were as those described in Table 1.

to [HCN] observed in plant tissues (Table 1), the relation becomes $v_2 = V \times [HCN]/K_m$. At steady state, $v_1 = v_2 = V \times [HCN]/K_m$. By rearrangement we obtain

$$[HCN] = (v_1/V) \times K_m$$

The above equation predicts that as the ethylene production rate increases, [HCN] will increase linearly, if V (or the amount of L-3-cyanoalanine synthase) and K_m remain unchanged.

Various enzymes are known to be sensitive to HCN inhibition (Solomonson, 1981), including cytochrome oxidase. The concentration of HCN which gives 50% inhibition of cyanide-sensitive respiration in plant tissues has been estimated to be 10–20 μM (Theologis and Laties, 1976). Thus, for the plant tissue to maintain [HCN] below the safe level of 1 μM, V (L-3-cyanoalanine synthase activity) should be at least 500 times larger than v_1 (ethylene production rate), if the in vivo K_m value for L-3-cyanoalanine synthase is similar to the reported in vitro value of 500 μM. In the experiment of Table 3 with ripe apple fruit, the in vivo v_1 (ethylene production rate) was 3.3 nmol/g-h, whereas in vitro V (extractable L-3-cyanoalanine synthase activity) was 1650 nmol/g-h. By applying the above equation, [HCN] can be estimated to be 1.0 μM, which is 5 times higher than the [HCN] value of 0.2 μM experimentally determined (Table 3). This discrepancy indicates that either the in vivo K_m value for L-3-cyanoalanine synthase was much lower than the in vitro value of 500 μM or the in vitro L-3-cyanoalanine synthase activity was underestimated due to the inactivation or incomplete recovery of the enzyme activity during the extraction procedures.

It should be pointed out that the estimated [HCN] represents the average concentration in the tissue. It is generally thought that ethylene is synthesized in the cytoplasm, whereas L-3-cyanoalanine synthase is localized mainly in mitochondria (Akapyan et al., 1975; Wurtele et al., 1985). Thus, the different compartmentation of these two enzymes would result in an uneven distribution of HCN within the cell.

Physiological Consequence

Ethylene has been established as an important plant hormone influencing a wide spectrum of physiological events. However, little information has been obtained concerning the metabolism of its coproduct cyanide in the ripening process. Ripening is a developmental process, and it is expected that fruit tissues during evolution would acquire the capability to detoxify this source of cyanide. The remaining question would be how plant tissues cope with HCN from ACC in somewhat unexpected situations such as environmental stress and hormonal changes, which are known to induce ethylene biosynthesis and thereby HCN production.

Cyanide Resistant Respiration

Though not the case with animals, in many plant tissues the poisoning of cytochrome oxidase by respiration inhibitors such as cyanide cannot slow down the respiration rate. This is due to the operation of an alternative route of electron transport also known as the cyanide resistance respiration. The alternative route allows transport of electrons from ubiquinol to a flavoprotein to the oxidase, which has a lower affinity for O_2. Since this cyanide resistance respiration does not couple to ATP formation, it is generally believed to serve as an overflow mechanism to remove electrons when the cytochrome pathway becomes saturated by rapid glycolysis and Krebcycle activity.

In the ripening fruits such as apple, avocado, and tomato, the rise in ethylene biosynthesis is accompanied by an increase in respiration in the tissue. This climacteric rise can be hastened in the preclimacteric stage by ethylene and HCN. Because of this coincidence, many researchers (Muzutani et al., 1987; Pirrung and Brauman, 1987) have alluded to the possibility that the increase in respiration may be due to the coproduct cyanide that resulted from the increased ethylene biosynthesis and that the increase in respiration is probably due to the operation of the cyanide-resistant electron transport. However, neither of these speculations are supported by experimental results. Yip and Yang (1988) reported that during the ripening process of apple and avocado, the steady concentration of cyanide in tissues could never exceed 1 μM, an amount unlikely to trigger any physiological changes of the tissues. Theologis and Laties have examined the engagement of the alternative respiration in intact fruit, such as avocado and banana, reaching the conclusion that the alternate path is not required to sustain the elevated respiration rates which characterize the climacteric stage in these fruits. Laties and his associates (Theologis and Laties, 1978; Tucker and Laties, 1985) have also revealed that the respiration in either preclimacteric or climacteric avocado fruit is mediated by the cytochrome respiratory path and that there is no engagement of the cyanide-resistant path in either tissue.

Auxin-Induced Cyanide?

Auxins, another prominent plant hormone, are found to induce ethylene production via the ACC pathway in many plant tissues. Therefore, many physiological effects originally regarded as the auxin-induced are actually via the action of ethylene. Examples include root and root hair initiation and leaf epinasty, abscission, and dehiscence. The studies concerning the induction of ethylene by auxins are well documented in the field of ethylene research. Much emphasis is on two major systems: tomatoes and mungbean hypocotyls. In tomatoes, nine different members of ACC synthase genes have been identified. Three of them, LE-ACS2, LE-ACS3, and LE-ACS5, are to some extent induced by auxins. In the mungbean system, auxin and cytokinin are known to have synergistic effects on ethylene production via the induction of ACC synthase. As in the case of tomato, more than one ACC synthase gene has been found to be related to elevated ethylene production. Since ACC oxidase is generally not the limiting step in the ethylene biosynthesis pathway, exogenous application of auxin and ACC would usually increase ethylene production and thereby HCN production in plant tissues. As we have shown that mungbean hypocotyl can be made cyanogenic when incubated with exogenous ACC (plus AOA, which acts as a cyanide metabolic inhibitor). We expect that mungbean hypocotyl if treated with auxin would also induce ACC and thereby HCN production; however we cannot actually make the mungbean cyanogenic by adding in auxin (plus AOA) because AOA can also inhibit ACC synthase activity (Yang and Hoffman, 1984).

Growing evidence suggests that some auxinic herbicide, such as quinclorac, 2,4-D, picloram, or clopyralid would exert its effect via the induction of cyanide (from the ACC pathway). Quinclorac effectively controls weed (both monocot and dicot) proliferation in rice fields. Apart from rice, wheat and oilseed rape also demonstrate high tolerance to quinclorac whereas barnyard grass (a monocot) and tomato (a dicot) are extremely susceptible to this chemical. Root application of quinclorac to barnyard grass caused inhibition of shoot growth accompanied by chlorosis and necrosis. Grossmann and Kwiatkowski (1995) showed that quinclorac induces ethylene biosynthesis via the ACC pathway and elevates cyanide concentration in barnyard grass shoot tissue up to a maximum of 40 μM. They also observed an increase in MACC content and L-3-cyanoalanine synthase activity in the shoot tissue. Furthermore, exogenous application of HCN could mimic the phytotoxic effect of quinclorac, but root application of 2-chloroethylphosphonic acid (an ethylene releasing compound) could not; AVG (an ACC synthase inhibitor) if applied prior to the application of quinclorac, alleviated herbicide-induced ethylene formation and the phytotoxic effects on shoots. All these data imply that the phytotoxic effect of quinclorac on barnyard grass is probably via the induction of ACC-dependent cyanide. Quinclorac also caused reduction of shoot growth, leaf epinasty, and chlorosis in young tomato plants when treated hydroponically with 10^{-7} M of the chemical. Grossmann and Schmulling (1995) reported that ethylene biosynthesis inhibitors could alleviate the effect of quinoclorac on tomato and that transgenic antisense-ACC synthase (LE-ACS2) tomato plant proved quinclorac resistant.

Future Research

If the argument about auxin type herbicides exerting their action via the ACC-dependent HCN production stands, the issue about some other forms of stress ethylene and its physiological consequences remains unresolved. The key may be to rely on the capability of the metabolism of HCN in those tissues being stressed. That may explain why some plant species are more susceptible to stress than others. At present, only one enzyme, L-3-cyanoalaine synthase is found to detoxify cyanide in plants. A more thorough study of this enzyme on the physiological, biochemical, and molecular levels should be conducted in plants under various stress conditions. This may include studies on how plants metabolize wound-induced cyanide, chilling induced cyanide, and others.

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