VERSITA

INTERNATIONAL
Agrophysics
www.international-agrophysics.org

Int. Agrophys., 2012, 26, 347-353
doi: 10.2478/v10247-012-0049-z

Stress responses of spring rape plants to soil flooding

T. Balakhnina[1]*, *R. Bennicelli*[2], *Z. Stępniewska*[2], *W. Stępniewski*[3], *A. Borkowska*[4], *and I. Fomina*[1,5]

[1]Institute of Basic Biological Problems, Russian Academy of Sciences, 142290 Pushchino, Moscow Region, Russia
[2]Environmental Protection Institute, Catholic University of Lublin, Kraśnicka 102, 20-551 Lublin, Poland
[3]Chair of Land Surface Protection Engineering, Lublin University of Technology, Nadbystrzycka 40B, 20-618 Lublin, Poland
[4]Institute of Agrophysics, Polish Academy of Sciences, P.O. Box 201, 20-290 Lublin, Poland
[5]Biosphere Systems International Foundation, Tucson, Arizona 85755, USA

Received September 26, 2011; accepted October 22, 2011

A b s t r a c t. Stress responses of spring rape to soil hypoxia were investigated during 8-days flooding. Soil air-filled porosity decreased from 25-30% to 0%, oxygen diffusion rate – from 2.6-3.5 to 0.34 μmol O_2 m^{-2} s^{-1}, and redox potential – from 460 to 150 mV within few hours. Alcohol dehydrogenase activity in roots increased up to 7-fold after one day of flooding and then decreased to 170% of control. Superoxide dismutase activity in roots increased by 27% during first 3 days and then dropped to 60% of control; in the leaves superoxide dismutase activity increased in average by 44%. Ascorbate peroxidase activity in leaves increased by 37% during first 3 days and then decreased to control value. Glutathione reductase activity increased by 45% in roots of flooded plants but did not change in leaves. Proline concentration in leaves increased up to 4-fold on the 3d day of flooding and then decreased to control value. Thus soil flooding induces increase of alcohol dehydrogenase activity and subsequent increase of superoxide dismutase and glutathione reductase activities in roots while the leaves display a few days increase of free proline concentration and ascorbate peroxidase activity, and a long-term increase of superoxide dismutase activity.

K e y w o r d s: spring rape, flooding, oxygen diffusion rate, redox potential, enzyme activity

INTRODUCTION

Soil flooding changes subsurface plant gas exchange. The low diffusion rate of oxygen in water-filled pore space of the soil results in limitation of oxygen availability for plant roots and soil microorganisms and leads to a switch of aerobic metabolism of plant roots into less efficient anaerobic fermentation, causing a fast depletion of carbohydrate reserves. Hypoxic conditions in soil cause also a decrease of Eh (Balakhnina *et al.,* 2009, 2010; Gliński and Stępniewski, 1985; Bailey-Serres and Voesenek, 2008). This, in turn, stimulates evolution of carbon dioxide, molecular hydrogen, hydrogen sulphide, ethylene, and methane and accumulation in the soil of reduced phytotoxins (Fe^{2+}, Mn^{2+}, sulfide and, at high concentrations, ammonium) which can have a negative impact on plants, causing, among others, growth retardation, reduction in leaf size, wilting of shoots and necrosis (Lucassen *et al.*, 2002; Smith and Restal, 2006).

Plant adaptation to soil hypoxia includes series of interconnected reactions directed to survival during the periods of hypoxic and anoxic conditions and to the homeostasis maintenance. Anatomical and morphological changes help to provide oxygen to the plant tissues (Colmer, 2003; Pederson *et al*., 2009; Vartapetian *et al.*, 2003).

In connection with the specificity of the effect of hypoxia on plants, of special interest are compensatory changes connected with transformations of respiration pathways. Under oxygen deficiency most plants exhibit intensification of glycolysis accompanied with accumulation of lactate and ethanol (Rocha *et al.*, 2010). Under prolonged and deep hypoxic stress the formation of ethanol prevails. Production and utilization of this phytotoxic product is connected with functioning of alcohol dehydrogenase (ADH; EC 1.1.1.1.). Activity of this enzyme under hypoxia or anoxia increases significantly through induction of the alcohol dehydrogenase gene expression. The ADH gene family consists of one to four members, depending on the plant species. The developmental expression and tissue-specific responses of each gene member to hypoxic stress was demonstrated on various species (Garnczanska 2002; Preiszner *et al.*, 2001; Wignarajah *et al.*, 2006).

*Corresponding author's e-mail: tbalakhnina@rambler.ru

Formation of reactive oxygen species (ROS) appears to be an unspecific plant response to different stress factors, including hypoxia. In particular ROS may be formed in electron transport chains, because of $NADP^+$ limitation. Due to this oxygen becomes an alternative electron acceptor. Induction of such ROS as superoxide radical (O_2^-) and hydrogen peroxide (H_2O_2) initiates peroxidation of lipids, proteins, pigments, and other cell compounds (Arbona *et al*., 2008, Balakhnina *et al*., 2009, 2010) and leads to serious damage of cells and of the entire organism. Plants possess an evolutionary formed defensive system against oxidative destruction. It consists of low molecular antioxidants (ascorbic acid, reduced glutathione, tocopherols, and others) and antioxidant enzymes decomposing ROS). Among antioxidant enzymes, superoxide dismutase (SOD; EC 1.15.1.1.) has been identified as an essential component in the organism defense mechanism. Besides SOD, superoxide can be scavenged directly by ascorbate or glutathione. Neutralization of formed H_2O_2 occurs in ascorbate-glutathione cycle with participation of ascorbate peroxidase (AsP; EC 1.1.1.1.), glutathione reductase (GR; EC 1.6.4.2), and other enzymes (Asada, 2006; Blokhina *et al*., 2002).

The pertinent literature presents data on correlation of the degree of resistance to extreme temperatures, salinity, drought and other stress factors with the free proline concentration in the plant tissues. According to different authors the proline accumulation in plant should be considered as one of the links of the common chain of biochemical adaptation mechanisms functioning in stressed plants (Radyukina *et al*., 2008; Shevyakova *et al*., 2009). Some reports showed an increase in proline concentration in plants under soil flooding (Yordanova and Popova, 2007). It was found that, under the waterlogging conditions, the number of new leaves, root length, plant height and plant biomass and content of soluble protein in roots of *Malus* species declined, while the content of malondialdehyde (MDA) and proline, the generation rate of superoxide radical, relative membrane permeability, and the activities of SOD and peroxidase in roots significantly increased (Bai *et al.,*2008).

The aim of this paper was to test whether spring rape can be cultivated in soil with elevated water content and subjected to the short term flooding.

MATERIALS AND METHODS

The spring rape is widely adapted, and performs well in a range of soil conditions, providing that moisture and fertility levels are adequate (Biology Document BIO1994-09). Seedlings of spring rape (*Brassica napus* var. *Oleifera* f. *annua* cv. Lisonne) were grown in a field conditions in Hajdów (Lublin, Poland) experimental field (Eutric Histosol, pH 7.2 in 0.01 mol KCl; C_{org} – 32.6%) during the first four weeks from sowing. After that the seedlings of the same size were carefully transplanted into the pots. To eliminate variability and the effect of transplantation stress we used 3 pots as replications and 3 plants per pot (together 9 plants per each measurement day and treatment; 12 pots *ie* 36 seedlings per treatment) and seven days acclimation period to the growth chamber conditions. Each pot (25, 35, and 25 cm in height) was filled with the same soil to the height of 15 cm (soil volume in the pot was about 13.13 dm^3). Before experiment soil samples from the field were dried up to stable mass to test water content. The pots (27 in total) were put into a growth chamber, weighed every day and watered up to 40-45%. By this soil moisture tension was maintained at 15-20 kPa and air-filled porosity, Eg, was 25-30%. In the growth chamber the air temperature was 20±2°C during the day and 16±2°C during the night. The light period was 12 h, the light intensity was 950 μmol photons m^{-2} s^{-1}. Relative air humidity was 45±5/70±5% during the day/night. After 7 days of plant acclimation to the experimental conditions in the growth chamber the pots were divided into two treatments: 12 pots were kept under the control conditions, 12 pots were irrigated until full saturation of the soil in each pot and excess of 15-20 mm of water permanently present on the soil surface during entire experiment. Three extra pots were used for initial plant analysis (0 days after treatment). Soil and plant measurements were performed after 1, 3, 5 and 8 days of the differentiation of soil conditions in the pots. Three pots of the flood treatment and three pots of the control treatment were used on each measurement day (after having performed the soil aeration measurements) for plant analysis. Three plants per pot were pooled and used as one replicate.

The soil aeration status *ie* porosity (Eg), oxygen diffusion rate (ODR) and redox potential (Eh) was determined. The ODR values were measured by a device with an automatic adjustment of an effective reduction voltage (Malicki and Walczak, 1983) using seven platinum electrodes (0.5 × 4 mm) placed in the middle of the soil layer, and polarized for 4 min to a voltage of -0.65 V with respect to saturated calomel electrode. The amount of O_2 reduced per electrode surface (μmol m^{-2} s^{-1}) was calculated from the reduction current (Gliński and Stępniewski, 1985). Redox potential was measured using seven similar platinum electrodes (0.5 × 4 mm) placed at the middle part of the pot *vs*. saturated calomel electrode with a portable Orion 404 ion analyzer (Orion, Boston, Ma 02129).

To examine specific plant reaction to soil flooding ADH activity was measured in the roots. Unspecific reactions *ie* intensity of peroxidation process estimated by concentration of thiobarbituric acid reactive substances (TBARs), and plant antioxidant potential evaluated by the levels of SOD and GR activities, were determined in the roots and leaves. AsP activity and proline concentration was measured in the leaves. The analyses were performed from the crude homogenates and enzymes extracts of control and treatment plants. All the results were presented per one gram of root or leaf dry mass. Leaf and root samples were dried at 95°C till

stabilization of the dry mass.The middle part of roots or fully developed last but one leaf without midrib (0.5 g) were homogenized manually in a mortar with 4.5 ml of cooled (2-4°C) 30 mmol K/Na phosphate buffer, pH 7.4, containing 0.1 mM EDTA and 2% PVP. The homogenates were filtered through a nylon cloth. A part of the filtrate defined by us as crude homogenate was used for assessment of the intensity of peroxidation process on the basis of TBARs concentration. The second part of the filtrate was centrifuged at 15 000 g for 20 min. In the supernatant, described as enzymatic extract, the activities of SOD, GR, and AsP were determined. For ADH activity measurements the root homogenate was prepared by addition of 12 ml of 0.1 mol Tris-HCl buffer, pH 8.5, and 2 mmol dithiotreitol to plant material (1 g) and ground in a mortar filled with sand. The homogenate was centrifuged at 10 000 g for 20 min. Enzyme activity was measured in the supernatant.

The TBARs concentration, activity of AsP and activity of GR were assessed according to Balakhnina *et al.*, 2010. The absorbance of TBARs was measured at 532 and 600 nm using Shimadzu UV-VIS 160A (Kyoto, Japan) spectrophotometer. Concentration of TBARs was calculated using coefficient of extinction equal to 1.56 10^{-5} mol cm^{-1}. The activity of AsP was determined by oxidation of ascorbic acid. The measurements were performed at 290 nm and 2.8 $mmol^{-1}$ cm^{-1} coefficient of extinction was used for calculations. The activity of GR was determined by glutathione dependent oxidation of NADPH The measurements were performed at 340 nm, 6.22 $mmol^{-1}cm^{-1}$ coefficient of extinction was used for calculations. ADH activity was determined by ethanol dependent reduction of NAD^+ (Benz *et al.*, 2007). The measurements were performed at 340 nm. The coefficient of extinction equal to 6.22 $mmol^{-1}$ cm^{-1} was used for calculations. Total SOD activity was measured according to Giannopolitis and Ries (1977). One unit of SOD activity was defined as the amount of enzyme that can cause 50% inhibition in the rate of NBT reduction. Proline concentration was determined in leaf homogenates prepared by manual grinding of 0.5 g of the leaf tissues in 10 ml of 0.3% aqueous sulfosalicylic acid. Homogenate was filtered through Whatman # 2 filter paper (Bates *et al.*, 1973). Two ml of the filtrate reacted with 2 ml acid ninhidrin and 2 ml of glacial acetic acid in a test tube for 1 h at 100°C, and the reaction was terminated in an ice bath. The reaction mixture was extracted with 4 ml toluene, mixed vigorously with a test tube stirrer for 15-20 s. The chromophore containing toluene was aspirated from aqueous phase, warmed to room temperature and the absorbance read at 520 nm using toluene for a blank. The proline concentration was determined from a standard curve and calculated.

For statistical analysis, the data were divided into two treatments (control and flooding) and one-way ANOVA for 8 days of experiment time was performed. Levels of significance for differences between mean values ($P<0.05$, $P<0.01$, $P<0.001$) are shown in Table 1.

RESULTS AND DISCUSSION

After soil flooding porosity decreased from 25-30 to 0% and Eh decreased from 430-480 to 150 mV within few hours. Average ODR in soil under control conditions ranged at 2.6-3.5 μmol O_2 m^{-2} s^{-1} and for the flood period declined to 0.34 μmol O_2 m^{-2} s^{-1}.

T a b l e 1. Effects of soil flooding on alcohol dehydrogenase (ADH) activity, thiobarbituric acid reactive substances (TBARs) concentration, superoxide dismutase (SOD), ascorbate peroxidase (AsP) and glutathione reductase (GR) activities and proline concentration of *Brassica napus* expressed as p-values (probability values for rejection of the null hypothesis) from ANOVA test

Parameters	Time (days)			
	1	3	5	8
	Roots (control/flooding)			
ADH	0.001***	0.001***	0.001***	0.001***
TBARs	0.009**	0.002**	0.001***	0.001***
SOD	0.005**	0.301	0.556	0.001***
GR	0.121	0.002**	0.002**	0.001***
	Leaves (control/flooding)			
TBARs	0.54	0.001***	0.002**	0.323
SOD	0.001***	0.001***	0.001***	0.001***
AsP	0.003**	0.002**	0.351	0.856
GR	0.088	0.042*	0.553	0.139
Proline	0.001***	0.001***	0.001***	0.186

Level of significance: *P < 0.05, **P < 0.01, ***P < 0.001. Effects were tested with one way ANOVAs (GLM 1). Average various traits are given in Figs 1-6.

The ADH activity in the roots of control plants did not show significant changes (Fig. 1) during the experiment. After the 1st day of flooding ADH activity in the roots increased up to 7 fold of the control and remained at the same level till the 3rd day. Then the ADH activity in flooded plants decreased but remained significantly higher (170%) than that in control plants (Fig. 1, Table 1).

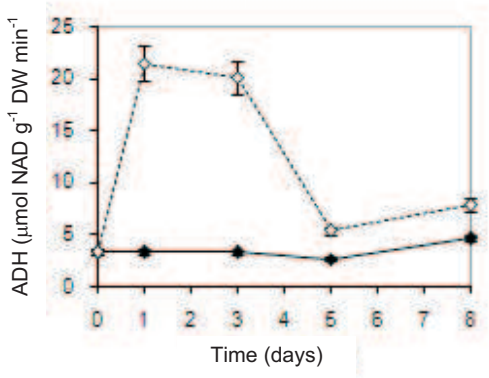

Fig. 1. Alcohol dehydrogenase (ADH) activity in roots of *Brassica napus* grown under optimal soil watering (control – solid line, flooding – dashed line). The values are means of three replicates (pots) ± standard deviations (d.m.). SD.

The concentration of TBARs in the roots of the control plants showed a gradual increase from 0.25 to 0.38 mol g^{-1} d.m. during the entire experimental period (Fig. 2a). In the leaves of the control plants the TBARs concentration was by one order of magnitude higher and varied from 2.0 to 2.5 mol g^{-1} d.m. (Fig. 2b). Under flood conditions a considerable increase of the TBARs concentration in the roots was observed during experimental period (Fig. 2a), the relative values being 138, 175, 152 and 155% of the control after 1, 3, 5, and 8 days, respectively. In the leaves the TBARs concentration increased under flood conditions with a certain delay compared to the roots. It reached 128 and 136% of the control at the 3rd and 5th days of the experiment, respectively (Fig. 2b). Then it decreased to control level at the 8th experimental day.

The SOD activity in the roots of control plants was 6 times lower than that in the leaves (Fig. 3). During 8 days of the experiment the SOD activity in the control increased gradually by 43 and 30% in the roots and in the leaves, respectively. Under flooding conditions the SOD activity in the roots increased significantly (Fig. 3a) during the first 3 days (130, 124% of the control after 1, 3 days, respectively) and later gradually decreased down to 60% of the control. The SOD activity in the leaves of flooded plants increased up to 150% of the control after 3 days and then remained 40-44% higher than the control value until the end of the experiment (Fig. 3b).

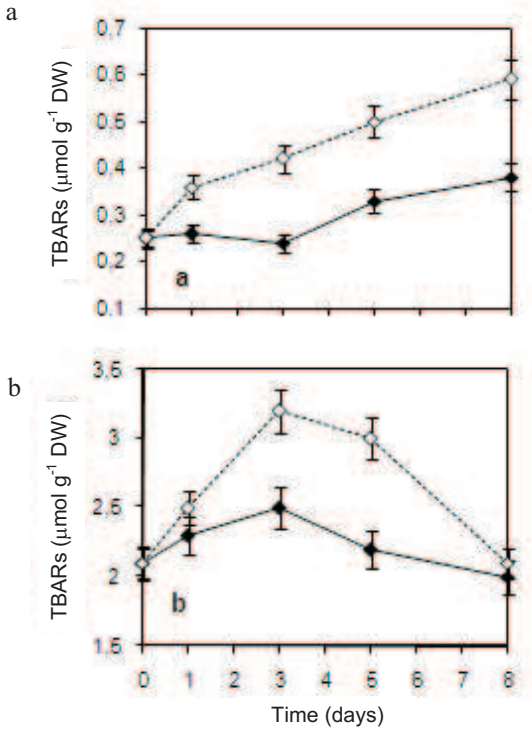

Fig. 2. Thiobarbituric acid reactive substances (TBARs) in: a – roots, b – leaves of spring rape grown under optimal soil watering and flooding. Explanations as in Fig. 1.

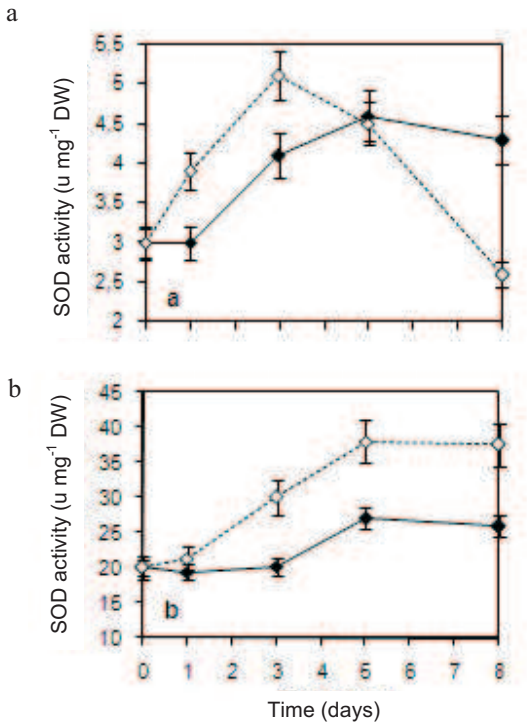

Fig. 3. Superoxide dismutase (SOD) activity in: a – roots, b – leaves of spring rape grown under optimal soil watering and flooding. Explanations as in Fig. 1.

The leaf AsP activity of the control plants remained at a level of 24.0 μmol AsA g^{-1} d.m. min^{-1} during the experiment (Fig. 4). In the flooded plants the AsP activity increased significantly (Table 1) starting from the 1st day. It reached 137% at the 3rd day, and then dropped to the control level.

The GR activity in the control roots (Fig. 5) decreased from 3.4 to 2.3 μmol NADPH g^{-1} d.m. min^{-1} during 8 days of experiment. Activity of GR in the roots of the flooded plants oscillated around 3.5 μmol NADPH g^{-1} d.m. min^{-1} during the entire stress period and starting from the 3rd day of flooding it was by 45% higher than that in the control plants. In the leaves of the control plants the GR activity was about 9.35 μmol NADPH g^{-1} d.m. min^{-1} during the experimental time and did not differ significantly under flood conditions.

The concentration of free proline in the leaves of flooded plants showed a significant increase after the first day of the experiment (Fig. 6, Table 1) and rose up to four times of the control value at the 3rd day. Then proline concentration in flooded plants decreased to the control level.

The soil measurements performed in the flood treatment showed that the hypoxic conditions in soil developed as rapidly as in our earlier studies on barley and bean (Balakhnina *et al.*, 2009, 2010). These demonstrated suppression of plant growth, pigment accumulation and biomass production under soil flooding. Devkota and Jha (2011) showed that total dry mass of *Centella asiatica* decreased up to one fourth from the control with exposure to extensive water (125%) as a result of a less by half in chlorophyll content. The specificity of the present investigations is connected with integrated studying of the specific and unspecific biochemical responses of spring rape plants to soil flooding.

Oxygen deficiency induces anaerobic respiration resulting in ethanol accumulation in plant tissues. Some authors concluded that plant tolerance to hypoxia depends on their ability to multiply activity of ADH, the main enzyme of ethanol oxidation. Really, activity of ADH was shown to be raised in the roots of various plants exposed to soil hypoxia (Garnczanska, 2002; Wignarajah *et al.*, 2006; and others).

Plant tolerance to soil hypoxia was also shown to be dependent on root morphology. In particular, Benz *et al.* (2007) showed that different genotypes of *Piriqueta caroliniana* can respond to hypoxic soils by producing oxygen-conducting aerenchymous tissue or through induction of ADH. The authors assumed that aerenchyma development is an effective strategy in habitats subject to persistent flooding, while elevating activity of enzymes for ethanolic fermentation is effective only under ephemeral flooding. In our experiments, the ADH activity in the roots of flooded spring rape multiplied up to 7 fold after one day of flooding. The following decrease in ADH activity observed after the third

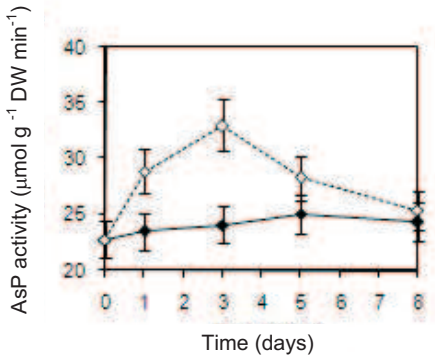

Fig. 4. Ascorbate peroxidase (AsP) activity in leaves of spring rape grown under optimal soil watering and flooding. Explanations as in Fig. 1.

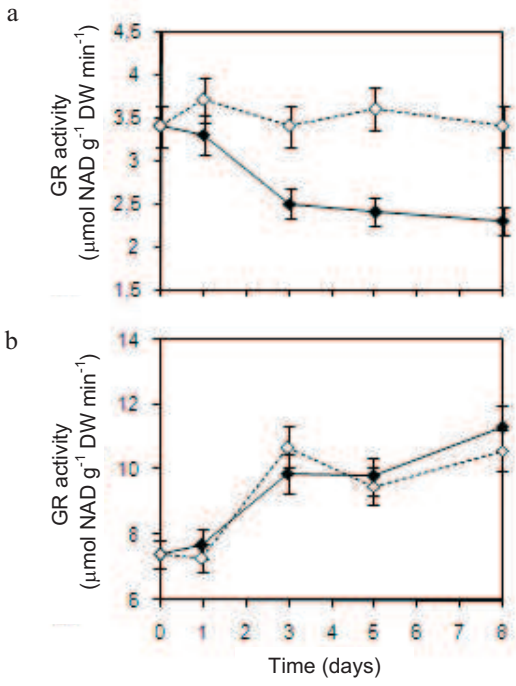

Fig. 5. Glutathione reductase (GR) activity in: a – roots, b – leaves of spring rape grown under optimal soil watering and flooding. Explanations as in Fig. 1.

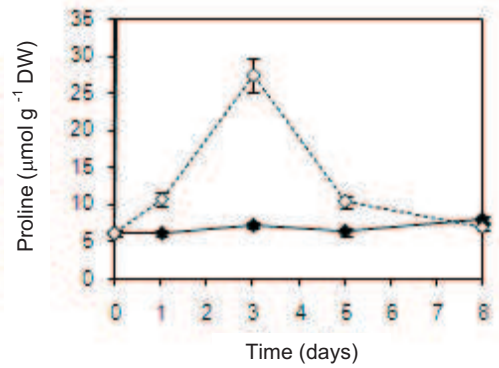

Fig. 6. Proline concentration in leaves of spring rape grown under optimal soil watering and flooding. Explanations as in Fig. 1.

day of the experiment is in agreement with papers cited above. But in our case the ADH activity in flooded plants remained higher than in the control ones during the entire experimental period.

Intensification of peroxidation process was shown to be a result of increased ROS concentration under stress conditions (Asada, 2006; Blokhina *et al*., 2002). In the present study the intensity of oxidative destruction processes as an unspecific response to stress development was examined by concentration of TBARs as the main products of peroxidation of lipids, carbohydrates, proteins and other compounds. According to Bakh-Engler theory, peroxidation processes occur permanently and unavoidably in the cells of living organisms. The relationship between the concentrations of hydrogen peroxide and MDA in roots and leaves of various plants exposed to soil flooding was repeatedly demonstrated (Asada, 2006) and the existence of a direct relationship between stress sensitivity and the early accumulation of MDA was shown (Arbona *et al*., 2008). Usually soil hypoxia increased the MDA concentration, especially in intolerant species (Chen and Qualls, 2003; Yordanova and Popova, 2007). In our experiments the TBARs concentration increased and then remained at a high level in roots of flooded spring rape but in the leaves an initial increase in the TBARs concentration changed to a decrease at the end of the experiment.

Early stages of oxidative stress development can be controlled by low molecular weight antioxidants and enzymes scavenging ROS (Asada, 2006). The positive antioxidant response (activities of SOD, AsP, catalase, and GR) in leaves and roots of citrus genotypes was proposed to be responsible for a higher tolerance to flood stress (Arbona *et al.*, 2008). Wang and Jiang (2007) assumed that SOD and AsP are mainly involved in waterlogging-induced antioxidant responses, and the partial waterlogging could also significantly affect root antioxidant activities, particularly in waterlogging-sensitive cultivars. Blokhina *et al.* (2002) summarized literature data on different response of SOD and other antioxidant enzymes to oxygen deprivation stress and concluded that increased antioxidant capacity did not always correlate positively with the degree of plant protection.

It is suggested that the efficiency of stress protection depends, in the first place, on specificity of a plant species, stage of stress development, and subsequent induction of SOD (scavenging superoxide radicals), peroxidases (scavenging H_2O_2), and GR (Balakhnina *et al.*, 2009, 2010). In present study, the SOD activity in spring rape leaves was found to increase after 3 days of flooding and maintained at the high level during the experiment. This fact together with dynamic of TBARs concentration (decrease after initial increase) shows that the leaves are well defended against indirect action of soil flooding. In spring rape roots induction of SOD was started earlier, after 1 day of flooding, but then, after 5 days, it displayed a gradual decrease below the control which correlated with stress intensification (increased TBARs concentration).

Ascorbate-glutathione cycle involving several enzymes, including AsP and GR, is known as an important and efficient defense system for decomposing H_2O_2 (Blokhina *et al.*, 2002). Duration of stress, plant species, and plant organs are the factors influencing AsP activity associated with waterlogging tolerance (Wang and Jiang, 2007). In spring rape leaves of flooded plants AsP activity increased almost on 40% after 3 days of flooding (Fig. 3a, Fig. 4).

GR is required to maintain a high ratio of GSH/GSSH in the presence of NADPH (Blokhina *et al.*, 2002). Decrease of GR activity in bean leaves after 2 days of soil hypoxia was considered (Balakhnina *et al.*, 2010) to be reflection of the increased content of reduced forms of antioxidants of ascorbate-glutathione cycle. In the present work the GR activity in spring rape leaves under flood conditions did not differ significantly from control value (Table 1) but in the roots it remained about 36-50% higher than control (Fig. 5, Table 1). Comparison of these results with the data on SOD activity in leaves and roots (Fig. 3a,b) supports conclusion that stress tolerance correlates well with consequence high levels of CuZn - SOD and GR activities.

Concentrations of free proline in the leaves of spring rape increased synchronously with SOD and AsP activities in the beginning of soil flooding experiment. Shevyakova *et al.* (2009) observed on iceplant that changes in SOD activity and proline accumulation in response to paraquat treatment combined with NaCl revealed an opposite dependence to accumulation of proline: the more proline accumulated in leaves, the lower activity of the enzyme. Moreover, exogenous proline decreased not only the rate of lipid peroxidation and content of superoxide radical but also SOD activity (almost five-fold) in leaves of adult plants (Shevyakova *et al.*, 2009). Radyukina *et al.* (2008) concluded that proline antioxidant effects in common sage are manifested only after 12 h of stressor action, whereas antioxidant enzymes are involved in ROS scavenging during the earlier stage of damaging factor action.

Proline accumulation is known to be related to non-enzymatic detoxification of free radicals (superoxide, peroxide or hydroxyl) that are generated excessively under stress (Radyukina *et al.*, 2008). The authors explain such ability of this amino acid by the presence of tertiary carbon which can form stable radical tearing off free radical reactions induced by ROS (Radyukina *et al.*, 2008). Our data show that increase of free proline concentration and antioxidant enzymes activities can occur simultaneously in the leaves of spring rape during 3 days of soil flooding. It should be noted that at further stress development, free proline concentration and AsP activity in spring rape leaves decreased with decrease in TBARs concentration to control levels while SOD activity continued to increase till 5th day of flooding and then remained high.

CONCLUSIONS

1. The increase of alcohol dehydrogenase activity is a specific stress response to hypoxic conditions induced by soil flooding in spring rape roots.

2. The unspecific stress responses in roots involve concurrent high levels of superoxide dismutase and glutathione reductase activities: superoxide dismutase activity increases at the beginning of stress development and then decreases dramatically when glutathione reductase activity maintained at the level above control.

3. The monotonic increase of thiobarbituric acid reactive substances concentration indicates that the roots of flooded plants survive strong oxidative stress.

4. The leaves of flooded plants survive indirect action of soil hypoxia and display adaptive reactions involving a few days increase of free proline concentration and ascorbate peroxidase activity, and stable high level of superoxide dismutase activity.

REFERENCES

Arbona V., Hossain Z., López-Climent M.F., Pérez-Clemente R.M., and Gómez-Cadenas A., 2008. Antioxidant enzymatic activity is linked to waterlogging stress tolerance in citrus. Physiol. Plant., 132, 452-466.

Asada K., 2006. Production and scavenging of reactive oxygen species in chloroplasts and their functions. Plant Physiol., 141, 391-396.

Bai T.H., Li C.Y., Ma F.W., Shu H.R., and Han M.Y., 2008. Physiological responses and analysis of tolerance of apple rootstocks to root-zone hypoxia stress. Sci. Agric. Sinica, 41, 4140-4148.

Bailey-Serres J. and Voesenek L.A.C.J., 2008. Flooding stress: acclimations and genetic diversity. Ann. Rev. Plant Biol., 59, 313-339.

Balakhnina T., Bennicelli R., Stępniewska Z., Stępniewski W., and Fomina I., 2010. Oxidative damage and antioxidant defense system in leaves of *Vicia faba major* L. cv. Bartom during soil flooding and subsequent drainage. Plant Soil, 327, 293-301.

Balakhnina T.I., Gavrilov A.B., Włodarczyk T.M., Borkowska A., Nosalewicz M., and Fomina I.R., 2009. Dihydroquercetin protects barley seeds against mould and increases seedling adaptive potential under soil flooding. Plant Growth Regul., 57, 127-135.

Bates L.S., Waldren R.P., and Teare I.D., 1973. Rapid determination of free proline for water-stress studies. Plant Soil, 39, 205-207.

Benz B.R., Rhode J.M., and Cruzan M.B., 2007. Aerenchyma development and elevated alcohol dehydrogenase activity as alternative responses to hypoxic soils in the *Piriqueta caroliniana* complex. Amer. J. Bot., 94, 542-550.

Blokhina O., Virolainen E., and Fagerstedt K.V., 2002. Antioxidants, oxidative damage and oxygen deprivation stress: a review. Annals Bot., 91, 179-194.

Chen H. and Qualls R.G., 2003. Anaerobic metabolism in the roots of seedlings of the invasive exotic *Lepidium latifolium*. Environ. Exp. Bot., 50, 29-40.

Colmer T.D., 2003. Long-distance transport of gases in plants: a perspective on internal aeration and radial oxygen loss from roots. Plant Cell Environ., 26, 17-36.

Devkota A. and Jha P.K., 2011. Influence of water stress on growth and yield of *Centella asiatica*. Int. Agrophys., 25, 211-214.

Garnczanska M., 2002. Hypoxic induction of alcohol and lactate dehydrogenases in lupine seedlings. Acta Physiol. Plant., 24, 265-272.

Giannopolitis C.N. and Ries S.K., 1977. Superoxide dismutases. I. Occurrence in higher plants. Plant Physiol., 59, 309-314.

Gliński J. and Stepniewski W., 1985. Soil Aeration and Its Role for Plants. CRC, Boca Raton, FL, USA.

Lucassen E.C.H.E.T., Bobbink R., Smolders A.J.P, van der Ven P.J.M., Lamers L.P.M., and Roelofs J.G.M., 2002. Interactive effects of low pH and high ammonium levels responsible for the decline of *Cirsium dissectum* (L.) Hill. Plant Ecol., 165, 45-52.

Malicki M. and Walczak R.A., 1983. A gauge of the redox potential and the oxygen diffusion rate in the soil with an automatic regulation of cathode potential (in Polish). Zesz. Probl. Post. Nauk Roln., 220, 447-451.

Pederson O., Rich S.M., and Colmer T.D., 2009. Surviving floods: leaf gas films improve O_2 and CO_2 exchange, root aeration, and growth of completely submerged rice. Plant J., 58, 147-156.

Preiszner J., Vantoai T.T., Huynh L., Bolla R., and Yen H., 2001. Structure and activity of a soybean ADH promoter in transgenic hairy roots. Plant Cell Reports, 20, 763-769.

Radyukina N.L., Shashukova A.V., Shevyakova N.I., and Kuznetsov V.V., 2008. Proline involvement in the common sage antioxidant system in the presence of NaCl and paraquat. Russian J. Plant. Physiol., 55, 649-656.

Rocha M., Licausi F., Araújo W.L., Nunes-Nesi A., Sodek L, Fernie A.R., and van Dongen J.T., 2010. Glycolysis and the tricarboxylic acid cycle are linked by alanine aminotransferase during hypoxia induced by waterlogging of *Lotus japonicus*. Plant Physiol., 152, 1501-1513.

Shevyakova N.I., Bakulina E.A., and Kuznetsov V.V., 2009. Proline antioxidant role in the common ice plant subjected to salinity and paraquat treatment inducing oxidative stress. Russian J. Plant Physiol., 56, 663-669.

Smith K.A. and Restall S.W.F., 2006. The occurrence of ethylene in anaerobic soil. Eur. J. Soil Sci., 22, 430-443.

Vartapetian B.B., Andreeva I.N., Generozova I.P., Polyakova L.I., Maslova I.P., Dolgikh Yu.L., and Stepanova A.Yu., 2003. Functional electron microscopy in studies of plant response and adaptation to anaerobic stress. Annals Bot., 91, 155-172.

Wang K. and Jiang Y., 2007. Antioxidant responses of creeping bentgrass roots to waterlogging. Crop Sci., 47, 232-238.

Wignarajah K., Greenway H., and John C.D., 2010. Effect of waterlogging on growth and activity of alcohol dehydrogenase in barley and rice. New Phytol., 77, 585-592.

Yordanova L.Y. and Popova L.P., 2007. Flooding induced changes in photosynthesis and oxidative status in maize plants. Acta Physiol. Plant., 29, 535-541.