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# Improvement of tolerance to paraquat and drought in barley (*Hordeum vulgare* L.) by exogenous 2-aminoethanol: effects on superoxide dismutase activity and chloroplast ultrastructure

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**Abstract**

In pot and field experiments conducted over several years, the influence of 2-aminoethanol on growth and yield of barley (*Hordeum vulgare* L.) under conditions of different water supply was studied. Under drought stress, 2-aminoethanol pre-treatment increased the grain yield of barley by 5–30%. The effects of application of the plant constituent, 2-aminoethanol on biomass formation, the content of chlorophyll and protein and the activities of superoxide dismutases (SOD) were studied in drought stressed barley plants. When plants were treated with 2-aminoethanol, and were exposed to water deficit, growth inhibition and chlorophyll content losses diminished. Analyses of SOD activity by native PAGE indicated the presence of two Cu/Zn–SOD isoenzymes in barley shoots. The activity of Cu/Zn–SOD II, the major isoenzyme in barley shoots, was strongly stimulated by 2-aminoethanol. Drought stress also induced a strong increase in the activity of Cu/Zn–SOD II, but the combined effect of 2-aminoethanol application and drought resulted in the highest Cu/Zn–SOD II activity. We studied further the protective effects of 2-aminoethanol treatment on chloroplast ultrastructure by electron microscopy. Here, paraquat was used as a causal agent of oxidative stress. At sublethal paraquat doses (0.1–1 mM), 2-aminoethanol could prevent the membrane deterioration. The results indicate a role of 2-aminoethanol pre-treatment in the protection against oxidative stress in plants.


*Keywords:* *Hordeum vulgare*; Barley; Stress tolerance; Drought; Paraquat; Superoxide dismutase; Membrane damage; Electron microscopy

## 1. Introduction

Environmental factors such as water, temperature and nutritional status affect the biochemical responses of plants to stress. Plants have genetically controlled mechanisms that allow them to live and grow under stress [1]. One of the most important environmental factors is the availability of water. Salinity or other stressors cause a similar physiological stress response and thus drought is a general stress equivalent [2,3]. Sufficient water supply is the most important abiotic factor that land plants depend on for growth [2]. Water deficit inhibits photosynthesis via stomatal closure and a lack of $CO_2$ and leads to the formation of reactive oxygen species (ROS) [4–8], which in turn, cause membrane damages by lipid peroxidation [9,10]. Changes in the concentration of antioxidant molecules (e.g., ascorbic acid, glutathione, carotenoids) and antioxidative enzymes (e.g., superoxide dismutase [SOD], peroxidases [POD]) have been related with water deficiency [2,10]. ROS detoxification involves dismutation of superoxide radicals by SOD to hydrogen peroxide, a partially reduced ROS [11,12]. The enzymes catalase (CAT) and ascorbate POD detoxify the cellular hydrogen peroxide [11,13]. In plants, various types of SOD metalloproteins have been found in different cellular compartments. Cu/Zn isoforms are located in cytosol and chloroplast, Mn–SOD is mitochondrial and Fe–SOD is chloroplastic [11,12]. Chloroplastic and cytosolic Cu/Zn–SOD activities increase during water deficiency in *Pisum sativum* L. [14]. Drought tolerant *Sorghum* has a

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higher antioxidant capacity during drought stress than drought susceptible *Sorghum* varieties [15]. Drought stress can alter the oxidative balance of the plant cell, and adaptation to drought is generally correlated with keeping the ROS level relatively low by the antioxidant system [16].

Several important biogenic amines have been found in plants that have morphogenetic and stress-physiological significance. 2-Aminoethanol (2-AE), as used in this paper, is a naturally occurring compound in plant cells. It plays an important role in embryogenesis, organ development and the formation of intracellular membranes. It is well known that plant exposure to stress (e.g., drought, salt, heavy metals, etc.) results in disintegration of biological membranes and the decomposition of their phospholipids, and in the liberation of 2-aminoethanol and choline. The elevated level of amines due to the exposure to stress induces an alarm reaction that activates cellular resistance and tolerance mechanisms [17,18]. It has also been suggested that external 2-aminoethanol might function as a signal for initiating stress tolerance and may serve as a membrane stabilizer, too. One hundred fifteen field trails and 140 scale experiments on farms at 17 different sites in East-Germany with different climate and soil conditions have been carried out to investigate the influence of 2-aminoethanol and its metabolite, choline, on the yield and quality of barley, wheat, rye and potato. Under these field conditions and treatments, yield accessions of 5% (under low stress conditions) were increased to 20% (under severe stress conditions) [2]. Moreover, for several years, mechanism and reproducability of these effects have been studied in pot experiments under treatments with abiotic stressors [3,19–21]. Higher plant vitality was the main reason for the yield-improving effect of native amino alcohol application in unfavourable environments [2,19]. Moreover, the stronger physiological activity in tillers increased the transport of assimilates and N compounds to the root region [20]. Root growth has therefore also been stimulated by 2-aminoethanol or choline [22].

The herbicide paraquat (methyl viologen, 1,1′-dimethyl-4,4′-bipyrydinium dichloride) exerts its phytotoxic effects by catalyzing the transfer of electrons from photosystem I to molecular oxygen resulting in accumulations of superoxide radicals in chloroplasts that cause lipid peroxidation and membrane destruction. Paraquat can readily penetrate into leaf tissue through the cuticula. Shortly after spraying, photosynthetic activity decreases, membranes rupture, and the treated parts of the plant wilt due to the loss of turgor within hours [23,24]. In the final stage, necrotic lesions appear on the leaf surface and the plant dies.

In the present paper, barley plants were treated with 2-aminoethanol with the goal to increase their resistance to drought stress via the stimulation of SOD production. Furthermore, the protective effect of 2-aminoethanol to the oxidative damage of chloroplast membranes of paraquat treated barley plants was to be shown by electron microscopy.

## 2. Materials and methods

### 2.1. Plant material and growth conditions

Barley (*Hordeum vulgare* L. cv. Alexis) plants (10 plants each) were grown in pots with 2.5 kg quartz sand (particle size 0.1–0.63 mm, water capacity 18%). The following nutrients were added (amounts per pot): 1.0 g N as $NH_4NO_3$, 0.5 g P as $KH_2PO_4$, 1.0 g K as $K_2SO_4$, 0.6 g Mg as $MgCl_2$ and 3.0 g Ca as $CaCO_3$-powder, 27.2 mg Fe as $FeCl_3$ and 27.2 mg Fe as Fe-EDTA, 13.6 mg Mn, 6.8 mg Cu, 3.4 mg Zn, 1.36 mg B and 1.36 mg Mo. The experiment was carried out in a growth chamber with a light/dark regime of (16 h/22 °C and 8 h/16 °C). Light intensity was 100 μmol photon $m^{-2}$ $s^{-1}$.

#### 2.1.1. Drought as stressor

The amino alcohol 2-aminoethanol (0.5 mg 2-AE/plant) was applied 2 days before the stress period (DC 31). To evoke drought stress plants were cultivated under a drought stress regime (one period of 7 days at 25% of the water capacity of the sand) which started after tillering and was finished after the drought stress period. Well-watered plants (60% of the sand water capacity) were used as control. Four Mitscherlich pots with 10 plants each, were used per treatment.

#### 2.1.2. Paraquat as stressor

At the 3-leaf stage of the 2-week-old plants half of the pot cultures were spayed with aqueous 2-aminoethanol (Merck) solution (0.5 mg 2-AE/plant). Two days after 2-aminoethanol pre-treatment, all the potted plants were sprayed with solutions of 0, 0.1, 1.0 and 5.0 mM paraquat (Sigma, St. Louis, MO, USA) in sublethal concentrations and exposed to continuous light (100 μE $m^{-2}$ $s^{-1}$). The sublethal doses of paraquat had been determined in previous experiments. Eight hours after paraquat treatment, plants treated with paraquat only or with paraquat/2-aminoethanol were harvested for electron microscopy (TEM). Four replicates were used per treatment. Two days after paraquat application, barley plants were harvested.

#### 2.1.3. Harvest procedure

After harvesting, shoots and roots were separated. The plant material was frozen in liquid nitrogen and lyophilized for the determination of the dry weight. The dry material was ground in a stainless steel mill (IKA, A11 basic). The powder was then stored at −70 °C until analysis.

### 2.2. Preparation of the leaf material for electron microscopy

For ultrastructural examinations of chloroplasts, samples were taken from the middle part of the lower three to four leaves of barley plants. The segments were cut into small pieces (1–2 mm in length) and fixed in 4% (v/v) glutaraldehyde dissolved in 0.2 M potassium phosphate

buffer, pH 7.4. The samples were dehydrated in graded ethanol solutions and embedded in a low viscosity epoxy resin mixture [25]. The blocks were sectioned with a glass knife using a Reichert ultramicrotome. The sections were all cut to the same nominal thickness (70 nm). The grids were sequentially stained with uranyl acetate followed by lead citrate and examined in a Philips CM10 transmission electron microscope.

### 2.3. Determination of chlorophyll

Chlorophyll was extracted by homogenizing 100 mg powdered leaf tissue in 10 ml acetone solution (acetone, water and $NH_3$-solution [25%]; 80:15:5; v/v). After centrifugation at 4000 × *g* for 10 min, chlorophyll content in the supernatant was analysed spectrophotometrically at 480, 645, 647, 652, 663, 664 and 750 nm, as described by Schopfer [26].

### 2.4. Preparation of extracts for enzyme assays and protein determination

The lyophilized and ground shoot samples (500 mg) were homogenized on ice with an Ultraturrax (Jahnke & Kunkel, Stauffen) for 2 min in 3 ml of homogenizing solution containing 100 mM potassium phosphate buffer, 2% (w/v) polyvinylpyrrolidone and 2 mM dithioerythritol (pH 7.2) and extracted for 2 h at 4 °C under gently stirring. The homogenate was filtered through one layer of Miracloth (Calbiochem), centrifuged at 10,000 × *g*, 4 °C for 20 min. The supernatant was dialyzed against 4 l of 5 mM potassium phosphate buffer at 4 °C for 8 h (several changes of the buffer). The dialyzed extracts were cleared by centrifugation (10,000 × *g*, 10 min) and used for further analyses.

The protein concentration was determined according to the method of Lowry [27] using human serum albumin as a standard. Absorbance was determined using a UV–vis spectrophotometer.

### 2.5. Superoxide dismutase

The non-denaturing PAGE of the crude protein extracts was carried out on 12% polyacrylamide gels (120 mm × 110 mm × 1 mm) using a Biometra electrophoresis system (Biometra, Göttingen) according to the manufacturers specifications. Protein solutions (30 μg) were loaded on to the gel and separated at 4 °C, 120 V and 30 mA for 3 h.

Immediately after electrophoresis, the activity of SOD isoenzymes was visualized using the NBT staining procedure [3,28]. The gel was incubated in 2.5 mM nitroblue tetrazolium chloride solution (NBT) at 25 °C for 20 min and then soaked in 50 mM potassium phosphate buffer at pH 7.5 containing 0.028 mM riboflavin and 0.3% (v/v) tetramethylethylenediamine (TEMED) for 30 min in the dark. The gel was then illuminated to induce the photoreactive staining process caused by the SOD activity. The stained gels were scanned and analysed with a raytest system (Raytest Isotopenmessgeraete, Straubenhardt/Germany; scanning software AIDA 2.0).

SOD isoenzymes were differentiated by performing the activity stains gel previously incubated at 25 °C for 30 min in 50 mM potassium phosphate buffer at pH 7.5, containing 2 mM KCN or 5 mM $H_2O_2$. Cu/Zn–SODs are inhibited by KCN and $H_2O_2$; Fe–SODs are inactivated by $H_2O_2$ but resistant to KCN and Mn–SODs are resistant to both inhibitors [29].

### 2.6. Statistical analysis

The experiments were repeated several times, at least three times under the same conditions with essentially the same results. All measurements were subjected to analyses of variance (ANOVA) to determine the least significant difference. The significance in this paper refers to statistical significance at the $p < 0.05$ level.

## 3. Results

### 3.1. Stabilization of drought stressed plants in growth, protein and chlorophyll content by 2-aminoethanol

Biomass, protein, and chlorophyll content of barley plants was affected by drought stress and by pre-treatment with the stress tolerance activating amino alcohol (2-aminoethanol, 2-AE). The biomass produced under drought stress conditions, fell significantly in shoots and roots compared to well-watered plants (Table 1). A pre-treatment of stressed plants with 2-aminoethanol, however, resulted in a biomass increase of about 10% in shoots and 35% in roots relative to the control. With respect to chlorophyll and

Table 1
Protection of growth, chlorophyll and protein content from drought stress by exogenous 2-aminoethanol

| Treatment | Shoot biomass (mg dry wt./plant) | Root biomass (mg dry wt./plant) | Chlorophyll (shoot) (μg $g^{-1}$ dry wt.) | Protein (shoot) (mg $g^{-1}$ dry wt.) |
|---|---|---|---|---|
| Well-watered (no stress) | $92.1 \pm 3.7^a$ (100) | $33.4 \pm 4.6^a$ (100) | $2.95 \pm 0.21^a$ (100) | $108.0 \pm 7.5^a$ (100) |
| Well-watered + 2-AE | $99.2 \pm 2.9^b$ (108) | $49.4 \pm 11.1^b$ (148) | $2.81 \pm 0.15^a$ (95) | $116.5 \pm 5.2^b$ (107) |
| Drought stress | $77.0 \pm 1.3^c$ (84) | $20.7 \pm 3.3^c$ (62) | $2.46 \pm 0.21^b$ (83) | $92.6 \pm 6.5^c$ (86) |
| Drought stress + 2-AE | $86.6 \pm 2.6^a$ (94) | $32.3 \pm 2.1^a$ (97) | $2.41 \pm 0.12^b$ (82) | $111.3 \pm 6.5^a$ (103) |

The data are the mean value ± S.D. of three individual experiments. Different letters indicate significant differences ($p < 0.05$). Values in parenthesis are given in percent.

protein content, a stress tolerance inducing effect of 2-aminoethanol was more recognizable. In stressed plants pre-treated with 2-aminoethanol, the protein content was similar to that of the well-watered, whereas the drought stressed plants without 2-aminoethanol treatment had 14% less protein than the well-watered untreated plants.

### 3.2. Stimulation of the major Cu/Zn–SOD in barley by 2-aminoethanol

To test the hypothesis that 2-aminoethanol antioxidant capacity may be mediated through their scavenging properties, SOD isoenzymes were analysed as indicators of oxidative stress and/or as a protective effect of 2-aminoethanol against reactive oxygen species.

The protein extracts from barley shoots subjected to non-denaturating PAGE showed two distinct bands of SOD isoenzymes (Fig. 1A and B), which were identified as two Cu/Zn–SODs on the basis of their sensitivity to KCN and $H_2O_2$. Cu/Zn–SODs were designated as Cu/Zn–SOD I and Cu/Zn–SOD II, according to their increasing mobility in polyacrylamide gel. Cu/Zn–SOD II was the major isoenzyme in shoots. The major Cu/Zn–SOD in shoots occurs in chloroplasts [3,11]. Palatnik [30] also observed two Cu/Zn–SOD isoenzymes in *Hordeum vulgare* L. cv. Maris Mink. We could not detect a new isoenzyme in plants cultivated under water deficit (Fig. 1), but the quantitative ratio between the isoenzymes had been altered. Drought stress induced a strong increase in the activity of Cu/Zn–SOD II (about twofold compared to well-watered plants) whereas, Cu/Zn–SOD I was not affected by drought. These alterations correlate with the enhanced superoxide radical production. The activity of Cu/Zn–SOD II, the major isoenzyme in barley shoots, was strongly enhanced in well-watered plants by pre-treatment with 2-aminoethanol. We detected an increase of about 100% compared to untreated water sufficient plants. This 2-aminothanol-induced stimulation of SOD was further enhanced by water deficit. We observed a dramatic increase in activities of both SOD isoenzymes (SOD I about 50% and SOD II about 275%).

### 3.3. Stabilization of paraquat stressed plants in growth, protein- and chlorophyll content by 2-aminoethanol

The vigour of 2-aminoethanol pre-treated and not pre-treated barley plants one and 2 days after spraying a 1.0 mM paraquat solution is shown in Fig. 2. Aminoethanol pre-treated plants showed much less shoot and leaf damages.

Paraquat treatments higher than 0.1 mM caused decreases in growth and total chlorophyll concentrations (Figs. 3 and 4). The application of 2-aminoethanol to barley plants reduced the losses in fresh weight and total chlorophyll concentrations relative to treated plants. This applied to all plants pre-treated with the amino alcohol, irrespective of the various paraquat co-treatments.

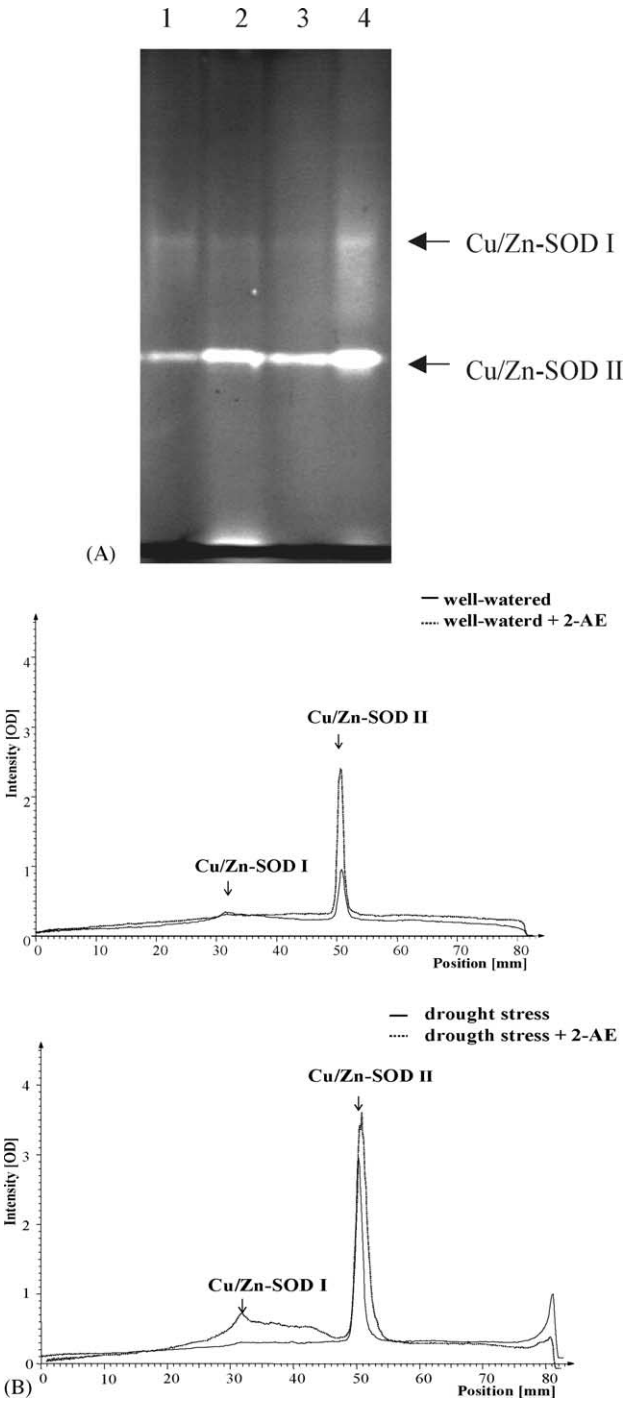


Fig. 1. Effects of drought and pre-treatment with 2-aminoethanol on SOD activities in shoots of *Hordeum vulgare* L. cv. Alexis. (A) Protein extracts resolved on non-denaturating polyacrylamide gels. Lane 1, well-watered; Lane 2, well-watered + 2-AE; Lane 3, drought stress; Lane 4, drought stress + 2-AE. (B) Quantification of SOD isoenzymes by densitometric determination.

### 3.4. Protection of the chloroplast ultrastructure against paraquat-induced oxidative stress by 2-aminoethanol

In our electron microscopy studies, the chloroplasts of the palisade layer were investigated. The paraquat-only treat-

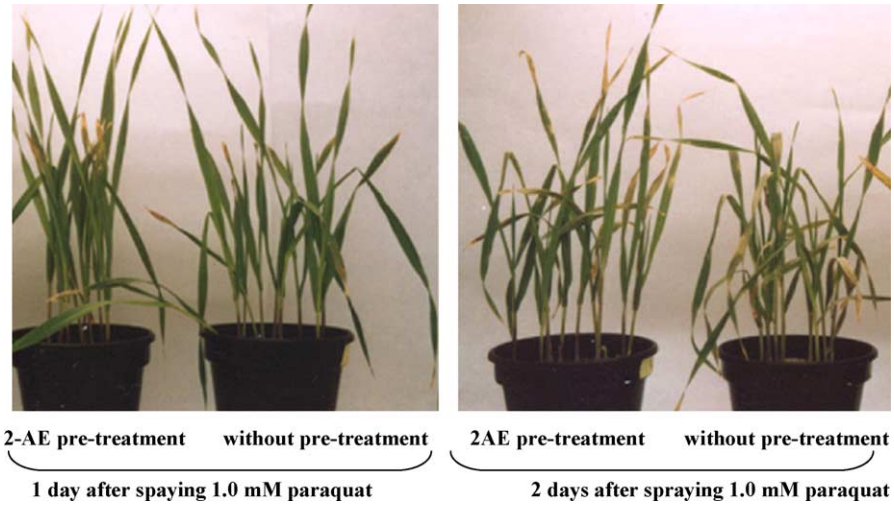


Fig. 2. Damage to 2-aminoethanol pre-treated and not pre-treated barley plants 1 and 2 days after spraying 1.0 mM paraquat.

ment led to a remarkable loss of turgor which was detectable at 0.5 mM concentration of paraquat (Fig. 5). All paraquat treated samples exhibited membrane deterioration in a time and concentration dependent manner. Also, the internal membrane structure was less organized, the parallel pattern of the lamellae disorganized and the orientation of the grana changed considerably (Fig. 5B and C). The highest paraquat doses induced complete disorganization and membrane disruption. Even the envelope of the chloroplasts was deteriorated. Finally, the lipid content of the membranes accumulated in dark droplets (Fig. 5D). At this stage, the effect of the herbicide could also be seen macroscopically as extensive wilting, chlorosis and drying of the leaves.

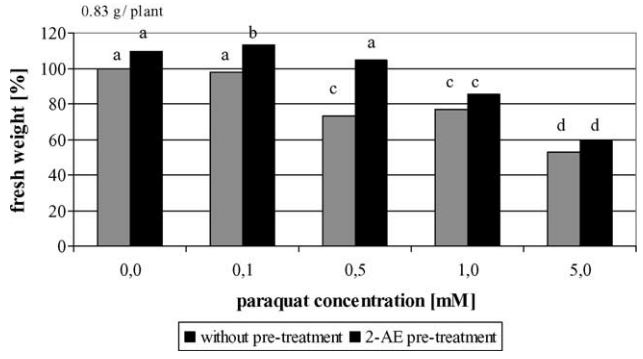


Fig. 3. Protective effects of 2-aminoethanol on the growth of photooxidatively stressed barley plants. The fresh weight of shoots was determined after a stress period of 2 days. Different letters indicate significant differences ($p < 0.05$).

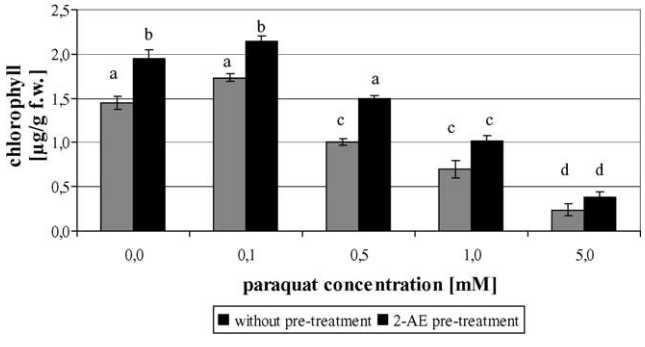


Fig. 4. Protective effects of 2-aminoethanol to chlorophyll in photooxidatively stressed barley plants. The chlorophyll content was determined in leaves treated with increasing paraquat concentrations (2 days after paraquat treatment). The Data are the mean value ± S.D. of three individual experiments. Different letters indicate significant differences ($p < 0.05$).

The paraquat/2-aminoethanol pre-treated plants, as compared to the paraquat-only treated ones, revealed a membrane-stabilizing effect associated with the presence of exogenous 2-aminoethanol (Fig. 5E and F). However, this depended on the actual concentration of paraquat and the duration of the treatment. At low paraquat doses pre-treatment with 2-aminoethanol prevented damage to the chloroplast. This was not so the case, however, with a 1.0 mM concentration of paraquat, although the extent of deterioration was less than that found in the paraquat-only treated samples. There was no protective action of 2-aminoethanol at 5.0 mM concentration of paraquat, the membrane structure of the chloroplasts became disrupted completely (not shown).

## 4. Discussion

Increasing evidence indicates that stress results in the oxidative deterioration of biological macromolecules, and therefore, at least in part, in the oxidative tissue destruction [31]. Stress conditions can be detected with physiological methods which measure antioxidative enzymes and membrane destruction [32,33].

According to the obtained results we assume that 2-aminoethanol acts as an exogenous stressor or ‘stress-tolerance-activating substance’ to plants, and induces an adapting mechanism similar to stress [3,8,34]. Increases in

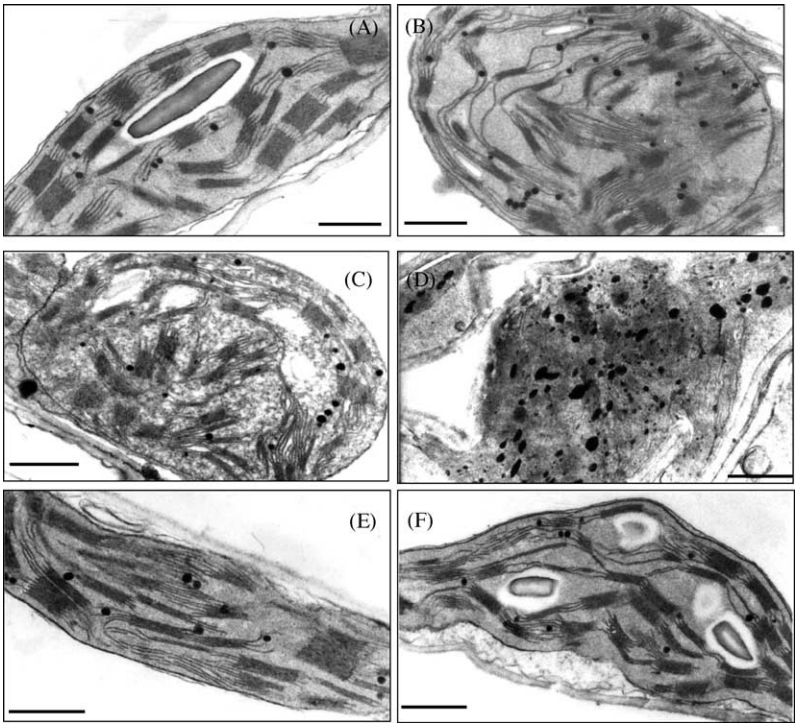


Fig. 5. Protective effect of a 2-aminoethanol pre-treatment to paraquat induced oxidative (membrane) damage of barley chloroplasts. Electron micrographs taken 8 h after paraquat application. (A) control (no paraquat and no 2-AE pre-treatment). (B) Treatment with 0.5 mM paraquat. (C) Treatment with 1.0 mM paraquat. (D) Treatment with 5.0 mM paraquat. (E) Pre-treatment with 2-AE and treatment with 0.5 mM paraquat. (F) Pre-treatment with 2-AE and treatment with 1.0 mM paraquat (scale bars 1 μM).

biomass, protein, and chlorophyll content of drought stressed plants in response to 2-aminoethanol may be related to the induction of the antioxidant responses that protect plants against damage. An important radical generating process is photooxidation. Water deficit induces inhibition of photosynthesis and leads to increased production of ROS in the chloroplasts [4,35,36]. The higher concentration of oxygen radicals during drought orginates from the diminution in $CO_2$ fixation, and results in higher leakage of electrons to molecular oxygen. Consequently, the thylakoid membrane electron leakage to molecular oxygen increased in plants after water deficit [16]. Changes in antioxidant molecules and enzymes are correlated with drought [2,3,37].

The ability of 2-aminoethanol to increase plant growth, and reduce the adverse effect of drought stress, may result from a lowering oxidative level. Similar to our results, Chowdhury and Choudhuri [38] postulated that the extent of drought-induced damage to membranes is negatively correlated with the capacity of drought-tolerant and sensitive plants to increase the total SOD- and CAT-activity. A lower level of destroyed membranes was also correlated with the accumulation of lower concentrations of ROS in the tolerant plant species.

Stress leads to an increased production of oxygen radicals which are reduced by a higher activity of Cu/Zn–SOD II (Fig. 1A and B). The strong enhancement in the activity of Cu/Zn–SOD II isoenzyme in response to 2-aminoethanol application in well-watered barley plants is a protective process against stress. Pre-treatment of plants with the native amino alcohol acts as a moderate exogenous stressor. The short-time response to this ‘eu-stressor’, its influence on growth, chlorophyll, and protein formation (e.g., SOD isoenzyme formation is comparable with the primary response to stress. The data presented here indicate that 2-aminoethanol pre-treated plants tolerate higher levels of stressors such as drought or paraquat treatment. These results are consistent with the fact that amino alcohol pre-treated plants are better adapted than untreated ones to respond to ROS, and suggest that this difference could be related to a higher superoxide detoxifying capacity. Plants with high levels of antioxidants, either constitutive or induced by stress tolerance activating substances, are more tolerant to oxidative damages. Moreover, increasing production of ascorbic acid in tomato plants treated with 2-aminoethanol [2] is congruent with the hypothesis that this amino alcohol promotes the decrease of the oxidative potential in plants.

Fig. 6 offers a simplified description of the biosynthetic pathway of 2-aminoethanol, choline, and glycine betaine in plants. The concentration of 2-aminoethanol, found in green spring barley plants, amounts to about 200 mg $kg^{-1}$ dry matter. The application of the amino alcohol increases the content to about 450 mg $kg^{-1}$ dry matter for a short time. It

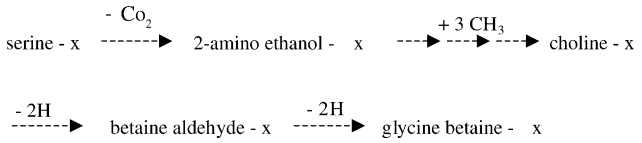


Fig. 6. Scheme of the biosynthetic pathway of 2-amino ethanol, choline, and glycine betaine in barley plants (according to [46]). X marks derivates of serine, 2-amino ethanol, and choline which include both membrane-bound phosphatidyl compounds and water-soluble phosphoryl bases.

decreases to a value of the control plants also without being influenced by precipitation with a week [39]. After the pre-treatment with 2-aminoethanol (labelled with $^{14}C$) the radioactivity was found in a similar specific activity in all parts of the plant shoots. Two weeks after the treatments, the radioactivity was detected in the original compounds, in phospholipids (membranes) and in the final metabolite, glycine betaine, in equal proportions [20]. We suppose that 2-aminoethanol applications result in higher endogenous concentrations of the amino alcohol (2-aminoethanol/choline) and lead to the stabilization of membranes.

In the next step, we used paraquat as tool for oxidative stress. Membrane destabilization is generally attributed to lipid peroxidation, due to a strong production of toxic oxygen species [40]. Our electron microscopic studies indicated that paraquat induced membrane damages in chloroplasts of barley plants (Fig. 5A–D). The pre-treatment with 2-aminoethanol reduced the oxidative stress-promoted membrane deterioration (Fig. 5E and F). The decrease in paraquat-induced damages of thylakoid membranes is a phenomenon caused by pre-treatments with amino alcohol, and is resulting in a stabilization of oxidatively stressed plants.

Transgenic plants that contain single transgenes for the expression of antioxidant enzymes (e.g., SOD, ascorbate peroxidase) in plant cells have been developed and studied. However, manipulation of a single antioxidant enzyme has provided only little improvement to stress tolerance. The level of SOD production against oxidative stress has been varied in transgenic plants [41]. For example, McKersie et al. [42,43] found improvements to oxidative stress tolerance, whereas Payton et al. [44] found no improvements. Kwon et al. [45] postulated an enhanced tolerance of transgenic tobacco plants which expressed both SOD and ascorbate peroxidase in chloroplasts against oxidative stress induced by paraquat. The reason for these differences is the complexity of the ROS detoxification, because changing one antioxidant enzyme may not change the capacity of the antioxidant pathway. We propose that 2-aminoethanol pre-treatment of plants leads to a higher capacity of the ROS detoxification. The strong increase in the activity of Cu/Zn–SOD II and the fortification of membranes in response to an amino alcohol pre-treatment indicate that this combination is a successful strategy in oxidative stress tolerance.

It can be concluded from our investigations that the protective action of 2-aminoethanol is efficient at 'lower strength of oxidative stress'. At higher concentrations of ROS, when the membrane deterioration is more pronounced, the protective action of 2-aminoethanol cannot be detected anatomically. In this stage 2-aminoethanol is unable to counteract the membrane-damaging action of oxygen species and to stabilize the membrane structure of the chloroplasts. The influence of the application time (stage of ontogenesis) of 2-aminoethanol on the formation of antioxidant systems and adaptation mechanisms against various abiotic and biotic stressors are subject of further investigations. In addition, more investigations on the characterization of different transcription factors in 2-aminoethaol pre-treated plants will be done.

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