Emission of water stress ethylene in wheat (*Triticum aestivum* L.) ears: effects of rewatering

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Abstract

In this work it has been found that ethylene production increased only slightly under conditions of a moderate or severe water stress. However, the rehydration of the plants at full turgor after desiccation caused a high emission of ethylene. The desiccation would not irreversibly inactivate the enzymes of the ethylene pathway, since rehydration made the synthesis recommence almost immediately. Water deficit also increased the free radical levels and the antioxidant scavengers, such as superoxide dismutase. Free radicals promote the conversion of 1-amino-cyclopropane-1-carboxylic acid to ethylene, then it is logical to think that both chemical species are involved in the phenomenon of the acceleration of the grain maturity before the plant collapses.

Abbreviations: SOD = superoxide dismutase; ACC = 1-amino-cyclopropane-1-carboxylic acid

1. Introduction

Ethylene synthesis increases in plants in response to stresses such as low and high temperatures, radiation, flooding, water deficit, insect attacks and many toxic substances [1]. The emission of ethylene as a result of these situations is known as stress ethylene [25].

The metabolic changes provoked by water deficits have attracted an intense investigation [3]. Ethylene increased after a water deficit in cotton [10], orange leaves [7], wheat leaves [9, 20] and any other species [12, 14]. The biochemical mechanism of ethylene synthesis under water stress is still unknown and most of the literature is controversial. Irigoyen et al. [14] found that pieces of alfalfa leaves produce more ethylene when they were subjected to a water deficit ($-1,6$ MPa) and ethylene evolution was even higher when the

drop in water-potential was severe ($-2,0$ MPa). Likewise, cut leaves and seedlings of wheat emanated more ethylene upon rapid water loss [20]. On the contrary, no significant increase in ethylene production was found when plants (bean, cotton, and roses sp.) were subjected to cessation of irrigation, not even when they were later rewatered [19]. Neither did Narayama et al. [20] nor Carbone et al [9] find a significant increase in the ethylene production of cut leaves of wheat plants, suffering a water stress. Moreover, a lower hydration level of the leaves decreased the emission as compared with turgid ones. The discrepancy among the findings could be due to the different methodology used in the experiments. Some stressed plants gradually, others used a shock treatment while there were others who rewatered the plants. In addition, the response might depend on the severity of the stress, the organ used

to measure ethylene and the time in which the turgor was recovered. The consensus is that the production of ethylene during a water stress episode followed by rewatering is far from being elucidated. It has also been found that the stress ethylene accelerates the processes of maturity of the wheat leaves and ears [6], concomitantly with an increase in the generation of free radicals during water deficit [22]. This phenomenon would allow the production of seeds before the complete desiccation and the consequent plant collapse.

The following hypotheses were used in this research:

1. Gradual water-stress may induce the synthesis of ethylene until extreme desiccation hinders the enzymes activity.
2. Rehydratation may restore the ethylene synthesis and diffusion to the atmosphere and also foliar conductance.
3. A stress situation may also promote the production of more free radicals and their oxidative activity may interfere with the plant metabolism.

This confirmed the existence of stress ethylene synthesis in response to a moderate drought stress in wheat plants, but, under a severe deficit, the plants ceased to emit ethylene. After rewatering and subsequent cellular rehydration, the plants maximized their emanation of ethylene to the atmosphere. The responses observed with anti-oxidants chemical species and the increase of free radicals during the stress, provide a significant support to the hypothesis mentioned above.

2. Material and methods

Wheat (*Triticum aestivum* L. cv. Buck Poncho) caryopsis obtained from Buck S.A. were sown in plastic 10 L pots filled with a mixture of soil and sand (3:1). After emergence, seedlings were thinned to one plant per pot. Plants were watered daily and fertilized every other day with half-strength Hoagland [13] solution. Neither symptoms of mineral deficiencies nor attacks of pests were observed. Plants were grown in a greenhouse from sowing in August 1993 to ear maturity and the experiment were done in December. Mean temperature was 29/20 °C (day/night), midday photosynthetic photon flux density was 1600 μmol m^{-2} s^{-1} in August and 2500 μmol m^{-2} s^{-1} in December, measured with a Quantum sensor Li-190SA Licor Radiometer. Flag leaves conductance was determined by a Porometer Licor Model LI-1600. Photoperiod

was the natural of the locality (La Plata: 34°54′ L.S.). Fresh air was continuously supplied to the greenhouse to prevent the accumulation of ethylene or depletion of carbon dioxide. The greenhouse atmosphere was sampled periodically and analyzed for CO_2 content with an infrared gas analyzer (IRGA, PORT. PHOTO-SYNTHESIS SYSTEM Licor Model LI 6200) and for ethylene by GC as described below.

After anthesis (at different stages: Early milk, Late milk, Soft dough and Hard dough stages [27]) plants were subjected to the following three treatments.

1. Controls, watered discretionary to maintain a water potential between −0.2 and −0.4 MPa. Water potential of controls plants varied between the limits of −0.2 and −0.4 MPa in response to the relative humidity and greenhouse temperature.

2. At different stages: Early milk, Late milk, Soft dough and Hard dough [27], a group of 12 plants were exposed to a severe water stress (−1.9 MPa) by withholding water for 7 days and later watered till the control water potential (−0.2 and −0.4 Mpa).

After rewatering the plants, ethylene emitted by the ears and the conductance by the flag leaves was periodically determined. Likewise, the generation of free radicals and the antioxidants superoxide dismutase and catalase species in the flag leaves of all the treatments at Late milk stage were measured.

The water potential was measured with a pressure chamber on 6 randomly sampled plants (flag leaves) per treatment. To measure ethylene production, entire ears were cut off, then 12 entire ears were individually put into 40 ml glass flasks (previously vented with a stream of chromatographic air: O_2 = 21% ± 1% and N_2 = 79% ± 1%; H_2O < 5ppm/v; CO_2 < 1ppm/v) containing 0.1 ml distilled water to maintain humidity to saturation. The flasks were sealed with screw caps fitted with rubber septa [18] and ethylene was allowed to accumulate at 27 °C in darkness. After 1 h incubation, a 1 ml gas sample was removed with a gas-tight syringe and injected into a gas chromatograph (Konik KNK-3000 HRGC) equipped with an activated alumina column (0.32×1.80 cm) and a flame ionization detector. Injection port, column and detector temperature were kept constant at 110°, 120° and 170 °C, respectively. Nitrogen was used as carrier at a flow rate of 35 ml min^{-1}. Ethylene concentration was expressed as nl g^{-1} FW h^{-1}. The minimum detectable concentration in the gas sample was 0.08 nl ml^{-1}.

Two experiments were conducted during 1993 and 1994 under similar conditions and in the same seasons. The data presented here are from the experiment

conducted in 1994 and are representative of the result obtained in 1993.

Reactive oxygen species generation

An *in vivo* assay was performed placing 100 mg of cut wheat leaves in 8 ml of 40 mM Tris-HCl buffer (pH 7.0), in the presence of 15 μM DCFDA (2',7'-dichlorofluorescein, Molecular Probes Inc, Eugene, OR) at 30 °C. The incubation medium was removed after 90 min and fluorescence monitored in a Hitachi spectrofluorometer with excitation at 488 nm and emission at 525 nm [23]. Correction for autofluorescence was made by the inclusion in each experiment of parallel blanks (assay mixture without leaf material).

Homogenate preparation

Cut leaves were homogenized with a Potter-Elvejhem homogenizer in 50 mm phosphate buffer (pH 7.0) filtered through four layers of cheesecloth and then centrifuged at 750 g for 10 min.

Enzyme assays

The homogenates were added with 1% (w/v) Polyvinylpyrrolidone (PVP). Superoxide dismutase activity was determined spectrophotometrically as the inhibition of xanthine oxidase-dependent reduction of nitrobluetetrazolium (NBT) [5]. The reaction mixture contained 0.1 mM nitrobluetetrazolium, 0.1 mM EDTA, and 50 μM xanthine and xanthine oxidase in 50 mM potassium phosphate buffer (pH 7.8). One unit of SOD is defined as the amount of enzyme that inhibits by 50% a control rate of NBT reduction (0.025 units of absorbance at 550 nm per min)[16].

Catalase activity was measured according to Aebi [2]. The reaction mixture contained 15 mM H_2O_2, up to 100 μL of homogenate (7 mg protein/mL) with 0.2% Triton X-100 in 50 mM potassium phosphate buffer (pH 7.0).

The protein content was assayed according to Bradford [8] utilizing bovine serum albumin as standard.

Statistical analysis

Data are expressed as mean ±SEM and statistically analyzed by the variance test (ANOVA) P< 0.05.

3. Results

Ears from plants grown under water potential below the control showed slightly increased ethylene synthesis in all the grain stages examined. However, it must be noted that during the Early milk and Late milk stages emitted more ethylene than the other ears under the same stress conditions. As the severity of the water stress rose the emission augmented and peaked at about −1.0 MPa. Thereafter, it began to decrease gradually till the flag leaves reached a water potential of −1.9 MPa. These reductions were also observed in all the grain stages, as it is shown in the Figure 1 (A, B, C, D).

The stomatal conductance of flag leaves at the Early milk stage was 147 ± 23 mmol H_2O m^{-2} s^{-1} in the control, 52 ± 17 mmol H_2O m^{-2} s^{-1} when the water potential was as low as −1.9 MPa and 139 ± 32 mmol H_2O m^{-2} s^{-1} after the plants had regained their leaf water potential to control levels. When the plants were rewatered, the ears, gradually, began to produce ethylene though in much higher amounts than the controls. So when the flag leaves had acquired a water potential of −0.4 MPa, about seven hours after the rewatering began, the ears emitted 5.00 nL g^{-1} FW h^{-1} at the Early milk, 5.45 nL g^{-1} FW h^{-1} Late milk, 0.68 nL g^{-1} FW h^{-1} in the Soft dough, and it was negligible at Hard dough.

We measured the presence of free radicals and Figure 2A shows that in leaves of plants subjected to a water stress (S) (−1.9 MPa) free radicals increased as compared to the control plants (C) (104 ± 21 and 65 ± 12 AU of fluoresceine mg^{-1} DW h^{-1}). Free radicals increased further a twofold after rewatering (S+R) at the Late milk stage (247 ± 45). The levels of SOD, were also higher in the plants under the same stress condition and in the same stage of maturity. When the plants were watered, SOD increased its activity till 36.2 U.mg^{-1} prot min^{-1} (Figure 2B), while, the catalase activity did not change under the same conditions and at tha same stage of maturity (Figure 2C).
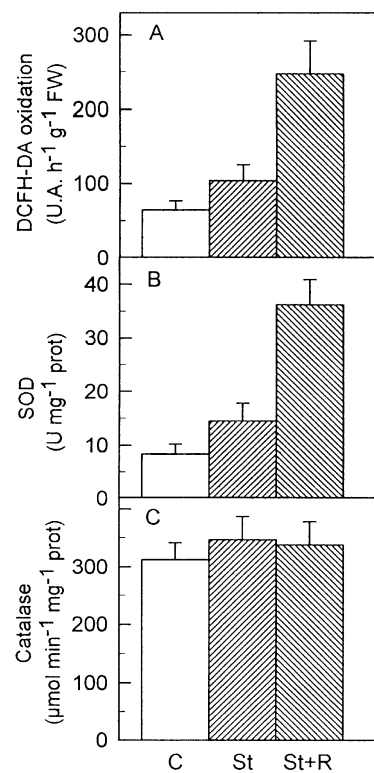
4. Discussion

The results of these experiments corroborate partially the findings of Irigoyen et al. [14] that plants suffering a severe water stress do not emit ethylene in significant amounts. Besides, they do not coincide with those published by Morgan et al. [19] who did not find an
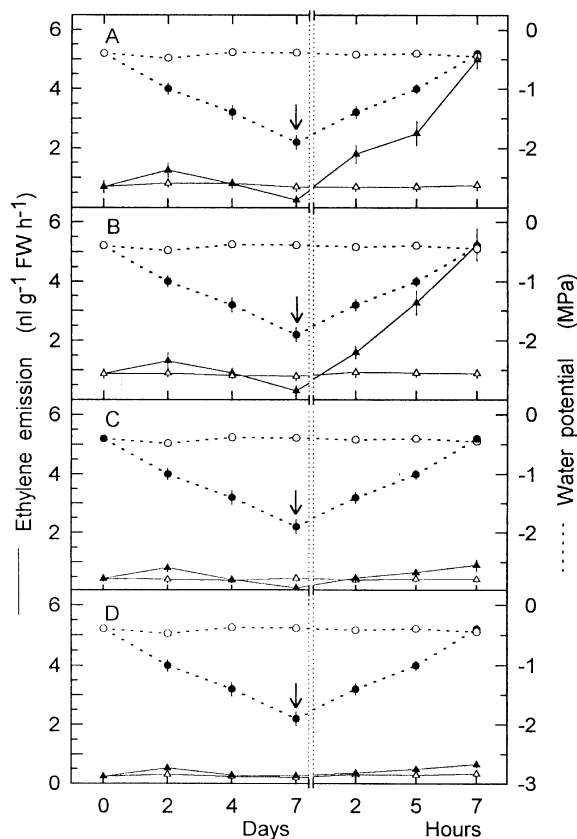
Figure 1. The effect of progressive soil drought (water stress) and rewatering on flag leaf water potential (Ψ = MPa, broken lines) and ethylene emission from wheat ears (nl g^{-1} FW h^{-1}, continuous lines) of Early milk (A), Late milk (B), Soft dough (C) and Hard dough (D) stages. Measured were made from 0 to 7 d after withholding water, and from 2 to 7 h after rewatering. Water potential (●); ethylene emission (▲) Water potential of control (well watered plants) varied between −0.2 and −0.4 Mpa (○) and ethylene emission (△) was almost constant from each stage experiment. After 7 d water potential of non irrigated plants (water stressed) decrease gradually and ethylene emission was almost negligible. Bars represent ±SE of the mean.

Figure 2. Effect of low water potential (water stress) and rewatering on the 2′7′-dichlorofluoroscein oxidation (A) and the activity of SOD (B) and catalase (C) as oxidative stress indicators of wheat leaves at Late milk stage. Cessation of irrigation occurred seven days before rewatering. Ψ_w of control plants was −0.4 MPa (C); Ψ_w of non irrigated plants (St) and non irrigated plus rewatering (St+R) was −1.9 MPa and −0.4 MPa, respectively. Data are means ±SE of the mean.

increase in the release of ethylene in bean and Rose hybrida L. cv Bluesette subjected to a water deficit.

Here we demonstrate that the stress ethylene synthesis occurred from the beginning of dehydration until the water content permitted a normal functioning of the enzymes of its pathway. This production increased until the water potential of the flag leaves reached a value of −1.0 MPa. From this water potential resulted in gradual lowering ethylene emission that almost ceased soon afterwards. It is possible that during the diminution of water in the cytosol, the protein molecules (enzymes) underwent changes in

their conformation preventing the synthesis of ethylene from proceeding. Moreover, even in this low water status some additional events may have occurred, for example lower stomatal conductance and, most probably an inhibition in the ethylene biochemical mechanisms. Our explanation is that the rewatering allowed the rehydrated proteins to recover their molecular folding resulting this in an abruptly increase of the liberation of this hormone. Under a water potential (−1.9 MPa) and a normal content of free radicals, ethylene production was not large and was not detected, most probably due to the low leaf conductance. When water reactivated the proteins structures after rewatering, the pathways of the ethylene synthesis recommenced to produce it, but in much more quantities, possibly, by the higher generation of free radicals [11, 24]. This higher free radicals production could be consequence of alterations and perturbations in the

most of the cytosol components occuring during water depletion and they were determined after rehidration (Figure 2A). The above explanation is based on the fact found by Mayac et al.[15] and McRae et al. [17] that the $.O^{-2}$ generated converts ACC to ethylene. The results obtained in the present experiments are coincident with the work done by Baisak et al. [4] about the water deficit on the levels on SOD and catalase. These authors found that drought did not alter the catalase activity, but the SOD activity was promoted, in spite of the fact that they did not subject the wilting plants to a treatment of rehydration. Moreover, in the present study there was a marked increase in free radicals only partially antagonized by the complex antioxidant system of the cell. The synthesis of ethylene ceased as the water deficit possibly modified the enzyme folding and, the equilibrium between free radical production and the enzymatic defense reactions [26]. This is in agreement with the idea that both ethylene and free radicals are involved in senescence [21]. We found that the levels of oxidative chemical species augmented in the flag leaves and they were even intensified after the recovering of the turgor. It is interesting that superoxide dismutase activity – a free radical scavenger – increased parallel to oxygen radical activity.

Our data suggest that the balance oxidants/antioxidants could be one of the most important natural process involved in the programmed event of the death.

Acknowledgments

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