Turkish Journal of Agriculture and Forestry

http://journals.tubitak.gov.tr/agriculture/

Research Article

Turk J Agric For
(2017) 41: 463-471

doi:10.3906/tar-1701-94

Antioxidant capacity of oilseed rape (*Brassica napus*) in different soil types

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Received: 20.01.2017 • **Accepted/Published Online:** 15.09.2017 • **Final Version:** 18.12.2017

Abstract: This work considers the results obtained by testing the parameters of antioxidant status in leaves and roots of oilseed rape at different growth stages (phase I: formation of leaf rosettes and preparing for winter hibernation; phase II: after the winter hibernation; phase III: flowering) grown in semicontrolled field conditions on different soil types. The experiment was conducted on four oilseed rape genotypes (Banacanka, Jasna, Kata, and Zlatna) in semicontrolled conditions in containers with three soil types: chernozem, solonetz, and solonchak. The germination failed on solonchak due to the excessive amount of salt, but results were obtained for plant material on chernozem and solonetz. The following parameters were examined: superoxide dismutase (SOD) enzyme activity, glutathione peroxidase (GPx) activity, lipid peroxidation (LP) intensity, glutathione (GSH) content, and total antioxidant capacity (by ferric reducing antioxidant power, FRAP method). The activity of all the tested parameters of antioxidant status in all genotypes, both in leaves and roots, was higher in plants grown on solonetz soil compared to plants grown on chernozem. Furthermore, the activity of all the tested parameters on both soil types was highest in the second phase of testing, the stage after the winter hibernation, because the plants were exposed to low temperatures in addition to salt stress. The vital thing to note is that GPx activity is important for the defense at the initial stages of growth, while in the later growth stages SOD assumes this role. The genotype Banacanka has been distinguished as the most tolerant, while the genotype Zlatna was the most sensitive.

Key words: Antioxidant enzymes, salinity, soil, stress tolerance, oilseed rape

1. Introduction

An important aspect of agriculture is the cultivation of plants for food, fiber, biofuel, medicine, and other products used to sustain and enhance human life. Agriculture was the key development in the rise of sedentary human civilization, whereby farming of domesticated species created food surpluses that nurtured the development of civilization (Ercisli, 2009; Erturk et al., 2010; Tahtamouni et al., 2016; Yeşil and Kara, 2016).

It is estimated that the high salinity affects 20% of the total arable land and 33% of irrigated agricultural land in the world. Furthermore, areas of saline soil are continuously increasing at a yearly rate of 10% for various reasons, including low rainfall, high temperatures, and the use of saline water for irrigation. In addition, it is estimated that by 2050 more than 50% of the arable land will be saline (Jamil et al., 2011).

Depending on the total concentration of soluble salts (electrical conductivity), the pH of the soil solution, and the exchangeable sodium percentage, saline soil includes three different types: saline, saline-alkali, and alkaline soils (Rasool et al., 2012). Slatinas are saline and/or alkaline soils, with adverse physical and chemical properties, exposed to frequent ground water moistening (Ćirić et al., 2012).

In optimal environmental conditions, each cell contains an appropriate balance between intercellular generation and neutralization of reactive oxygen species (ROS) (Dutilleul et al., 2003). However, under stressful situations, there is an imbalance between the production and the removal of ROS in specific cell compartments (Vellosillo et al., 2010; Karuppanapandian et al., 2011). It is considered that the increased production of toxic oxygen derivatives is a common characteristic of a stressful situation.

A large amount of cell ROS causes the inactivation of enzymes, cell organelle damage, cell membrane destruction, and degradation of pigments, proteins, lipids, and nucleic acids, which may eventually lead to cell death (Karuppanapandian et al., 2011). This situation in a cell leads to oxidative stress. In order to defend themselves against such conditions, plants developed various biochemical and molecular mechanisms (Mantri et al., 2012), which include activation of antioxidant enzymes, synthesis of certain compounds, changes in

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photosynthesis, and selective accumulation or exclusion of ions by the root system. There are numerous studies on the effects of salinity on plants' development, whereby salt stress conditions are simulated in the laboratory. On the contrary, very few authors used soil of the saline type in their experiments as a source of natural salt stress.

Oilseed rape (*Brassica napus*) does not require specific soil conditions, and many authors consider that this species is moderately salt-tolerant and can be grown in soils with a higher salt content. For this reason, it is often called the culture of the marginal land. However, salt tolerance of oilseed rape depends on many factors, primarily on the genotype and stage of development.

In order to provide an accurate understanding of physiological and biochemical processes in nature, this paper studies the effects of different types of soil on antioxidant status of oilseed rape in semicontrolled field conditions.

2. Materials and methods

The experiment was conducted on four oilseed rape genotypes, Banacanka, Jasna, Kata, and Zlatna, at the Institute of Field and Vegetable Crops, Novi Sad, Serbia, in semicontrolled conditions. Plants were grown in medium size Mitscherlich pots using 5–6 kg of soil in each pot. Three types of soils were used as a growing medium: chernozem, solonetz, and solonchak. Basic chemical properties and salinity indicators of the soils are shown in the Table.

In the first half of September, 20 seeds of oilseed rape were planted in each of the pots, and the number of seedlings was reduced to six per pot after emergence. During the growing season, the plants were exposed to environmental influences, without additional irrigation. In February plants were treated with an insecticide from the group of neonicotinoids. Samples were collected separately from leaves and from roots at three developmental stages: the phase of rosettes forming and leaf preparations for the winter cold, after the winter dormancy, and during the flowering stage. Samples were collected from each plant and constituted one group sample per pot. All analyses were conducted in three replicates.

2.1. Antioxidant enzymes and reduced glutathione

The superoxide dismutase (SOD) activity was measured according to Giannopolitis and Ries (1977), by measuring the ability of the enzyme extract to inhibit the photochemical reduction of nitro-blue tetrazolium. Glass test tubes containing the mixture were illuminated with a fluorescent lamp (Philips MLL 5000W). Identical tubes, which were not illuminated, were used as blanks. After the illumination for 15 min, the absorbance was measured at 560 nm. One unit of SOD was defined as the enzyme activity that inhibited the photoreduction of nitro-blue tetrazolium to blue formazan by 50%, and SOD activity of the extracts was expressed as SOD units per mg of protein.

The guaiacol peroxidase (GPx) activity was measured using the method of Kato and Shimizu (1987). The activity was calculated using the extinction coefficient of 26.6 mM^{-1} cm^{-1} at 470 nm for oxidized tetraguaiacol polymer. One unit of GPx activity was defined as the amount of the enzyme extract that consumes 1 μmol of H_2O_2 min^{-1} mg $protein^{-1}$. The content of GSH was determined with Ellman reagent at 412 nm according to the procedure of Punitha and Rajasekaran (2011).

2.2. Lipid peroxidation

Lipid peroxidation (LP) was estimated by TBA test, measuring malondialdehyde production using a spectrophotometric assay. The color intensity of the malondialdehyde–thiobarbituric acid complex in the supernatant was measured. The extinction coefficient at 532 nm of 153,000 mol^{-1} cm^{-1} for the chromophore was used (Ng et al., 2000).

2.3. Ferric reducing antioxidant power (FRAP)

Ferric reducing antioxidant capacity was estimated according to the FRAP assay (Benzie and Strain, 1999). The FRAP reagent was prepared by mixing acetate buffer (300 mM, pH 3.6), 2,4,6-tripyridyl-s-triazine reagent (10 mM in 40 mM HCl), and FeCl3•6H20 (20 mM) in the ratio of 3:1:1. A sample of 100 μL was mixed with 3 mL of working FRAP reagent and absorbance (593 nm) was measured after 4 min. The test was performed at 37 °C. The FRAP value was calculated using the following formula:

Table. Basic chemical properties and salinity indicators of the soils.

Soil type	Basic chemical soil properties		Indicators of salinity		
	$CaCO_3$%	Humus %	Water-soluble salts %	ECe 25 °C dS/m	pH water extract
Chernozem	11.68	2.15	0.06	1.27	7.84
Solonetz	0.25	3.14	0.15	1.40	7.87
Solonchak	12.09	1.54	0.69	11.90	9.94

FRAP value = ΔAsample (0–4 min)/ΔAstandard (0–4 min).

The 100 μM Fe^{2+} solution was used as a standard; 1 FRAP unit = 100 μM Fe^{2+}.

Total antioxidant capacity was expressed in FRAP units.

2.4. Statistical analysis

Statistical significance was performed using one-way ANOVA followed by comparisons of means using Duncan's multiple range test ($P < 0.05$). This analysis was done using the statistical software Statistica version 10 (StatSoft).

3. Results

The germination failed on solonchak, but results were shown for plant material obtained on chernozem and solonetz.

3.1. SOD enzyme

SOD activity in oilseed rape leaves grown on chernozem ranged from 124.24 to 186.7 U/mg of protein depending on the growth phase, while the activity on solonetz ranged from 132.2 to 202.3 U/mg of protein (Figure 1). During the examination at the stage of leaf rosettes (phase I), only in genotype Banacanka was leaf SOD activity higher on chernozem than on solonetz, while the other genotypes' activity was greater on solonetz. In all of the genotypes, except in genotype Banacanka, maximum activity of this enzyme was observed during the test after winter dormancy (phase II) in both soil types. SOD activity in oilseed rape roots grown on chernozem ranged from 69.79 to 104.7 U/mg of protein depending on the growth phase, while the activity on solonetz ranged from 66.8 to 100.3 U/mg of protein (Figure 1). In all of the genotypes, except genotype Kata, root SOD activity in the first phase was higher on solonetz than on chernozem. Similar results were observed in the second phase in all genotypes, except genotype Banacanka. In the final phase of testing, in genotypes Kata and Zlatna, the activity was higher on solonetz than on chernozem. Correlation analysis showed that the activity of this enzyme is directly related to plant parts (the leaf and the root) in all tested genotypes.

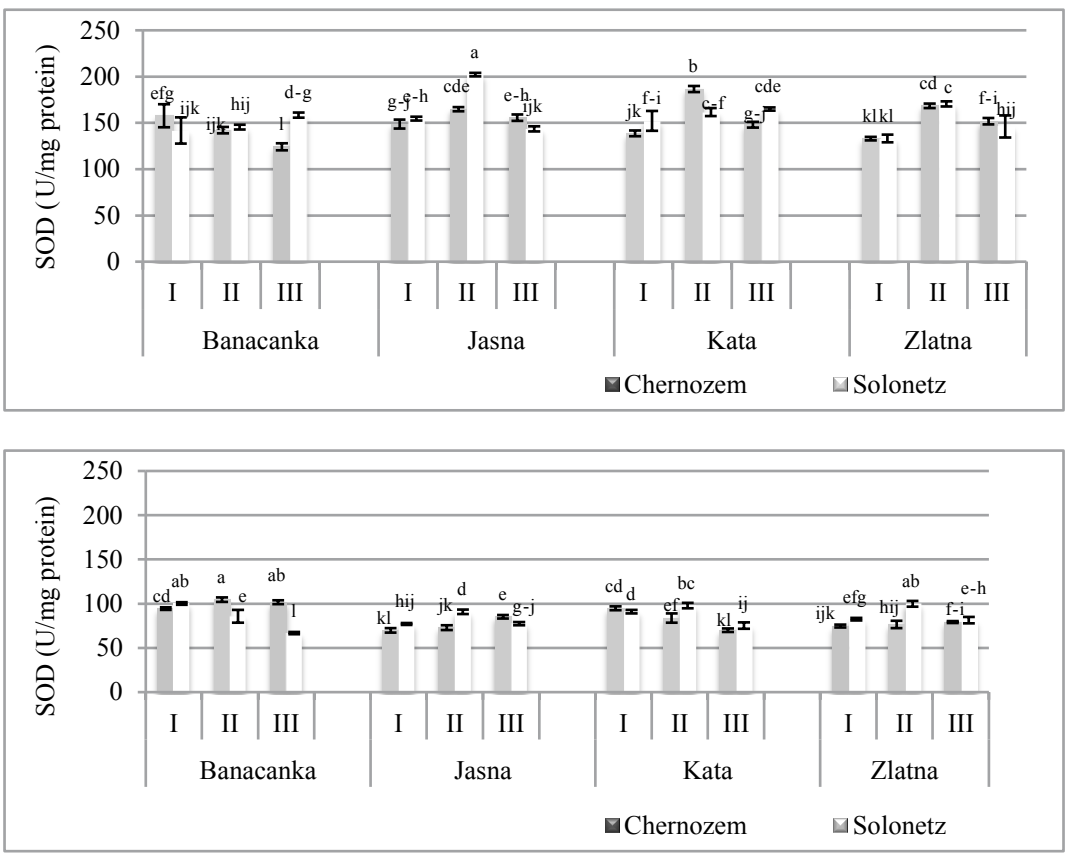

Figure 1. Activities of SOD in the leaves (top) and roots (bottom) of selected genotypes of oilseed rape grown on chernozem and solonetz at various development stages

I - Phase of formation of leaf rosettes and preparing for winter hibernation; II - phase after the winter hibernation, III - phase of flowering; each value with the same letter is at the same level of significance for the 95% interval compared by Duncan's multiple comparison test.

3.2. GPx enzyme

The activity of GPx in the oilseed rape leaves grown on chernozem ranged from 4.81 to 6.98 U/mg of protein depending on the growth phase, while the activity of plants grown on solonetz ranged from 4.87 to 7.18 U/mg of protein (Figure 2). The highest leaf GPx activity in all of the tested genotypes, regardless of the soil type, was observed in the second phase of testing. Genotype Zlatna showed the least difference between plants grown on these two soil types. GPx activity in the roots grown on chernozem was 15.52 to 18.00 U/mg of protein depending on the phase of growth, while the activity of plants grown on solonetz ranged from 15.65 to 19.92 U/mg of protein (Figure 2). In all genotypes, in all tested phases, except for genotype Jasna in phase III, the GPx activity in the oilseed rape leaves grown on solonetz was higher than on chernozem. As well as SOD activity, correlation analysis showed that the activity of this enzyme is directly related to plant parts (the leaf and the root) in all tested genotypes

3.3. GSH amount

The GSH amount in the leaves of oilseed rape grown on chernozem ranged from 8.50 to 10.40 µmol GSH/mg of protein depending on the growth phase, while the GSH amount in the leaves of oilseed rape grown on solonetz ranged from 9.08 to 11.36 µmol GSH/mg of protein depending on the growth phase (Figure 3). In all of the tested genotypes at all stages, except for genotype Kata in the third stage, the GSH amount in the leaves grown on the solonetz was higher compared to the values obtained on chernozem. The highest values in both soil types were identified in the second testing phase. The GSH amount in the roots grown on chernozem ranged from 10.84 to 14.49 µmol GSH/mg of protein depending on the growth phase, while the GSH amount in the roots grown on solonetz ranged from 11.74 to 14.90 µmol GSH/mg of protein depending on the phase (Figure 3). In all tested genotypes at all stages of the test, except for genotypes Jasna and Kata in the second phase, the GSH amount in

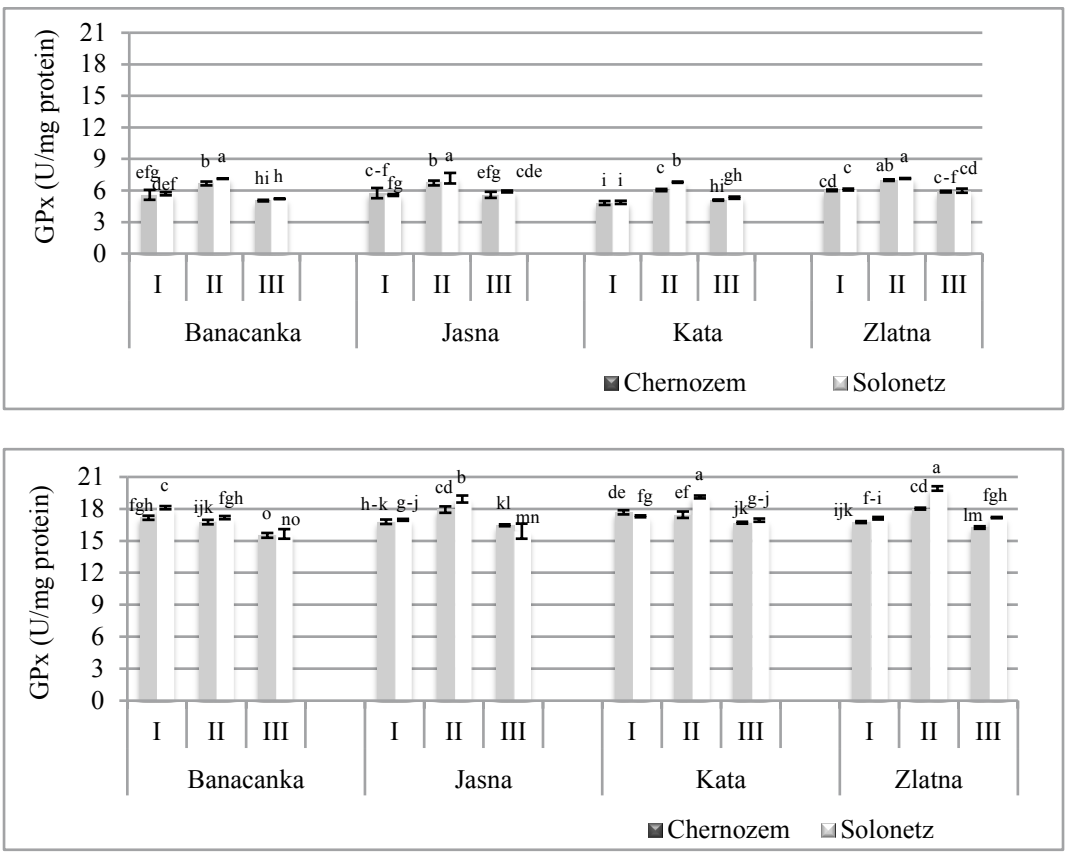

Figure 2. Activities of GPx in the leaves (top) and roots (bottom) of selected genotypes of oilseed rape grown on chernozem and solonetz at various development stages.
I - Phase of formation of leaf rosettes and preparing for winter hibernation; II - phase after the winter hibernation, III - phase of flowering; each value with the same letter is at the same level of significance for the 95% interval compared by Duncan's multiple comparison test.

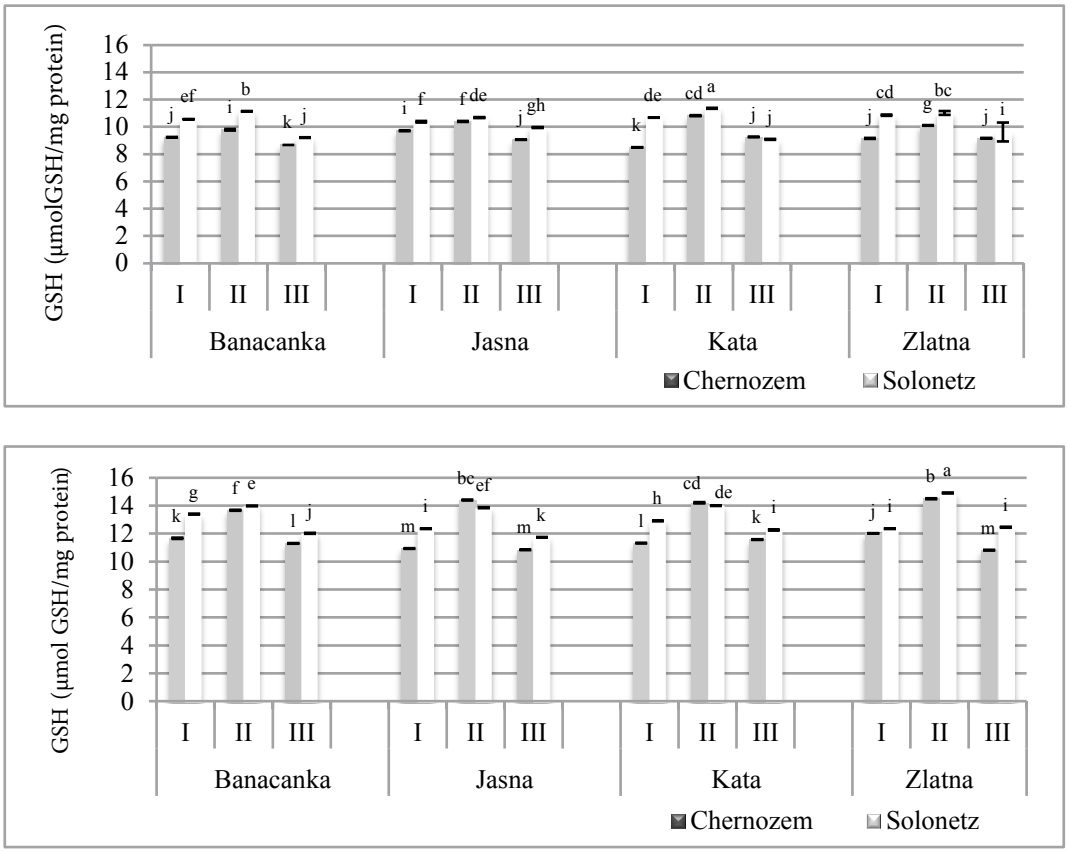

Figure 3. The amount of GSH in the leaves (top) and roots (bottom) of selected genotypes of oilseed rape grown on chernozem and solonetz in various development stages.
I - Phase of formation of leaf rosettes and preparing for winter hibernation; II - phase after the winter hibernation, III - phase of flowering; each value with the same letter is at the same level of significance for the 95% interval compared by Duncan's multiple comparison test.

the roots on solonetz was higher than the values obtained on chernozem. In the roots, the same as in the leaves, the greatest values of GSH were obtained in the second stage on both soil types. A strong correlation was observed between the GSH amount and the plant part in all the tested genotypes.

3.4. Lipid peroxidation intensity

The LP intensity in oilseed rape leaves grown on chernozem ranged from 86.32 to 179.49 nmol MDA/mg of protein depending on the phase, while the LP intensity in the leaves grown on solonetz ranged from 108.55 to 219.66 nmol MDA/mg of protein (Figure 4). In all of the tested genotypes at all stages, the LP intensity in the leaves grown on the solonetz was higher compared to the values obtained on chernozem. The LP intensity in the plant roots grown on chernozem ranged from 114.53 to 186.32 nmol MDA/mg of protein depending on the growth phase, while the LP intensity in the roots grown on solonetz ranged from 146.17 to 217.95 nmol MDA/mg of protein (Figure 4). In all genotypes at all tested stages, the LP intensity in the roots grown on the solonetz was higher compared to the values obtained on chernozem. In all genotypes, irrespective of the soil type, the highest values were in the second phase of testing, after the winter period. The correlation between LP intensity and plant parts, soil type, and testing phase was not significant in any of the tested genotypes.

3.5. Total antioxidant activity

Total antioxidant activity in the leaves of oilseed rape grown on chernozem ranged from 0.91 to 1.91 FRAP units depending on the growth phase, while the total antioxidant activity in the leaves grown on solonetz ranged from 0.93 to 2.31 FRAP units depending on the phase of growth (Figure 5). In the leaves of all genotypes in all growth phases, except for genotype Zlatna in the first and the last stages, the value of FRAP was higher on solonetz compared to chernozem. The highest values of total leaf antioxidant levels were observed in the first phase on both soil types. The total antioxidant activity in the roots grown on chernozem ranged from 0.83 to 1.91 FRAP

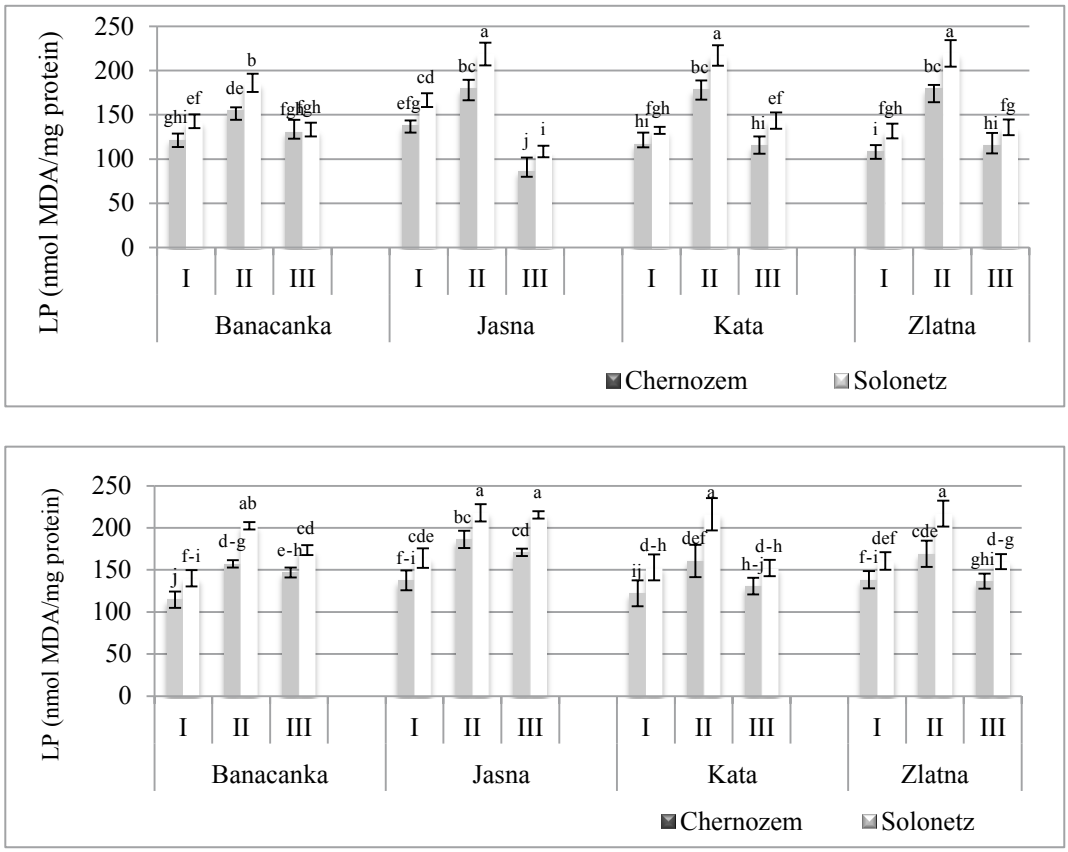

Figure 4. The LP intensity in the leaves (top) and roots (bottom) of selected genotypes of oilseed rape grown on chernozem and solonetz in various development stages.
I - Phase of formation of leaf rosettes and preparing for winter hibernation; II - phase after the winter hibernation, III - phase of flowering; each value with the same letter is at the same level of significance for the 95% interval compared by Duncan's multiple comparison test.

units depending on the phase of growth, while the total antioxidant activity in the roots grown on solonetz ranged from 1.13 to 2.00 units depending on the phase (Figure 5). Compared to chernozem, the values of FRAP in the roots of all genotypes in all phases of testing were higher on solonetz. The highest values of total antioxidant levels were observed in the first stage of examination. Significant correlation between the total antioxidant activity and testing phase for Jasna and Zlatna, as well as between this parameter and the soil type for Banacanka, was observed.

4. Discussion

Defense mechanisms against oxidative stress include preventive mechanisms, mechanisms for recovering from damage, physical defense, and antioxidant defense (Kaminski et al., 2012). Many studies have shown that major abiotic stresses such as salinity, acidity, and heavy metals increase the levels of free radicals and reactive oxygen species in the plant cells, which is directly related to changes in the activity of antioxidant enzymes.

Therefore, it is very important for cells to control the level of ROS, but at the same time not to eliminate them completely. Many studies have shown that increased activity of these enzymes is positively correlated with plant tolerance under stress conditions. Changes in enzyme activity also depend on the genotypes and the level of stress (Chen et al., 2007). It is known that antioxidant enzyme activities such as SOD, CAT, and Px influence the removal of ROS. For example, in species relatively tolerant to salinity, increased activity of some antioxidant enzymes was observed (Hernandez et al., 2000), while in susceptible species, Na^+ ions create a powerful inhibitory effect on certain forms of SOD (Hernandez et al., 1994). The role of antioxidant enzymes in the scavenging of ROS caused by salinity was determined as the main part of the defense mechanisms in halophytes (Shabala, 2013).

Increased activity of the enzyme SOD, intensity of LP, GSH content, and total antioxidant activity in plants grown in solonetz soil compared to plants grown in chernozem indicate the significant level of stress that the plants were

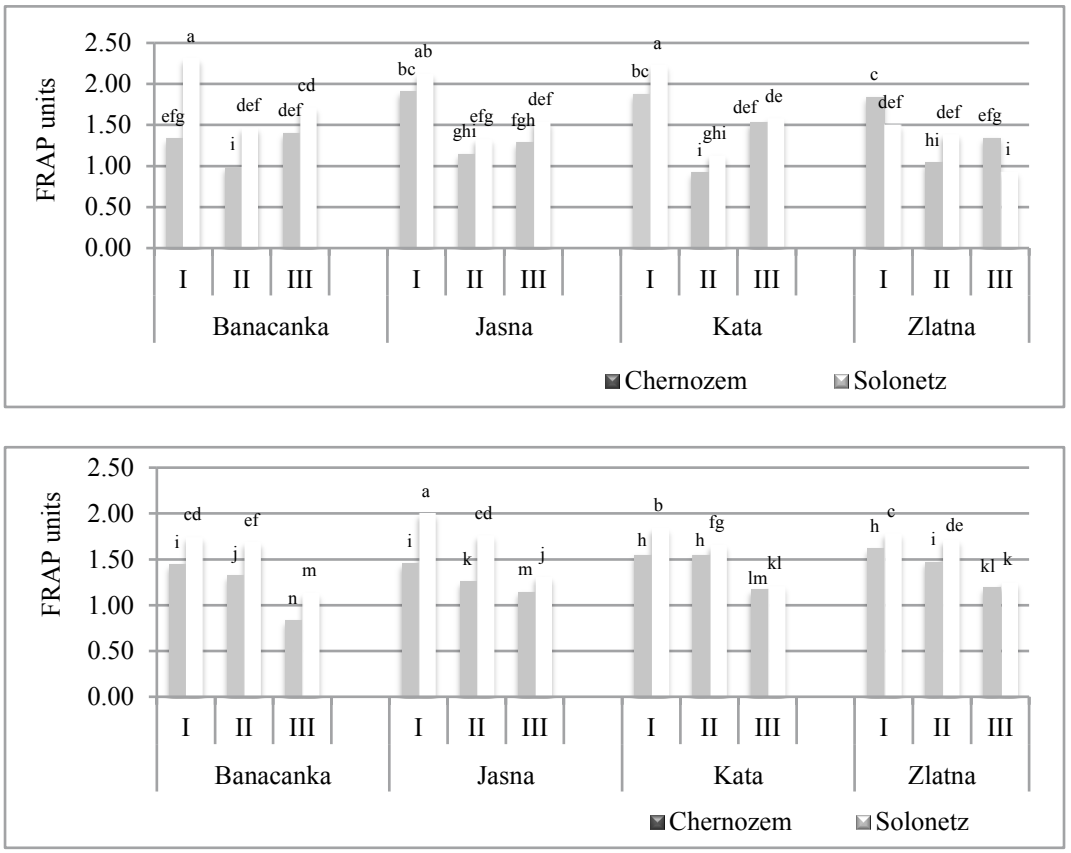

Figure 5. The total antioxidant activity (FRAP) in the leaves (top) and roots (bottom) of selected genotypes of oilseed rape grown on chernozem and solonetz in various development stages.
I - Phase of formation of leaf rosettes and preparing for winter hibernation; II - phase after the winter hibernation, III - phase of flowering; each value with the same letter is at the same level of significance for the 95% interval compared by Duncan's multiple comparison test.

exposed to. The values of GPx in leaves and roots were approximate in chernozem and solonetz, indicating that the activity of this enzyme was not a significant part of the plant defense mechanism against salt stress. The same situation is observed in the Zlatna genotype when it comes to SOD activity on both soil types and in all tested stages. By examining the effect of NaCl on the growth of wheat cultivars, Bhutta (2011) concluded that the scavenging of superoxide radicals by SOD had not been done perfectly. However, there are numerous studies that prove that this enzyme directly affects the reduction of oxidative damage during salt stress (Jalali-e-Emam et al., 2011; Muchate et al., 2016).

In some genotypes, particularly in the Zlatna genotype, significant differences of SOD and GPx activity between the leaves and roots of plants grown in these two soil types were observed. Zlatna showed the highest activity of these enzymes in the leaves, which indicates a high level of stress, but the difference in values between soil types was not significant. The differences in the activity of these enzymes in the roots between chernozem and solonetz were more apparent. Excessive salt concentration in the soil causes the accumulation of the salt in the root of the plant (Schuch and Kelly, 2008). In relation to the existence of different ways of plant response in the leaves and the roots to inadequate environmental conditions, Kaminski et al. (2012) stated that one of the plant strategies to combat salt stress is inhibition in the root: Kaspari's strips prevent the passage of ions through the xylem, so they do not move from the apoplast to symplast through the membrane.

In addition to the large quantities of salt in solonetz, which certainly has an effect on the activity of antioxidant enzymes, an increased level of oxidative stress in plants grown on marginal lands affects the level of availability of chemical elements that depend on a number of environmental characteristics of soil: pH, water potential of the soil solution, and organic matter content (Sundareshwar et al., 2003). This is supported by the fact that on the solonchak, whose pH and EC values are several times higher than those of chernozem and solonetz

(Table), the seed did not even germinate. Unfavorable physicochemical properties and the accumulation of sodium salts in solonetz make it unsuitable for the growth and development of plants (Dimitrijević et al., 2011), increasing the oxidative stress in all tested genotypes.

The increased amount of sodium in soil of this type also affects the ion balance, especially in metabolic processes. Moreover, the SOD and CAT activity is related to the concentration of the elements Na, Ca, Fe, Zn, Cu, Mn, Pb, and Cd, which indicates the complexity of the antioxidant system with the simultaneous involvement of chemical elements in the stimulation and modification of lipid peroxidation and the activity of these enzymes (Kaminski et al., 2012).

LP has major implications on the normal functioning of the cells, such as reducing the permeability of membranes, damage to membrane protein molecules that occurs through specific channels with increased leakage of transmission under normal conditions, and inactivation of receptors, enzymes, and ion channels (Gill and Tuteja, 2010). Damage to the membrane leads to an increase in cell loss, rapid desiccation, and death of the cell (Repetto et al., 2012). Lower LP intensity in different genotypes and species that are salt-tolerant indicates that the plants are better protected against oxidative stress in saline conditions (Tayefi-Nasrabad et al., 2011). On both soil types, in both leaves and roots, genotype Banacanka was distinguished with the lowest intensity of LP. Furthermore, the highest intensity of LP in all genotypes on both soil types was in the second phase, confirming that low temperature is a significant factor that increases oxidative stress in plant cells.

The activity of all the tested parameters of antioxidant status in all genotypes, both in leaves and roots, was higher in plants grown on solonetz soil compared to plants grown on chernozem. Additionally, the activity of all the tested parameters on both soil types was highest in the second phase of testing, at the stage after the winter hibernation, because the plants were exposed to low temperatures in addition to salt stress. The vital thing to note is that GPx activity is important for the defense at the initial stages of growth, while in the later growth stages SOD takes over this role.

Based on the analyzed parameters, it can be concluded that salinity as the primary stress factor and low temperature stress as a secondary factor cause different biochemical reactions in genotypes. The differences show that there was an activation of the antioxidant defense system, emphasizing its importance in stressful conditions.

Acknowledgment

This research is a result of research project TR 31025, "Development of new varieties and production technology improvement of oil crops for different purposes", funded by Ministry of Education, Science, and Technological Development of the Republic Serbia.

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