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# ETHYLENE AND THE RESPONSES OF PLANTS TO STRESS

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## Introduction

The suggestion that ethylene is involved in the responses of plants to stress is of no recent date and there are a multiplicity of publications over the last thirty years which tend to support this view. It must be said however that, almost in their entirety, the conclusions in such publications rest on correlations and inferences, which, no matter how logical, do not of themselves constitute proof of causal relationships.

In the same context, while it may be a relatively easy task to show that imposition of a stress leads to, say, increased ethylene biosynthesis, it is much more difficult to demonstrate that these effects are specific. Equally, it is difficult to prove that the effect of stress-induced ethylene has an “adaptive” or “survival” value for the plant in relation to the stress.

The purpose of this article is to provide a brief overview of the field and to attempt to assess whether, and to what extent, ethylene is involved in the responses of plants to stress.

## Stress and Ethylene Biosynthesis

It appears that almost any environmental perturbation can increase rates of ethylene biosynthesis, for example drought (El Beltagy and Hall, 1974), waterlogging (Kawase, 1972; El Beltagy and Hall, 1974), wounding and mechanical impedance (Goeschl et al, 1966) and salinity (El Beltagy et al., 1979).

The effects can be transitory (hours) or relatively long lived (days), may vary considerably with species and are complicated by the fact that ethylene biosynthesis shows a diurnal rhythm (El Beltagy et al., 1976), and that the growth regulator can control its own biosynthesis via autocatalysis or autoinhibition.

Such is the ubiquity of these effects that the first question which arises is “how specific are the mechanisms?”.

The pathway of ethylene biosynthesis in higher plants is now well defined. Starting with the conversion of methionine to S-adenosyl methionine, the latter is then converted to aminocyclopropane carboxylic acid (ACC) by ACC synthase and in turn the ACC is converted to ethylene by ACC oxidase. Only the last two enzymes are specific to the ethylene pathway; ACC synthase is inducible and ACC oxidase may be constitutive or inducible.

Early on in studies on ACC synthase the curious fact emerged that the enzyme existed in multiple isoforms, isoforms moreover which showed low homology except in their catalytic domains. It soon became clear that, at least to some extent, these differences related to the nature of the inducing stimulus.

There are at least three groups of genes, one group of which is induced by auxin, another group by wounding and a third group classified as “ripening signal inducible” (Nakagawa et al., 1991; Nakajima et al., 1990). The situation is rendered even more complex by the observations that auxin-induced expression of the genes is positively modulated by cytokinin but negatively by abscisic acid and ethylene whereas wound-induced expression is positively modulated by auxin and abscisic acid and negatively by ethylene; the repening signal induced expression is positively modulated by ethylene (Imascki, pers. comm.)

Although definitive proof is lacking, it is possible that other isoforms are induced in response to environmental stimuli, alternatively, the wound-induced forms may constitute those which are induced in response to environmental perturbations generally. The fact that, as noted above, other hormones can regulate ethylene biosynthesis adds a further dimension – and further complications, since there is evidence that some stresses at least can modify their endogenous levels also. Since ACC oxidase has only recently been rigorously characterised the effects of stress, if any, on this enzyme are unknown.

The results with ACC synthase do however go some way to explaining why stress affects ethylene biosynthesis and, even at this stage are suggestive of a degree of specificity. There is one further matter which must be raised in this context. It has been known for some time (Smith and Russell, 1969) that waterlogging or soil compaction can lead to increased ethylene concentrations in the soil atmosphere (to levels well within the range where physiological effects are observable). Such increases are due to the activities of facultative anaerobes such as *Mucor hiemalis* (Dowdell et al., 1972) and place ethylene in a different category with respect to stress than other plant growth regulators since in this context it must be looked upon as an environmental factor itself, and since, as noted, there is no doubt whatever that it can control its own biosynthesis, the stimulus for such control can arise both externally and internally.

## Stress-induced Ethylene and Development

It would be surprising indeed if marked changes in the concentration of a plant hormone, however induced, did not result in changes in plant development and there is ample evidence to suggest that this is indeed so.

Thus, many of the effects of stress on development are identical or very similar to those induced by application of ethylene. Examples would be the abscission of leaves and inhibition of stem extension in response to drought and leaf epinasty in response to waterlogging. Such correlations, while compelling, do not however prove causality.

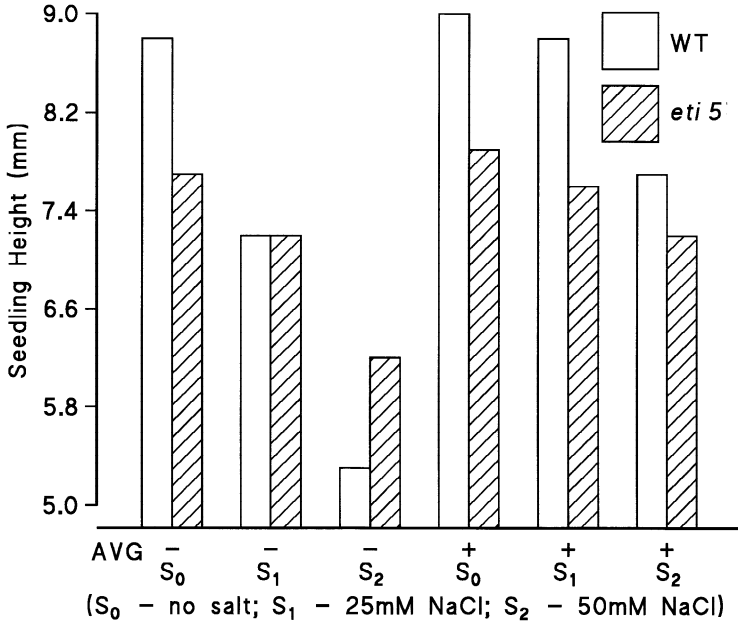
One of the earliest successful attempts to do so is the work on lacuna formation in maize roots in response to anaerobiosis (Drew et al, 1981). Thus, both ethylene and waterlogging (anaerobiosis) can induce lysogenous lacunae in maize roots. It was shown however that in the presence of $Ag^+$, a relatively specific inhibitor of ethylene effects that lacuna formation was prevented. While this did not constitute absolute proof the data went a long way further than the usual correlation.

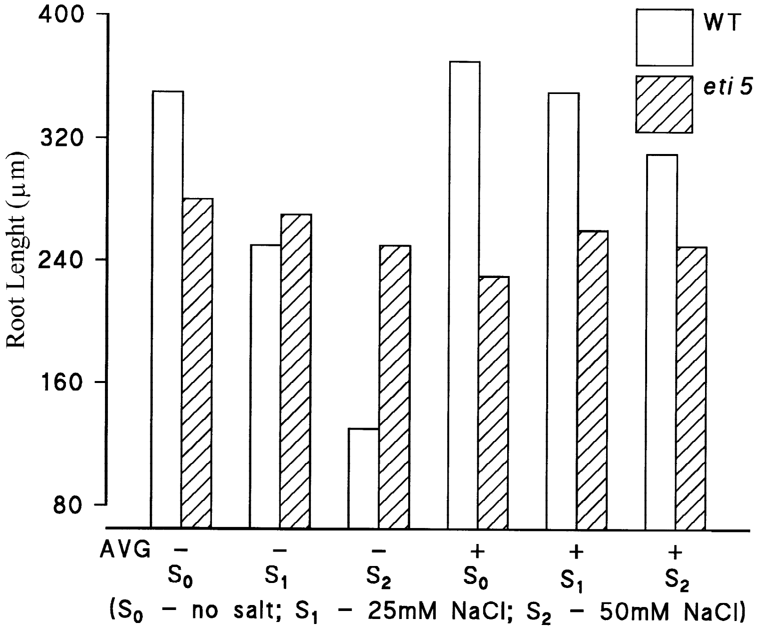
We have used two other approaches to this problem in the context of plant responses to salinity. Thus, there are available a number of inhibitors of ethylene biosynthesis of which the most specific is aminoethoxyvinylglycine (AVG). Clearly, if the developmental responses observed in response to stress are indeed a result of increased ethylene production, then inhibition of such an increase should lead to a diminution in the response.

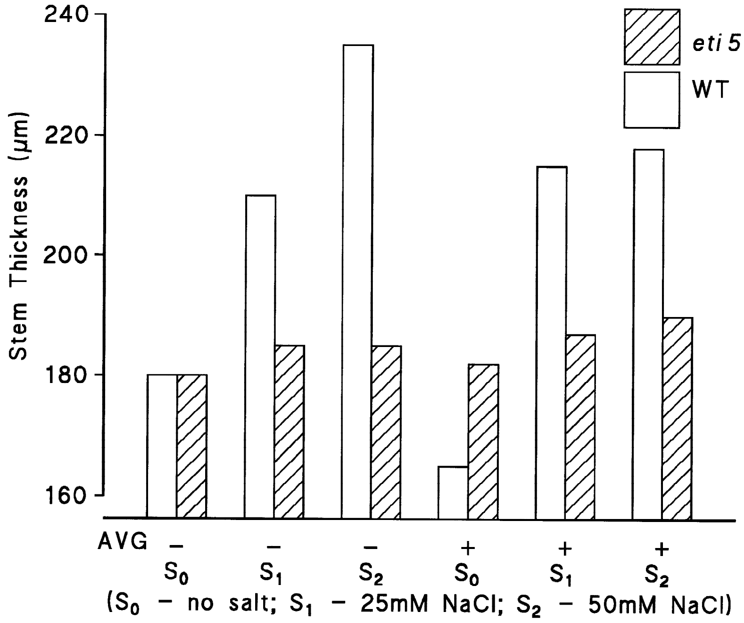
Fig. 1 shows the effect of AVG upon various growth parameters in *Arabidopsis* seedlings subjected to two levels of salinity. As noted previously, saline stress increases ethylene production and in this case there is a doubling in the rate of synthesis at 25 mM NaCl compared to controls and a further doubling at 50 mM. Application of ethylene to *Arabidopsis* seedlings increases hook closure, inhibits stem and root extension and increases seedling thickness. Examination of Fig. 1 (A-D) shows that all of these effects are mimicked by saline stress. In the presence of AVG these effects are diminished or greatly reduced, with the exception of stem thickness where the effect is marginal.

The results of the other approach are also shown in Fig. 1. Thus, we have available a range of *Arabidopsis* mutants with varying degrees of sensitivity to ethylene (Harpham et al., 1991). *eti* 5, the mutant shown in Fig. 1 (A-D) is completely insensitive to ethylene at concentration up to 10,000 > $\mu l\, l^{-1}$. Again, if the effects of saline stress are due to ethylene then these should not be apparent in the mutant. It is apparent that, with the exception of seedling height, salinity stress does not indeed affect significantly the growth parameters of the mutant. Unsurprisingly, additions of AVG have little if any further effect, except in the case of seedling height where there is a reduction in the inhibitory response.

Both the inhibitor and mutant approaches point in generally the same direction, namely that ethylene produced in response to the stress is indeed responsible, at least in part, for the developmental responses observed.







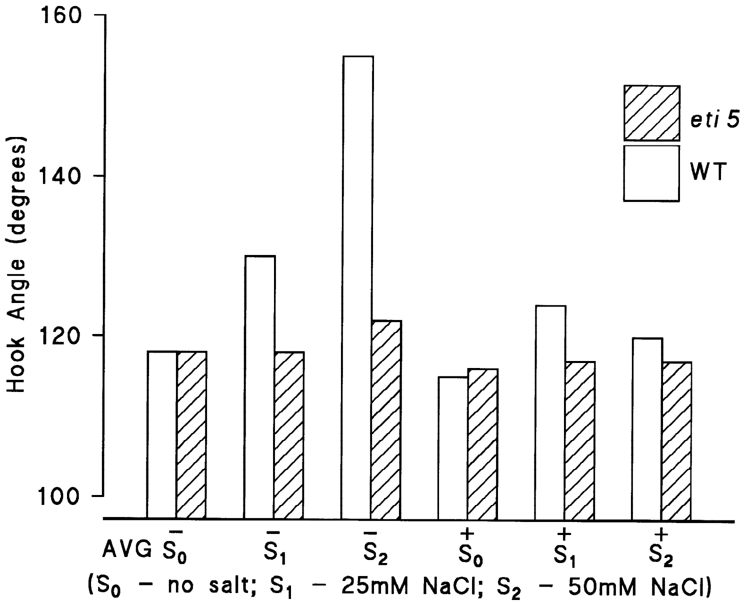


**Fig. 1.** Effect of aminoethoxyvinylglycine (AVG) and salinity upon growth and development of wild-type and *eti* 5 mutant *Arabidopsis* seedlings. Germinated seedlings were treated with a range of concentrations of NaC1 for 6 d in the dark in the presence or absence of 0.1mM AVG.

a) Epicotyl height, b) Root length, c) Stem thickness, d) Plumular hook angle

## Ethylene and Adaptation to Stress

While the foregoing demonstrates effects of stress-induced ethylene on developmental processes it does not illuminate the question as to whether these responses have significance in relation to survival of that stress. It is however possible to use these approaches to probe such possibilities.

Thus, it is known that the mechanical impedance of the soil, as a seedling emerges through it, induces increased ethylene biosynthesis by the seedling; equally, ethylene is known to maintain hook closure and stem thickening. It has been suggested that both of these responses have adaptive significance (Goeschl et al., 1966) firstly by protecting the stem apex and secondly by increasing the force exerted by the seedling and hence promoting emergence.

While these are attractive propositions they are again both correlative and teleological. If true however, then the capacity for a seedling to emerge from a soil should be related to its sensitivity to ethylene.

*Arabidopsis* seedlings respond to ethylene treatment in terms of the two growth parameters described, in the classical fashion (Table 1). In the same table the responses of the various *eti* mutants are shown, demonstrating a range from complete insensitivity to partial sensitivity.

**Table 1**.Effect of ethylene upon hook angle and stem thickness in *Arabidopsis* seedlings*. Seedlings were treated with 10,000 ml $l^{-1}$ if ethylene for values are means of 20 plants±s.e.

| | Hook angle (degrees) | | Hypocotyl width (µm) | |
|---|---|---|---|---|
| | – ethylene | + ethylene | – ethylene | + ethylene |
| Wild type | 105 ± 5 | 266 ± 4 | 196 ± 3.6 | 293 ± 5.1 |
| *eti* 3 | 102 ± 7 | 206 ± 16 | 199 ± 4.9 | 268 ± 3.7 |
| *eti* 5 | 76 ± 4 | 78 ± 5 | 182 ± 3.2 | 188 ± 4.0 |
| *eti* 13 | 91 ± 7 | 111 ± 33 | 182 ± 3.4 | 209 ± 9.4 |
| *eti* 10 | 82 ± 3 | 105 ± 5 | 181 ± 4.0 | 200 ± 3.8 |

*Adapted from Harpham et al. (1991)

Germinated seedlings of wild type and mutants were buried to a depth of 4 mm with sand and emergence monitored over a three week period (Fig 2). It is clear that emergence is variable and is directly related to the relative sensitivity of the mutants to ethylene shown in Table 1. This constitutes definitive evidence that the ethylene produced in response to a stress (in this case mechanical impedance) does indeed have an adaptive role.

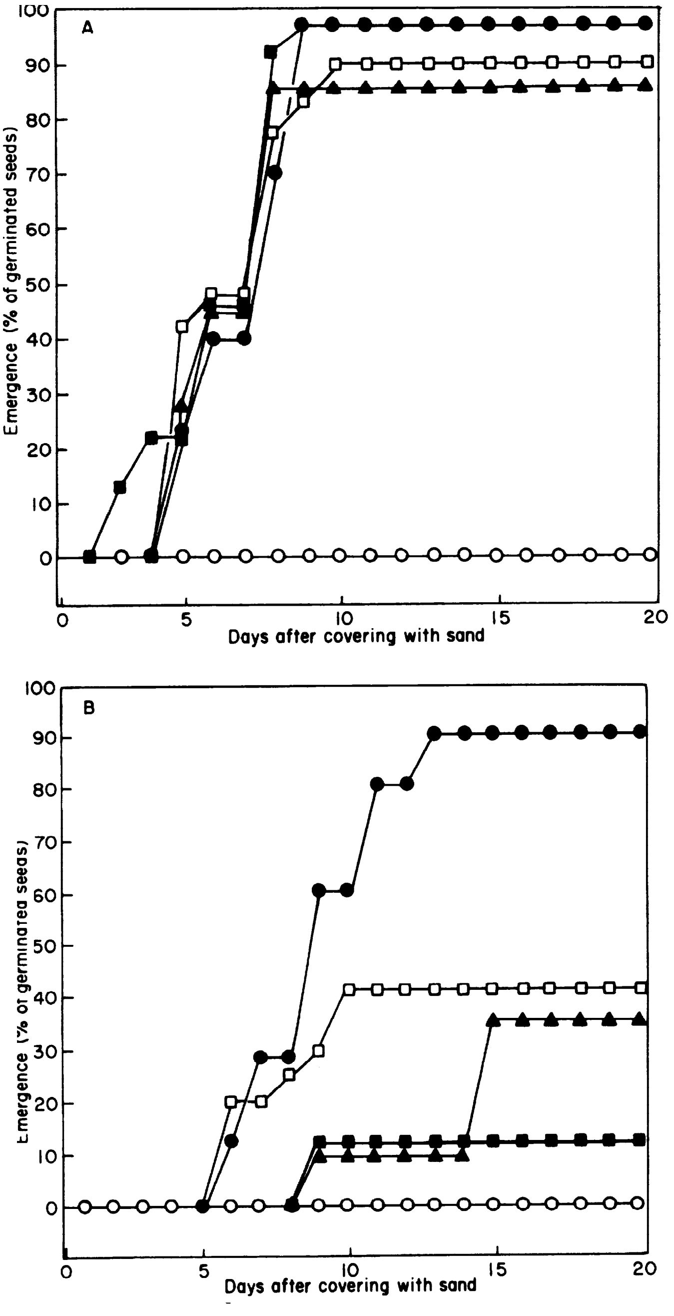


**Fig. 2.** Effect of burial in sand upon seedling emergence in wild-type and mutant *Arabidopsis* seedlings.
Seeds were buried to a depth of 2 mm (A) or 4 mm (B). Wild type (●), *eti* 3 (□), *eti* 5 (○), *eti* 10 (■), *eti* 13 (▲). From: Harpham et al. (1991)

## Conclusions

There is no doubt that ethylene biosynthesis is responsive to environmental stress of whatever kind. There is also increasing evidence of a degree of specificity in such responses in that particular genes may respond to particular stimuli.

It is also clear that many of the developmental responses to stress are in fact transduced by the increased rates of ethylene biosynthesis induced by the stress. In one case at least, namely mechanical impedance, it can be demonstrated that the induction of increased ethylene biosynthesis does have adaptive (survival) value. It would be naive however to assume that all stress-related-ethylene controlled phenomena have adaptive value and each case must be judged on its merits. In any case, it is doubtful if most responses to stress, adaptive or otherwise are controlled by a single growth regulator, or indeed by growth regulators alone at all. The definitive results with seedling emergence outlined here probably indicate that in this case ethylene is the single most important factor but do not exclude the possible intervention of others. Similar arguments could be advanced for abscisic acid whose role in the control of stomatal aperture is undisputed but where other factors also intervene.

Nevertheless, the evidence is compelling that ethylene does have a role in mediating the responses to stress and as more work is performed at the biochemical and molecular levels it is likely that a clearer understanding will emerge.

## References

Dowdell, R.J., K. A. Smith, R. Crees, S. W. F. Restall, 1972. Field studies of ethylene in the soil atmosphere: Equipment and preliminary results. Soil Biol. Biochem, 4, 325.

Drew, M.C., M.B. Jackson, S.C. Giffard, R. Campbell, 1981. Inhibition by silver ions of gas space (aerenchyma) formation in adventitious roots of *Zea mays* subjected to exogenous ethylene or to oxygen deficiency. Planta, 153, 217-224.

El Beltagy, A.S. Hall, M.A. Hall, 1974. Effect of water stress upon endogenous ethylene levels in *Vicia faba*. New Phytol., 73, 47-59.

El Beltagy, A.S., J. A. Kapuya, M.A. Madkhour, M.A. Hall, 1976. A possible endogenous rhythm in internal ethylene levels in the leaves of *Lycopersicon esculentum* Mill. Plant Sci. Lett., 6(3), 175-180.

El Beltagy, A.S., M.M. Khalifa, M.A. Hall, 1979. Salinity in relation to ethylene. Egypt. J. Hort., 6, 269-271.

Goeschl, J.D., L. Rappaport, H.K. Pratt, 1966. Ethylene as a factor regulating the growth of pea epicotyls subjected to physical stress. Plant Physiol., 41, 877-884.

Harpham, N.V.J., A.W. Berry, E.M. Knee, G. Roveda-Hoyos, I. Raskin, I.O. Sanders, A.R. Smith, C.K. Wood, M.A. Hall, 1991. The effect of ethylene on the growth and development of wild type and mutant *Arabidopsis thaliana* (L.) Heynh. Ann. Bot., 68, 55-61

Kawase, M., 1972. Effect of flooding on ethylene concentration in horticultural plants. J. Am. Soc. Hort. Sci., 97(5), 584

Nakagawa, N., H. Mori, K. Yamazaki, H. Imaseki, 1991. Cloning of a complementary DNA for auxin-induced 1-aminocyclopropane-1-carboxylate synthase and differential expression of the genes by auxin and wounding. Plant Cell Physiol., 32, 1153-1163.

Nakajinia, N., H. Mori, K. Yamazaki, H. Imascki, 1990. Molecular cloning and sequence of a complementary DNA encoding 1-aminocyclopropane-1-carboxylate synthase induced by tissue wounding. Plant Cell. Physiol., 31, 1021-1029.

Smith, K.A., R.S. Russell, 1969. Occurrence of ethylene and its significance in anaerobic soil. Nature, 222, 769-771.