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A Role for Ethylene in Barley Plants Responding to Soil Water Shortage

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Abstract. The influence of water shortage and ethylene (ethephon) application on ear fertility and tillering of barley plants were compared. In both cases, highest sensitivity was observed during jointing and pre-anthesis (Feekes 7–9). The ear initial with the surrounding tissue was identified as the site of ethylene action. Treating this region of barley plants with AVG before wilting partly prevented drought effects. These results, in connection with rising ethylene values in ear-bearing stem segments of wilting barley plants (more obvious in increasing ACC and MACC levels), especially in the drought-sensitive stages, favors a role for ethylene in the development of cereal plants under drought.

Water shortage has been reported by several authors to stimulate ethylene production in isolated tissues of higher plants (Aharoni 1978, Apelbaum and Yang 1981, El-Beltagy and Hall 1974, Graves and Gladon 1985, Hoffman and Yang 1983).

There are conflicting results concerning the drought-induced ethylene synthesis in intact plants (El-Beltagy and Hall 1974, Hubick et al. 1986, Morgan et al. 1990, Narayana et al. 1991, Wright 1981). The biochemistry and regulation of this process has been studied in considerable detail (Kende 1989, Yang and Hoffman 1984); however, the physiological function of the drought-induced ethylene burst in intact plants is not understood. The ethylene enhancement in droughted cereal leaves (McKeon et al. 1982, Wright 1977) is particularly well documented, although the physiological significance is still obscure. We therefore sought functions for the extra ethylene in droughted barley plants. The work suggests a role for ethylene in regulating aspects of grain yield development under water-deficient conditions.

Materials and Methods

Seeds of spring barley (*Hordeum vulgare* L. cv. "Trumpf") were sown in enamelled pods ("Mitscherlich pots") 20 cm in diameter and 6 dm^2 in volume. Each pot contained 4.5 kg sand (0.1–0.3 mm) and 2.0 kg soil (residual soil of shell lime) to which were added 0.6 g N as NH_4NO_3; 0.53 g P as $CaHPO_4 \times 2\ H_2O$; 0.4 g K as K_2SO_4; 0.3 g Mg as $MgSO_4 \times 7\ H_2O$; 2 ml of a 10% $FeCl_3$ solution and 1 ml of a 1:20 diluted Hoagland A–Z micronutrient solution. Fifteen plants per pot were grown. Pots were watered daily to predetermined levels of water availability expressed as percent of field capacity. Daily watering to 55% field capacity (daily ranse 35–55%, control) was sufficient to avoid water deficiency in the plants. Daily watering to 25% field capacity (max. fluctuation 13–25%) was the drought treatment. The respective soil water potentials were −0.1 MPa (55% field capacity) and −0.3 MPa (25% field capacity). To avoid uncontrolled watering, pots were moved into an open-sided glasshouse during rainfall. Stages of plant development are according to the Feekes scale (Feekes 1–5 = tillering −, Feekes 6–10 = shooting −, Feekes 10.1–10.5 = heading −, Feekes 11.1–11.4 = ripening period; see Briggs 1978).

Morphological and yield parameters were determined at seed maturity. Main shoots were marked during early tillering to facilitate certain estimation at later developmental stages. Aqueous solutions of ethephon (chloroethanephosphonic acid) (5×10^{-2} mol $\times\ l^{-1}$) and AVG (aminoethoxyvinylglycine) (4×10^{-3} mol $\times\ l^{-1}$) were applied in different ways. Shoots or shoot parts were sprayed to run off. The volume required increased from 6 ml/pot at Feekes 2 up to 12 ml/pot at Feekes 7 and all following stages. When only the base of the shoot was treated, volumes supplied were 4 ml/pot. Aqueous solutions of ethephon (5×10^{-2} mol $\times\ l^{-1}$) or AVG (1.6×10^{-2} mol $\times\ l^{-1}$) with Tween 20 (0.05%) added were pipetted behind the last sheath at the region of the main stem bearing the ear initial in both. The volumes applied were: ethephon 1×20 µl and AVG 2×25 µl. For analysis of ethylene, aminocyclopropane-1-carboxylic acid (ACC), and malonyl-ACC (MACC), stem segments containing the ear initial at the boot stage were excised below the last node of the main stem and trimmed to 5–9 cm. To measure ethylene emanation five segments were kept in a sealed container (50 ml) for 2 h and ethylene concentration of the headspace gas determined by injecting 1 ml into a GCHF 3 gas chromatograph (formerly VEB Chromatron, Germany) linked to a Pye 104 amplifier (Pye Unicam Ltd., England) equipped with an alumina column (2

m × 4 mm) and a flame ionization detector. Ten ears containing stem segments (3–6 g fresh wt) were frozen in liquid N_2 immediately after harvest and homogenized in 80% ethanol. The homogenate was filtered, the alcoholic extract evaporated in vacuo, redissolved in water, and filtered again. The aqueous extract obtained was passed down a cation exchange column (Dowex WX8, H^+-form) to separate conjugated ACC (effluent) from free ACC (eluated with 2 N NH_4OH). The conjugated ACC (presumed to be MACC) was purified by an anion exchange column (Dowex 1 × 4, OH^--form) eluted with 2 N HCl, the eluate evaporated to dryness and hydrolyzed in 10 N NaOH at 100°C for 3 h.

The free ACC fraction was purified by passing through a reversed-phase cartridge (Separon SGX-C 18, Chemapol, CSFR). The ACC content of the purified ACC fractions and of the MACC hydrolysates was determined according to Lizada and Yang (1979). Total losses determined by adding [^{14}C]-ACC or [^{14}C]-MACC to the extracts were found to be 20% for free ACC and about 50% (mainly losses of hydrolysis) for MACC (for more detail, see Bergner et al. 1990). Statistical evaluation was based on 5% confidence limits according to Sachs (1982).

Results

Comparisons of Responses to Water Stress and Ethephon

Reducing water availability at different stages of development decreased grain yield, especially if the water supply was diminished during the period of jointing and anthesis (Feekes 7–10.5; Fig. 1). The reduced grain yield was accompanied by a smaller number of kernels per ear of the main shoot associated with shoot stunting, decreased ear fertility, and outgrowth of mostly infertile tillers. The first two of these responses could be partly reproduced by applying ethephon at jointing and anthesis. Effects of ethephon on main shoot fertility and additional tillering resembled that caused by drought. The period of high susceptility to water stress overlaps considerably with that of greatest sensitivity to ethephon. Seven repeated experiments established unequivocally the time of highest sensitivity to both drought and ethephon was Feekes 7–9 (jointing, pre-anthesis). In contrast to water stress, ethephon did not decrease total grain yield per pot. This was because the additional tillers induced by this treatment were mostly fertile, while those on droughted plants were infertile.

Site of Ethylene (Ethephon) Action

Effects of ethephon (Fig. 2) were obtained by spraying all parts of jointing barley plants. To identify the site of action, barley plants were applied with ethephon in several ways (Table 1). When only the main shoot was sprayed, effects on ear fertility were much more pronounced then treating only the tillers. Tillering was stimulated by both kinds of ap-

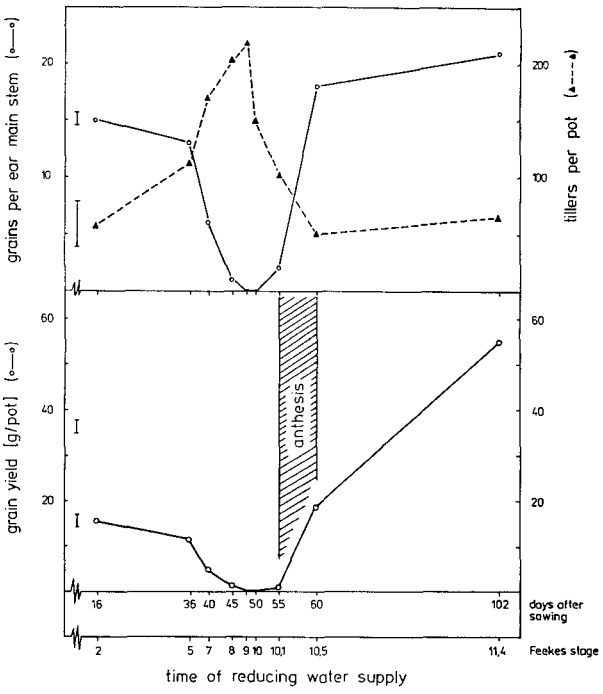

Fig. 1. Influence of reducing water availability at different stages of development on grain yield (below), number of tillers, and ear fertility of the main shoot (above). Level of watering was reduced from 55% to 25% field capacity at different stages of development. Vertical bars at left represent ± SE.

plications. Spraying ethephon to the lower (tiller bearing) nodes gave no significant effect on main shoot fertility and increased tiller formation only slightly. In contrast, upper parts of the main shoot responded to 20 µl of ethephon solution applied behind the outer leaf sheath near the developing ear initial. This was the most effective treatment for reducing ear fertility and increasing tillering.

Thus, the segment of the main culm bearing the last node and the ear initial in boot was probably the main site of ethylene (ethephon) action.

Effects of Aminoethoxyvinylglycine (AVG)

Effects of water stress on the ear fertility of the main shoot and additional tillering could be partly overcome by treating the plants with AVG. The inhibitor was given only to the region of the main shoot bearing the last node and the ear initial in boot (see Materials and Methods). It was shown in preliminary tests that this AVG treatment effectively inhibited auxin-stimulated ethylene formation of stem segments. Ear fertility of the main shoots of droughted plants was significantly increased by AVG at Feekes 7 and 9 (Fig. 3); additional tillering

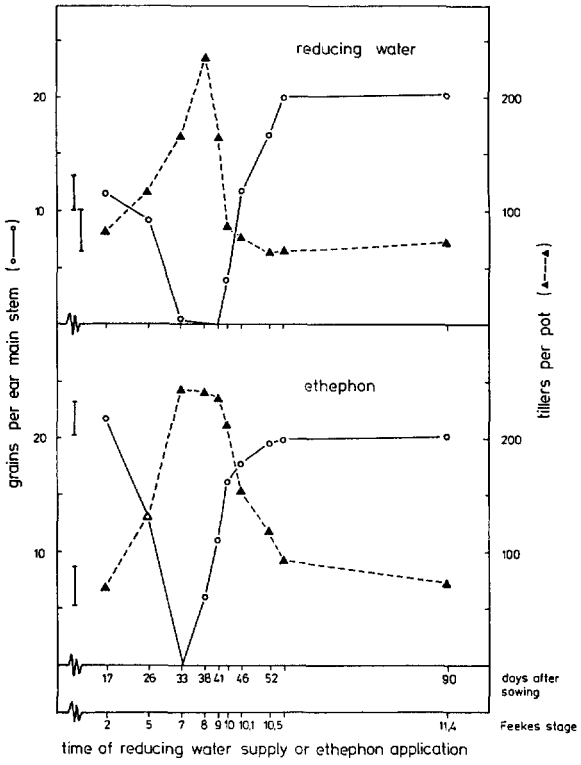

Fig. 2. Comparison of drought and ethephon action on tillering and main shoot fertility of barley. Water supply was reduced (above) or ethephon (5×10^{-2} mol $\times$ l^{-1}) was sprayed to well-watered plants (below) at different stages of development. Vertical bars at left represent ± SE.

was only prevented at Feekes 7, not at Feekes 9 (Fig. 4). Similar results were obtained in two further experiments (data not shown). In contrast, in these experiments decreased tillering caused by AVG was not statistically significant.

Ethylene Formation During Drought

To study the effects of droughting on the ethylene formation of the developing ear and the surrounding tissue, water supply was stopped at various stages of development. As water availability from the soil declined, leaf blades and apical stem segments of the main shoots were extracted for free and conjugated ACC (putative MACC); rates of ethylene emanation were also obtained. While the rate of ethylene production increased several-fold when excised primary leaves were wilting (data not shown), the influence of droughting whole plants on ethylene emanation was minimal. This applied not only to leaf blades but also to apical stem segments. When at Feekes 7, the ethylene formation in the 4th and 5th leaf blade (counting from the shoot base) was compared with that of the apical segments, the ear-bearing stem segments respond more to the stress than leaf tissue (data not shown).

Figure 5 comprises the combined results of three independent experiments carried out in different years. A decline in water availability lower than 25% field capacity led to an increase of the ACC and MACC contents in the developing ear and the surrounding tissue. This tendency was clearest at Feekes 7 and 9 but less pronounced at Feekes 6. Thus, during the jointing period of barley plants, ethylene precursor ACC and its conjugate rise in the ear initials as the soil dries. The response in terms of ethylene production was less clear, since stimulation of ethylene emanation was observed in only a few cases.

Discussion

Water stress during pre-anthesis depresses grain yield formation in cereals (O'Toole and Chang 1979). This was confirmed in the present experiments with a typical spring barley cultivar of central European origin. The causes will be multifarious. One contributing factor could be ethylene through its inhibitory effects on shoot extension and ear fertility. How processes leading to the loss of grain yield could be regulated also needs to be explained. The following hypothesis is to be proved: Shoot stunting and ear sterility occurring in barley plants water stressed at jointing stages are at least partly mediated by ethylene. This possibility was examined experimentally. Jackson (1987) proposed general criteria for implicating a hormone in the regulation of a naturally occurring developmental phenomenon. Experimental results demonstrated in this paper are evaluated in the light of these criteria. The first observation, leading to more detailed studies, was that of duplication. Ethephon applied to well-watered plants reproduced the effect of drought on main shoot fertility. The stages of highest sensitivity to ethephon coincided with that of drought susceptibility. Ethephon was also applied to different parts of the plant to localize ethylene release. Supplying ethephon to the developing shoot apex, which is enclosed by the sheathes of the upper leaves, was highly effective in reducing grain production at Feekes 7–9. Duplication of drought effects by ethephon was also observed with tiller formation. Plants normally cease tillering at Feekes 5. The drought regime caused new tillers to form at later stages than Feekes 5. This effect could also be duplicated by applying ethephon to well-watered plants. It may be that additional late tillering is a consequence of weakening the dominating shoots.

Table 1. Influence of ethephon on main stem fertility and tillering.

Mode of ethephon application	Stage	mg Ethephon per plant[a]	Grains per ear (main stem)	Tillers per pot
Spraying main shoots	Feekes 9	3–9	0	237
Spraying tillers	Feekes 9	3–9	89.4	391
Spraying basal plant parts	Feekes 7	0.5–1.4	93.7	127
Spraying basal plant parts	Feekes 9	0.5–1.4	85.3	124
Spraying upper plant parts	Feekes 7	1.8–5.3	0	418
Spraying upper plant parts	Feekes 9	1.8–5.3	6.8	395
Drop-application near apex	Feekes 7	0.14	7.3	153
Drop-application near apex	Feekes 9	0.14	2.1	203

The values represent the percentage to control (= 100%).
[a] Losses of the sprayed solution due to drifting were estimated to be 25–75% (Bergner and Otto 1974).

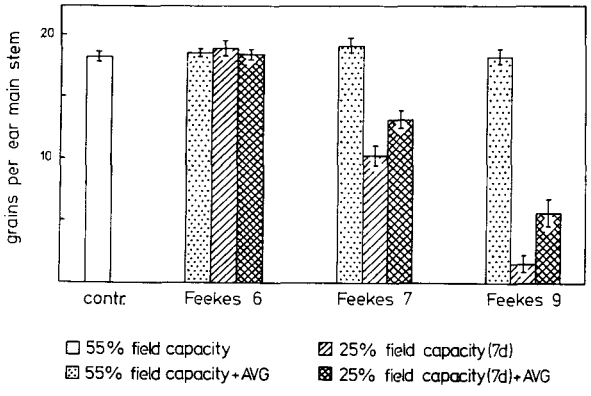

Fig. 3. Influence of a transient drought regime (reducing water availability from 55% to 25% field capacity for 7 days) ± AVG (20 µl/plant; 1.6×10^{-2} mol $\times$ l^{-1}) applied to the apical region of the main shoot on main shoot fertility of barley plants. Vertical bars represent ± SE.

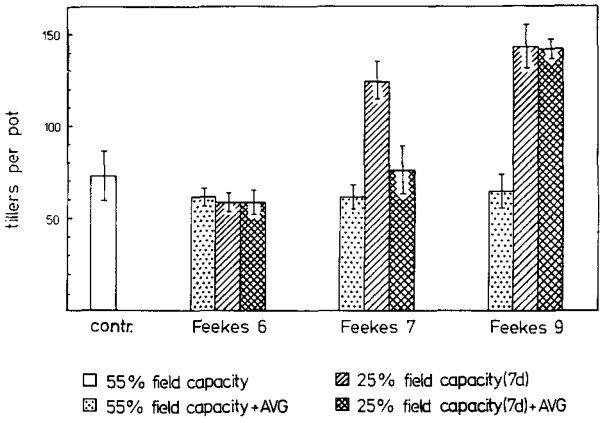

Fig. 4. Influence of a transient drought regime (reducing water availability from 55% to 25% field capacity for 7 days) ± AVG (20 µl/plant; 1.6×10^{-2} mol $\times$ l^{-1}) applied to the apical region of the main shoot, on the number of tillers of the barley plants. Vertical bars represent ± SE.

If an increase of endogenous ethylene is responsible for shoot stunting, ear sterility, and additional tillering, then inhibiting ethylene formation should prevent these stress effects. Accordingly, applying AVG, an effective inhibitor of ethylene biosynthesis (Yu and Yang 1979), improved grain yield of the stressed main shoot. The activity of AVG in preventing the stress effect was significant but restricted to a partial reversion of drought-induced ear sterility. It might be possible that the dosage or method of application (AVG was only given to the main shoot apex, while water stress would affect the whole plant) was not optimal and thus unable to reverse the effect of drought on sterility completely. On the other hand, it seems more likely that the loss of ear fertility under drought conditions is not entirely due to ethylene, and this cannot be completely prevented by inhibiting the biosynthesis of this plant hormone. There were conflicting observations concerning AVG effects on additional tillering. In contrast to the results shown in Fig. 4, the inhibitor failed to prevent late tiller formation in several other experiments (data not shown). This failure was observed in all experiments with limited efficiency of AVG on main stem fertility and reflects a close relationship between main stem fertility and tillering.

One of the most important criteria to verify the implication of ethylene in the regulation of drought-induced ear sterility and additional tillering should be a correlation between endogenous hormone content in the putative target tissue and the occurrence of the phenomena under consideration. Reducing water availability to a level at which ear fertility of the main shoot normally decreases should be accompanied by an increase of the rate of ethylene formation in the respective apical stem segments. In

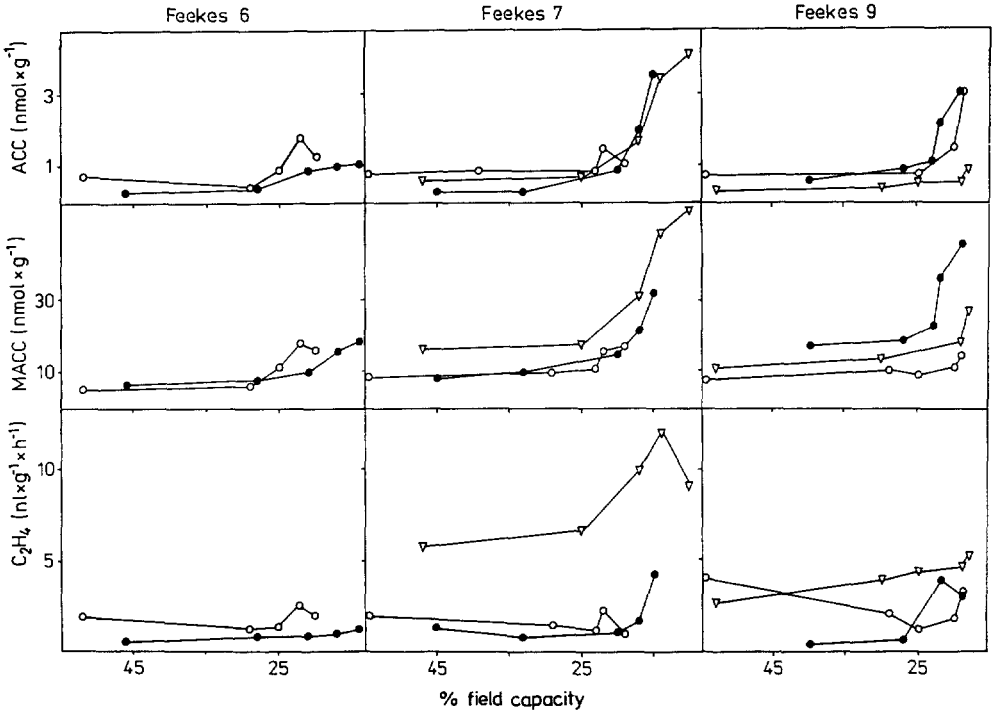

Fig. 5. Influence of declining water availability in the soil on ACC and MACC contents and ethylene emanation of apical stem segments of barley. Data were obtained in three independent experiments (--●-- 1986, --○-- 1987, --▽-- 1989), and represent mean values of three repetitions.

the experiments described, ethylene formation and endogenous ACC and MACC levels increased as soil moisture content declined. As the rate of evapotranspiration differed during the three experiments reported, soil moisture values do not entirely reflect the water stress of the plant tissue. This may explain the individual differences in the ACC and MACC contents between the different experiments. Nevertheless, it seems clear that at Feekes 7 and 9 ACC as well as MACC levels increase under drought. But it is surprising that the rate of ethylene formation often failed not to correlate with the content of its precursors in the tissue. Several explanations could be drawn into consideration: (1) Drought-induced enhancement of ethylene emanation could be masked by wound ethylene since ethylene production was measured after static collection. (In contrast stem segments were frozen immediately after harvest to extract ACC and MACC.) (2) The rate of ethylene emanation measured does not reflect the active ethylene titer within the cell. (3) ACC is partitioned off from the ACC oxidase enzymes in the cell and thus not readily available for conversion to ethylene. That water stress may promote such partitioning is worthy of further research. (4) Morgan et al. (1991) and Narayana et al. (1991) reported that water deficit does not promote ethylene production in whole plants. In our case apical stem segments were isolated from intact water stressed plants. The water stress induced ethylene burst can perhaps only be observed in excised organs but not in intact plants. These and other possibilities should be investigated.

This work gives limited support to the view that cereal fertility is reduced in droughted plants as a result of ethylene action. Certainly the ethylene precursor and its conjugate increase by drought. The production of the hormone was less dramatic. However, the action of AVG in restoring some fertility suggests that the drought-induced extra ethylene may have been detrimental to grain set.

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