THE EFFECT OF SALINITY STRESS ON THE ENZYMATIC ELEMENTS OF THE ANTIOXIDANT DEFENSE SYSTEM

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Summery of Ph.D. thesis

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2002

INTRODUCTION

Nowadays a substantial proportion of plant physiology research is made up of stress research. This is due partly to the changes occurring in the biosphere:

- change of global climate,
- increase in UV-B radiation because of the depletion of stratospheric ozone,
- presence of saline soils,
- growing coverage of arid (desert) areas;

and partly to the fact that the response mechanisms of the plants, i.e. how they adapt to various stress conditions, have not yet been fully clarified. Economic factors are also responsible for the intensive spread of such investigations, since unfavourable conditions cause great qualitative and quantitative losses in agricultural crops, leading to food supply problems.

It can be established, following the occurred changes in the environment, that some of the processes are going on unchangeable. We can't do anything else in these cases than try to adapt to the altered circumstances. This is valid for the plants also and the plant breeders have to keep it in mind. Considering all these facts the farther aim of the experiments can be the production of new varieties with higher resistance using the results of the studies with the antioxidant defense mechanisms. This is the reason why plants of agricultural importance, wheat, maize and sunflower were used in these studies.

All stress effects have an oxidative component, so plants are exposed to oxidative stress through the external factors influencing them. This is manifested in an increase in the concentration of free radicals and oxygen-containing reactive molecules. A complex antioxidative system consisting of enzymatic and non-enzymatic elements has developed to provide protection against these effects. The effect of changes in the external environment can be

efficiently measured through the activity of the enzymatic components of this system and these measurements can be used to determine the sensitivity of the plant to the given stress factor, and the effect of the stress factor on the plant.

OBJECTIVES

The fact that the concentration of the free radicals and active oxigen species increases in plant organisms under different environmental conditions was well known. An effective antioxidant defense system, where the activity of the enzymatic elements changes under external stressfactors, have been developed against the damaging effect of these molecules. The sensitivity of the plant to the stressfactor and its effect on the plant can be discovered by following these activity changes. Since the concentration of active oxygen species also rises after saline (NaCl) treatment (Hernández et al., 1993), we studied the effect of salt stress through the enzymatic components of the antioxidant defence system.

• The activity of the glutathione reductase enzyme was examined in order to determine whether a diurnal rhythm could be detected in the enzymatic defence system, and whether this was altered by treatment with various concentrations of NaCl.

• Among the external environmental factors, in addition to salt, the effect of various nitrogen sources (nitrate, ammonium) was also tested through the glutathione reductase, superoxide dismutase, guaiacol peroxidase, catalase and glutathione-S-transferase enzymes in order to determine how these factors affected the activity of the defence system and whether they strengthened or weakened each other's effects. To this end, changes in enzyme activity and the

presence of isoenzymes were investigated in plants grown under different conditions.

• Although it is not an antioxidant, the aldehyde oxidase enzyme has also been investigated in recent years in research on stress effects, since it has been shown to have a number of functions linked to stress responses, and had been implicated in the synthesis of two important phytohormones: indole acetic acid and abscisic acid. Since more and more information is being published on this enzyme, examinations were made on its localisation within the cell in the hope of isolating it in the mitochondria or peroxisomes of maize roots.

MATERIALS AND METHODS

Plant material and growth conditions

In the course of the experiments plants were raised in liquid culture, which facilitated studies on the effect of various treatments (50, 100, 150 mM NaCl) and nitrogen sources (NO_3, NH_4NO_3, NH_4). Plants of agricultural importance, wheat, maize and sunflower were used in the studies. We used both of the roots and leaves during the analyses.

Determination of enzyme activities

Catalase (EC 1.11.1.6) activity was assayed by following the decomposition of H_2O_2 at 240 nm (1 EU = 1 μmol H_2O_2 decomposed in 1 min) (Upadhyaya et al., 1985).

Guaiacol peroxidase (EC 1.11.1.7) activity was determined by monitoring the increase in absorbance at 470 nm due to guaiacol oxidation (1 EU = 1 μmol guaiacol oxidized in 1 min) (Upadhyaya et al., 1985).

Glutathione reductase (EC 1.6.4.2) was assayed according to the method of Smith *et al*. (1988) by following the increase in absorbance at 412 nm due to DTNB reduction by GSH generated from GSSG. One unit equalled the reduction of 1 μmol oxidized glutathione per min.

Superoxide dismutase (EC 1.15.1.1) activity was measured by the photochemical method as described by Beauchamp and Fridovich (1971). One

unit of SOD activity was defined as the amount of enzyme required to cause 50% inhibition of the rate of NBT reduction at 560 nm in the presence of riboflavin in the light. Blanks were kept in the dark and the others were illuminated for 15 min.

Glutathione-S-transferase (EC 2.5.1.18) activity was measured by following the changes in the absorbance at 340 nm. EU = the amount of enzyme that catalyses the formation of 1 μmol of S-2,4-dinitrophenylglutathione min^{-1} (Mannervik and Guthenberg, 1981).

Polyacrilamide gelelectrophoreses (PAGE)

The separation of the samples was carried out with non denaturing (native) PAGE. From the different samples the same amount of protein (45 μg) was loaded on the gels. We worked with discontinous buffer system. The gels were run with 20 mA/gel for 5 hours in the case of GR, SOD, Cat and AO and for 16 hours in the case of GPx. The temperature was constant 5 ºC.

Preparation of cell organelles

The separation of the different cell organelles was performed by 25-57 % linear sacharose gradient columns from maize roots. To identify the peroxisomes catalase activity was measured while for the separation of the mitochondria fractions cytochrom oxidase were used. The determination of AO activity was carried out in gels.

Mathematical analysis

For calculation of different periodicities, Fourier analysis was carried out by a radix-2 fast Fourier transform algorithm implemented in the MATLAB 4.0 programming environment.

Significant differences were determined using Student's *t*-test. Each treatment was analysed with 3 - 6 replicates depending on the experiment. Statistical analysis was performed with the help of the STATISTICA 5.0 program.

RESULTS AND CONCLUSIONS

The following results were obtained in the experiments:

- The activity of the glutathione reductase enzyme changed according to a diurnal rhythm, with a periodicity 15 and 20 hours. Beside the diurnal rhythm we were able to detect an ultradian rhythm with a shorter period.

- Salt treatment (NaCl) led to an increase in the activity of the glutathione reductase enzyme, but no linear correlation was found between the salt concentrations (50, 100, 150 mM NaCl) and the change in enzyme activity. The salt treatment did not effect the period time of the diurnal rhythm, but there was a change in the amplitude. The ultradian rhythm was sensitive to salt treatment.

- The enzymatic components of the antioxidative defence system were influenced not only by salt treatment, but also by the type of nitrogen source. Changes were observed in the activity of the glutathione reductase, superoxide dismutase, guaiacol peroxidase, catalase and glutathione-S-transferase enzymes and in the isoenzyme compositions of glutathione reductase, superoxide dismutase, guaiacol peroxidase and catalase. It was concluded from the results that the ammonium ion may serve as a stress signal responsible for activation of enzymes responsible for a number of early stress adaptation processes.

During the analyses of the distinct root zones, different effects of the diverse treatments on the antioxidant enzymes was observed in the tip than in the whole root. The different sensitivity of the metabolic processes can give a possible explanation for the phenomenon.

- Examinations on the localisation of the aldehyde oxidase enzyme within the cell showed it to be located in the cytosol.

The results of our study amplify the knowledge about the antioxidant defence system and conduce to a better understanding of the working mechanisms of the plant defence systems. The farther goal of this kind of researches is to provide additional information that is useful to develop breeding strategies for higher stress resistance in plants.

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