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Salt-induced changes in the antioxidant system and viability of oilseed rape

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Abstract

This work was aimed to investigate the effect of salt (NaCl) stress on three oilseed rape (*Brassica napus* L.) cultivars: 'Banacanka', 'Jasna' and 'Kata'. The following parameters were determined: antioxidant enzymes superoxide dismutase (SOD) and guaiacol peroxidase (GPX), reduced glutathione (GSH) quantity, lipid peroxidation (LP) intensity, radical-scavenging capacity (DPPH-RSC), hydroxyl radical scavenging ('OH radicals) and ferric reducing antioxidant power (FRAP). Seed viability was determined using the standard laboratory test for germination as well as the seed accelerated aging test measuring the germination, shoot and root length. The plants were subjected to three salt treatments: 0, 100, 150 and 200 mmol dm⁻³ NaCl for 7 days. All three cultivars responded with an increase of lipid peroxidation in seedlings and with a decrease of GPX activity. 'Banacanka' was found to be the most vulnerable cultivar, with an increase of 237% of LP intensity in comparison to the control at the highest NaCl concentration. The induction of the SOD activity was observed for all three cultivars by different NaCl concentrations. The antioxidant capacity measured by FRAP, DPPH-RSC and 'OH radicals was also induced, especially FRAP at the highest concentration in the cultivar 'Jasna' (by 282%). Germination, shoot and root length decreased for all investigated cultivars. Our results showed that cultivar 'Jasna' is the most adapted to salt stress because of the highest SOD activity and accumulation of antioxidants. Furthermore, this research suggests that some of the antioxidant parameters can be useful biochemical markers for the selection of oilseed rape tolerant cultivars under the conditions of salinity.

Key words: accelerated aging, antioxidant enzymes, *Brassica napus*, germination, lipid peroxidation, salt stress.

Introduction

Salinity is an important environmental factor that can severely inhibit plant growth and reduce yields of crops. High salt concentration plays a primary role in soil degradation causing significant yield reduction in a large number of crops. Moreover, during the last decades, the problem of salinity has been growing due to the reduced availability of fresh water, which has led to a high level use of inadequate water with high salt content in agricultural production (Shahbaz, Ashraf, 2013).

Salinity has a negative effect on plant growth by generating an osmotic potential in the environment that prevents normal water uptake. The second but no less important negative effect is the toxicity of excessive amounts of sodium ions, which leads to the lack of calcium and potassium and an imbalance of other nutrients. Also, the increased concentration of chloride

ions causes necrotic symptoms of toxicity due to the negative impact on the production of chlorophyll, thus significantly reducing growth and water use efficiency in plants (Hasanuzzaman et al., 2013). In other words, plant growth responses under saline conditions occur in two phases: 1) osmotic response, which is characterized by the reduction in growth caused by salt ions in the root zone, and 2) salt-specific response, which usually implies additional reduction in growth caused by toxic levels of salt ions in plant cells (Munns, 2005). As a consequence of these primary effects, salt stress also induces secondary effects such as oxidative damage and nutritional imbalance (Rameeh et al., 2012). In order to cope with negative effects of ionic stress, plants that grow on saline soils have developed different mechanisms (Turkan, Demiral, 2009).

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Many studies have shown that the antioxidant defence system plays a crucial role when plants start to be tolerant to salinity. It has been proven that plant species tolerant to salt increase the antioxidant enzyme activities and a number of non-enzymatic antioxidants in order to protect them from stressful conditions, whereas this cannot be observed in non-salt tolerant species (Turkan, Demiral, 2009).

An important step in crop production in adverse environmental conditions is the selection of high-quality seeds. In addition to the seed germination, as the most important parameters of quality seeds, quality evaluation is often based on seed viability testing. The seed viability or seed vigour is a set of characteristics that identify the activity and behaviour of seed lot of commercially acceptable germination in the different environmental conditions. This indicator does not show just the percentage of viable seeds in the sample, it also reflects the ability of seeds to establish normal seedlings/plants in unfavourable growing conditions that may occur in a field (ISTA, 2015). Considering the fact that the seed vigour depends on many factors, it is assumed that the cultivars which are better able to achieve high seed viability can show more efficient tolerance to stress conditions, such as salinity.

Nowadays, oilseed rape is the most important plant of moderate climates and the areas where it is grown occupy the third place among oil crops in the world (FAO, 2013). Oilseed rape is grown primarily for its seed that is high in protein and oil. This species has no particular requirements regarding soil type and many authors (Athar et al., 2009; Banuelos et al., 2013) believe that this plant species is moderately tolerant to salt stress and can be grown on soils with higher salt contents. Therefore, it is often referred to as the crop of marginal lands (Smith et al., 2013). However, the tolerance of oilseed rape to salinity depends on many factors, primarily on cultivars and stages of development.

Therefore, in the present research, the antioxidant status of seedlings from three oilseed rape cultivars: 'Banacanka', 'Jasna' and 'Kata', and the seed viability were studied for a better understanding how salt stress affects the antioxidant system in seedlings and also the mechanisms that enable tolerance to this type of stress.

Material and methods

The experiment was conducted with three oilseed rape (*Brassica napus* L.) cultivars: 'Banacanka', 'Jasna' and 'Kata', in 2015–2016.

Seed germination and growth parameters. Seed germination was determined by the standard laboratory method (SLM) to 400 seeds (four replicates of 100 seeds) and is expressed as a percentage (ISTA, 2015). As a medium for germination filter paper was used, moistened with distilled water in control and with three concentrations of NaCl – 100, 150 and 200 mmol dm⁻³ (Abdollahi, Jafari, 2012). The incubation period lasted 7 days, after which percentage of germinated seeds was determined evaluating seedlings with all essential structures, as well as the length of shoot and root. Seed viability of oilseed rape was studied by applying vigour test – a test of accelerated aging (AA) test. Seeds were kept in a water bath at 39°C and 100% relative humidity for a period of 72 hours after which 400 seeds in 4 replicates were sown in Petri dishes (Hampton, Tekrony, 1995).

In both tests, the seedlings were moistened with the same amount of NaCl solution (100, 150 and 200 mmol dm⁻³) during germination period. After seven days, using 10 seedlings from each treatment, growth parameters were determined.

Extraction procedures. One gram of fresh plant material was extracted with 25 ml 70% aqueous ethanol (0.1 M HCl) under 30 min sonication in an ultrasonic bath. After filtration under vacuum through a sintered glass funnel, extracts were kept refrigerated. These extracts were used for total antioxidant power determination by the ferric reducing antioxidant power (FRAP) method, radical-scavenging capacity (DPPH-RSC) and hydroxyl radical scavenging (•OH radicals). To determine the lipid peroxidation (LP) and antioxidant enzymes, 1 g of plant material was macerated with 50 ml 0.1 M K₂HPO₄ (pH 7.0) using mortar. Reduced glutathione (GSH) was extracted with 5% trichloroacetic acid and free proline with 3% sulphosalicylic acid. After 10 minutes of centrifugation at 4°C and 10,000 g, aliquots of the supernatant were used for enzyme and metabolite determinations as outlined below.

Lipid peroxidation (LP) was estimated by thiobarbituric acid, measuring malondialdehyde (MDA) production using a spectrophotometric assay. The colour 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).

Antioxidant enzymes and reduced glutathione (GSH). 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 which 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 which consumes 1 μmol of H₂O₂ min⁻¹ mg protein⁻¹. The content of GSH was determined with the Ellman reagent at 412 nm, according to the procedure of Punitha and Rajasekaran (2011).

Ferric reducing antioxidant power (FRAP). Ferric reducing antioxidant capacity was estimated according to the FRAP assay (Benzie, 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 × 6H₂O (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 minutes. The test was performed at 37°C. The FRAP value was calculated using the following formula:

$$\text{FRAP value} = \Delta A \text{ sample (0–4 min)} / \Delta A \text{ standard (0–4 min)}$$

The 100 $\mu\text{M Fe}^{2+}$ solution was used as a standard; 1 FRAP unit = 100 $\mu\text{M Fe}^{2+}$; total antioxidant capacity was expressed in FRAP units.

Radical-scavenging capacity (DPPH-RSC) assay was based on measurement of the change of the pink colour of 2,2-diphenyl-1-picrylhydrazyl into the yellow colour after reaction with the extract (Espin et al., 2000). Antioxidant capacity is in accordance with the decrease in the absorbance. Each extract (5, 10, 20 30 and 40 μl) was mixed with 90 $\mu\text{M DPPH}$ in methanol making up to the final volume of 3.0 ml. The mixtures were shaken vigorously and were stored in the dark for 30 minutes at room temperature. The decrease of absorbance of the reaction mixtures relative to the control was monitored at 515 nm. Hydroxyl radical scavenging ($\cdot\text{OH}$ radicals) was measured by the method of Halliwell and Gutteridge (1989) with minor changes. 200 μl of 2.8 mM 2-deoxy-2-ribose, 5 μl of 70% aqueous ethanol extract of oilseed rape, 400 μl of 200 $\mu\text{M FeCl}_3$, 1.04 mM EDTA (1:1 v/v), 200 $\mu\text{l H}_2\text{O}_2$ (1.0 mM) and 200 μl ascorbic acid (1 mM) were mixed to form a reaction mixture. After an incubation period of one hour at 37°C, the extent of deoxyribose degradation was measured by the thiobarbituric acid (TBA) reaction. Afterward, the reaction mixture was kept boiling with 1.5 ml of 2.8%

tri-chloroacetic acid (TCA) for 20 minutes at 100°C. RSC was calculated by the following equation:

$$\text{RSC} = ((A_0 - A_1)/A_0) \times 100,$$

where A_0 is the control, A_1 – the sample solution absorbance. Removal capacity of $\cdot\text{OH}$ radicals of the extract was expressed as a percentage of inhibition of deoxyribose degradation (%I).

Statistical analysis. Statistical significance was performed using a one-way analysis of variance (ANOVA) followed by comparisons of means using Duncan's multiple range test ($P < 0.05$). The correlation analysis and ANOVA were done using the software STATISTICA, version 10 (StatSoft Inc., USA). Because of the great differences in the absolute values of the particularly investigated parameters and for the sake of their mutual comparison, the results were expressed as the percentage of the control.

Results and discussion

Highly significant differences were found for all main sources of variation (salt stress level, cultivar) and interaction between them for SOD activity, GPX activity, LP intensity, DPPH-RSC, $\cdot\text{OH}$ radicals and FRAP (Table 1).

Table 1. Analysis of variance of main effects and interactions for oilseed rape cultivars

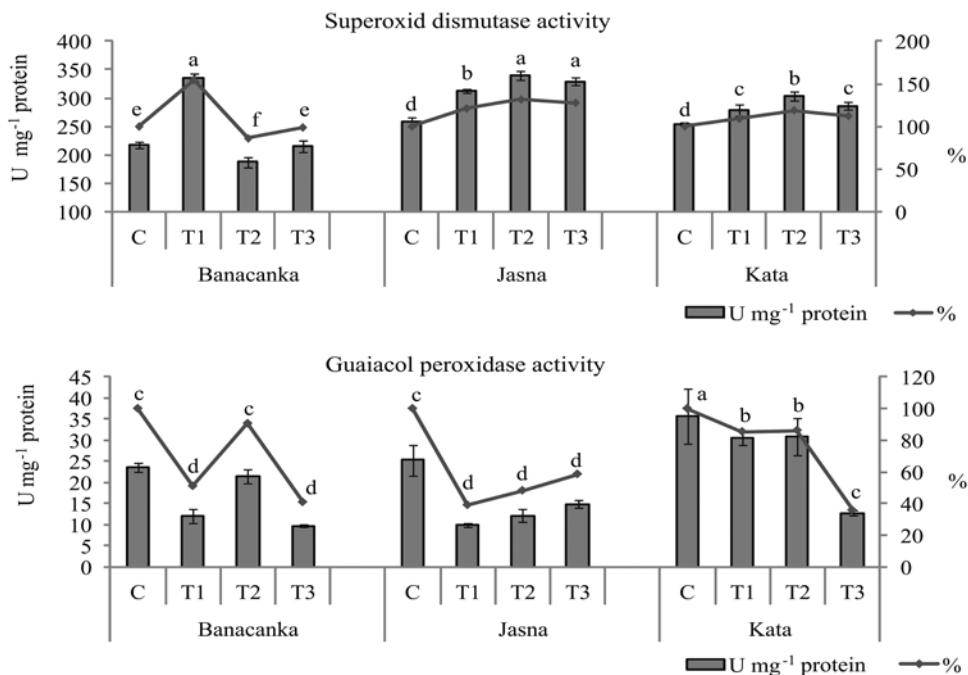
Source of variation	SS %	MS	P	SS %	MS	P	SS %	MS	P					
		SOD				GPX				LP				
Salt stress (S)	22.5	6419.2	0.000**	43	401.7	0.000**	93.9	75718.1	0.003**					
Cultivar (C)	35.6	1517.1	0.000**	37.2	521.4	0.000**	2.7	3206.4	0.000**					
S \times C	41.9	5961.1	0.000**	19.8	92.3	0.000**	3.4	1366.1	0.004**					
		GSH				$\cdot\text{OH}$ radicals				FRAP				
Salt stress (S)	74.4	18.2	0.000**	90.8	464.6	0.000**	71.9	7.1	0.003**					
Cultivar (C)	18.1	6.6	0.004**	7.8	59.1	0.000**	9.7	1.4	0.000**					
S \times C	7.5	0.9	0.001**	1.4	3.7	0.000**	18.4	0.9	0.000**					
		DPPH-RSC				SS – sum of squares, MS – mean squares; SOD – superoxide dismutase, GPX – guaiacol peroxidase, LP – lipid peroxidation intensity, GSH – reduced glutathione quantity, $\cdot\text{OH}$ radicals – hydroxyl radical scavenging, FRAP – ferric reducing antioxidant power, DPPH-RSC – radical-scavenging capacity; ** – $P < 0.01$								
Salt stress (S)	49.3	30.6	0.000**											
Cultivar (C)	34.3	31.9	0.000**											
S \times C	16.4	5.1	0.002**											

The activity of antioxidant enzymes. The values of SOD at different NaCl concentrations varied as following: 187.29–336.29 U mg^{-1} ('Banacanka'), 312.64–339.23 U mg^{-1} ('Jasna') and 277.90–303.45 U mg^{-1} ('Kata') of proteins (Fig. 1). In cultivars 'Jasna' and 'Kata' shoots, the activity of this enzyme was gradually increased by increasing the salt stress level. Furthermore, an increase in SOD activity was observed to a concentration of 150 mmol dm^{-3} NaCl, while it was reduced at the highest concentration. These two cultivars responded in the same manner to the same salt stress level. In contrast, cultivar 'Banacanka' had the highest values at the concentrations of 100 mmol dm^{-3} NaCl.

Studying the SOD activity in salt-tolerant and salt-sensitive oilseed rape varieties, Jalali-e-Emam et al. (2011) have shown that this enzyme plays an important role when it comes to salinity tolerance. Decreased SOD activity and other antioxidant enzymes at extremely high salt concentrations could be caused by difficult

absorption of sufficient amounts of water due to the high concentration of ions in the root zone (Dai et al., 2009). If a plant does not absorb water, the amount of salt in the plant will be reduced which inhibits the function of the antioxidant system. Another possible reason for the decrease in the activity of this enzyme at high levels of salt stress is a lot of toxic Na^+ ions which affect the generation of a large amount of reactive oxygen species (ROS) that the antioxidant defence system is unable to control. Also, the decrease of the SOD activity indicates that the scavenging activity of this enzyme may be distracted or results in plant adaptation to salt stress (Shahbazi et al., 2011).

The GPX activity in the shoots at different concentrations of NaCl kept going from: 9.68–21.44 U mg^{-1} ('Banacanka'), 9.90–14.85 U mg^{-1} ('Jasna') and 12.83–30.86 U mg^{-1} ('Kata') of proteins (Fig. 1). When it comes to the cultivars 'Banacanka', 'Jasna' and 'Kata', there was a significant decrease in the GPX activity with



Note. C – control, T1 – 100 mmol dm⁻³ NaCl, T2 – 150 mmol dm⁻³ NaCl, T3 – 200 mmol dm⁻³ NaCl; means with similar letter are not significantly different, compared by Duncan's multiple comparison test ($P = 0.05$).

Figure 1. Changes in the antioxidant enzymes – superoxid dismutase (SOD) and guaiacol peroxidase (GPX) – activity in oilseed rape cultivars after the treatment with different NaCl concentrations

a higher intensity of salt stress. The most prominent decrease appeared at the concentration of 200 mmol dm⁻³ NaCl in comparison to the control of the following cultivars – 'Kata' (64.17%) and 'Banacanka' (59.11%).

As previously noted, salinity leads to the formation of stress conditions which disrupt the system of electron transport by creating a large amount of ROS, which may cause significant damage to cells (Tanou et al., 2009). In these conditions, peroxidases play an important role because they catalyze the reactions in which phenolic substrates are oxidized, while the ROS are reduced to less harmful forms of oxygen. Regulating the availability of H₂O₂, peroxidases affect growth or cell elongation, as well as the connection of phenolic components in order to inhibit growth.

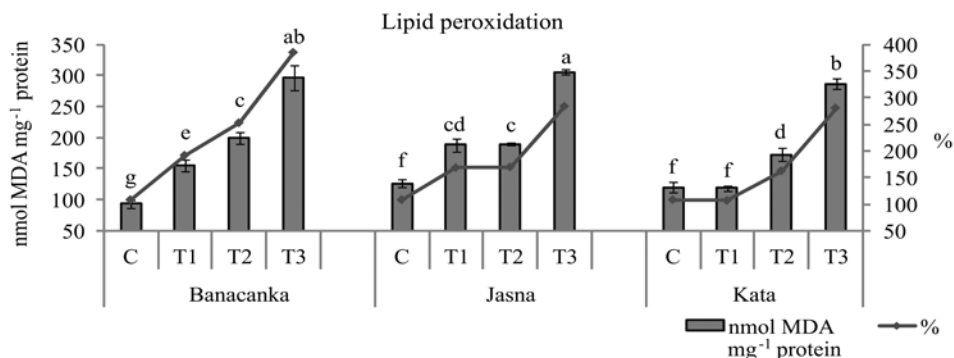
Tolerant species or varieties are characterized by increased activities of antioxidant enzymes and in regard to this, a higher SOD activity was found in this experiment in cultivars 'Jasna' and 'Kata'. Similar

increase of SOD activity was reported by Zare and Paknyat (2012) in fifteen oilseed rape cultivars, exposed to two levels of salt stress. Our research results confirm that the increased activity of antioxidant enzymes of salt-tolerant varieties indicate that scavenging of ROS might be a part of the overall adaptive plant strategy in response to stress conditions (Mittal et al., 2012).

Lipid peroxidation (LP). This parameter was predominantly influenced by the salt stress level (93.9%), while cultivar amounted to 2.7% and interaction salt stress level × cultivar to 3.4% of the total variability.

In all examined cultivars, there was a gradual increase in the intensity of LP with a higher concentration of NaCl. Cultivar 'Banacanka' showed the highest (237.21%) increase at the highest salt stress level, while 'Kata' showed the lowest (147.73%), compared to the control (Fig. 2).

Due to the extremely high vulnerability of lipids to oxidation, the main product of lipid peroxidation-



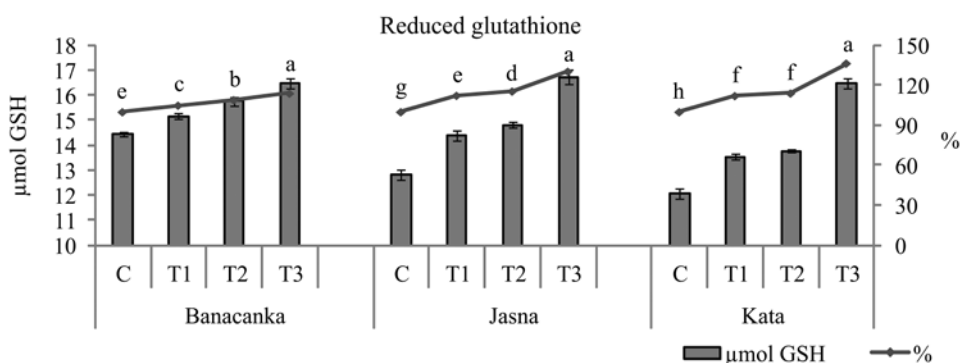
Explanations under Figure 1

Figure 2. Changes in lipid peroxidation (LP) in oilseed rape cultivars after the treatment with different NaCl concentrations

MDA could be considered as a potential marker for levels of oxidative stress *in vivo* (Popovic et al., 2013). Lower values of the LP intensity in salt-tolerant varieties and species indicate that the plants were better protected against oxidative stress in saline conditions (Tayefi-Nasrabadi et al., 2011). Although the capacity of the removal of hydroxyl radicals functions well, the intensity of lipid peroxidase was high in all examined cultivars during exposure to salt stress conditions. A strong negative correlative relationship between $\cdot\text{OH}$ radicals and LP was observed in all cultivars: 'Banacanka' ($r = 0.87$), 'Jasna' ($r = 0.81$) and 'Kata' ($r = 0.77$). Such a relationship may indicate the importance of membrane lipids and their peroxidation for normal metabolic process under stressful conditions. The accumulation of ROS in the examined cultivars was inhibited by its enhanced $\cdot\text{OH}$

radical-scavenging ability and thus it is protected from lipid peroxidation of membrane systems and oxidative damage under salt stress during germination and early seedling growth. The cultivar 'Kata' showed the highest values of this parameter pointing to intensive lipid degradation and a high level of oxidative stress.

Reduced glutathione (GSH). Like LP intensity, the amount of GSH was predominantly influenced by the salt stress level (74.4%), while cultivar amounted to 18.1% and interaction salt stress level \times cultivar to 7.5% of the total variability. In all cultivars, this parameter enhanced with the increasing NaCl concentration. There are no statistically significant differences between the concentrations of 100 and 150 mmol dm^{-3} NaCl in cultivars 'Jasna' and 'Kata'. The lowest value was observed in cultivar 'Kata' at all stress levels (Fig. 3).



Explanations under Figure 1

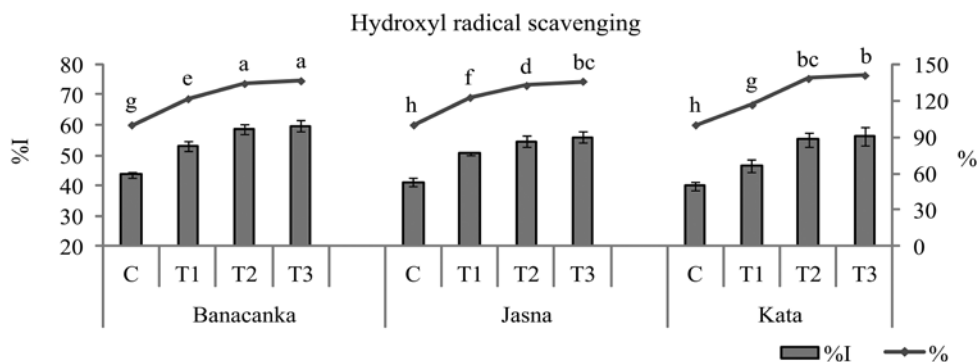
Figure 3. The amount of reduced glutathione (GSH) in oilseed rape cultivars after the treatment with different NaCl concentrations

GSH is the non-enzymatic ROS scavenging system which directly reacts with free radicals by detoxifying them. Based on these results, it could be suggested that salt stress leads to the increased amounts of GSH in oilseed rape seedlings in different cultivars, and it can be assumed that this parameter contributes to oxidative stress tolerance caused by high concentrations of NaCl. This antioxidant is involved in ROS detoxification, redox signaling and modulation of gene expression and regulation of enzyme activity (Foyer, Noctor, 2009). The importance of glutathione in plant growth and development is reflected in the fact that the reduced synthesis of glutathione is in direct correlation with growth disorders (Vernoux et al., 2000), and also

the fact that complete absence of glutathione synthesis causes plant death. Furthermore, the strong negative correlative relationship between the amount of GSH and the percentage of germination at both tests points to an important role of non-enzymatic antioxidants in the process of adapting to salinity. These results indicate that changes in the GSH content and thus in the glutathione-acrobats cycle lead to the reduction of oxidative damage, improving tolerance to oxidative stress.

Hydroxyl radical scavenging ($\cdot\text{OH}$ radicals).

The value of $\cdot\text{OH}$ radical scavenging activities at different concentrations of NaCl ranged as following: 53.11–59.60%I ('Banacanka'), 50.53–56.02%I ('Jasna'), 46.52–56.25%I ('Kata') (Fig. 4).



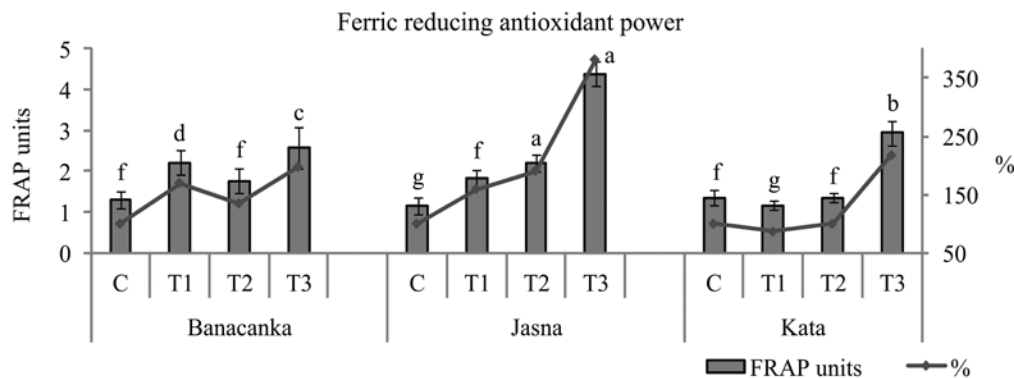
Explanations under Figure 1

Figure 4. Hydroxyl radical scavenging ($\cdot\text{OH}$ radicals) in oilseed rape cultivars after the treatment with different NaCl concentrations

In all cultivars, there was an increase in the capacity of $\cdot\text{OH}$ radicals scavenging activities with increasing concentrations of NaCl, but the difference between the concentrations of 150 and 200 mmol dm^{-3} was not statistically significant. Environmental stresses, including high salinity and drought, cause increased production and accumulation of ROS leading to oxidative stress and disturbance of normal cellular activity (Tanou et al., 2009). One of the most toxic ROS is the hydroxyl radical. In addition, there is no enzymatic reaction that can directly eliminate this highly reactive and toxic radical. Therefore, its accumulation inevitably leads to a reaction that damages the thylakoid membrane and

disturbs the functions of photosynthetic apparatus (Cruz de Carvalho, 2008). Due to the high reactivity of $\cdot\text{OH}$ radicals in biological systems, the ability of plant cells to neutralize the reactive radicals is of great importance for protection against oxidative stress. The data showed that all oilseed rape cultivars have the extensive capability to neutralize $\cdot\text{OH}$ radicals and that this capacity rises along with increasing levels of stress.

Ferric reducing antioxidant power (FRAP). In all cultivars, the highest value of this parameter was at the highest stress level, while 'Jasna' showed the most significant increase (281.85%), compared to the control (Fig. 5).



Explanations under Figure 1

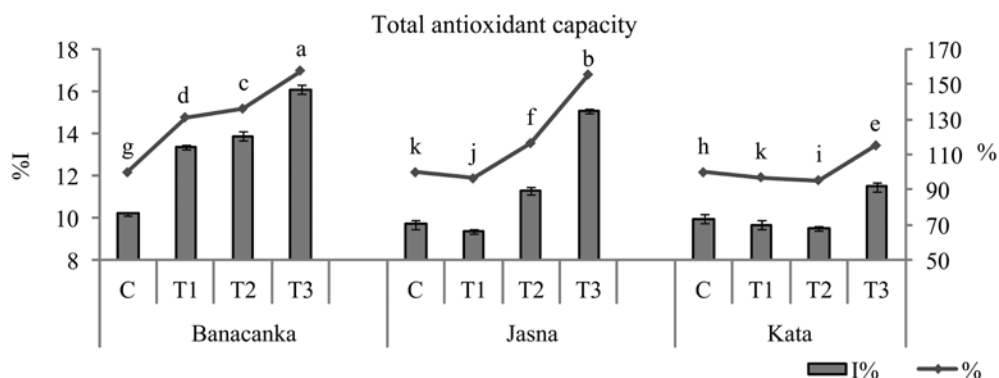
Figure 5. Ferric reducing antioxidant power (FRAP) in oilseed rape cultivars after the treatment with different NaCl concentrations

Cultivars 'Banacanka' and 'Jasna' showed an increase in the total antioxidant activity at all NaCl concentrations, compared to the control, while in 'Kata' this indicator was the lowest at the lowest level of stress. The obtained results indicated that the level of salt stress affected the total antioxidant activity in all cultivars. Since it is proved that an efficient antioxidant system can provide better protection against oxidative stress (Kumar et al., 2011; Stajner et al., 2013), the total antioxidant activity expressed in FRAP units has an important practical significance in the ranking of varieties and species according to tolerance to oxidative stress. In cultivar 'Banacanka' the increased value of FRAP was at the lowest NaCl concentrations, which may indicate that small amounts of NaCl have a stimulating effect on the activation of the antioxidant defence system.

Radical-scavenging capacity (DPPH-RSC).

This parameter was mostly under the influence of the salt stress level (49.3%) and cultivar (34.3%). As indicated in Figure 6, there was observed a gradual increase of DPPH scavenging activity with increasing concentrations of NaCl in cultivar 'Banacanka', and the increase was most significant at the concentration of 200 mmol dm^{-3} . In cultivar 'Jasna', the decrease of activity of this parameter was observed at the lowest level of stress, but at the maximum concentration, there is an increase of 55.57% in comparison to the control. In cultivar 'Kata', this parameter decreased at the lowest and moderate level of stress.

DPPH-RSC scavenging activity is determined by an indirect method which is based on the ability of the extract to reduce the activity of a moderately stable



Explanations under Figure 1

Figure 6. Radical-scavenging capacity (DPPH-RSC) in oilseed rape cultivars after the treatment with different NaCl concentrations

DPPH radical by an exchange of hydrogen atoms between the radicals and an antioxidant (Prakash, 2001). In salt stress conditions, cultivars 'Banacanka' and 'Jasna' compared to 'Kata' showed a better scavenging ability. These results may indicate that higher concentrations of NaCl (150 mmol dm⁻³) affect the imbalance between ROS generation and the process of neutralization. This is corroborated by the results of reduced LP and increased SOD activity in these two cultivars. On the other hand, Miljus-Djukic et al. (2013) reported that

plants have a higher capacity to resist salt stress at high NaCl concentrations such as 150 and 200 mmol dm⁻³ if the change in DPPH-RSC activity does not vary significantly with increasing stress level, as it is the case with cultivar 'Kata'.

Germination. Highly significant differences were stated only for salt stress level while cultivar and interaction between these two main sources of variation were not significant for seed germination at both (SLM and AA) tests (Table 2).

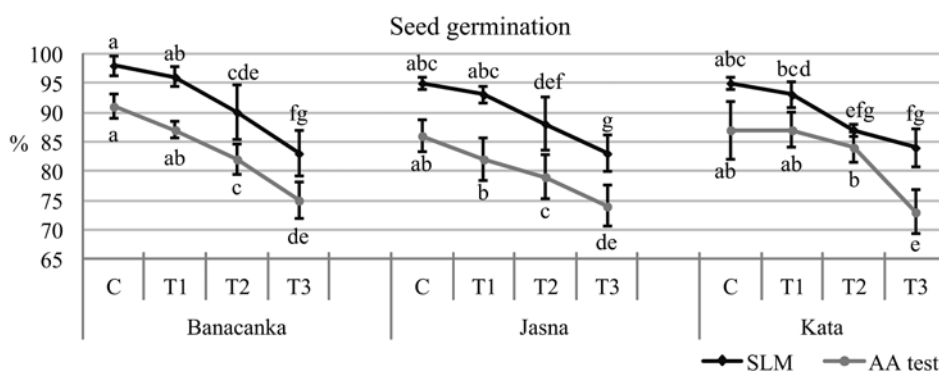
Table 2. Analysis of variance (ANOVA) of main effects and interactions for oilseed rape cultivars (germination, shoot length and root length) for SLM and AA tests

Source of variation	SS (%)	MS	P	SS (%)	MS	P	SS (%)	MS	P
Standard laboratory method (SLM)									
	germination			shoot length			root length		
Salt stress level (S)	94.6	299.0	0.000**	95.9	2140.5	0.000**	88.0	7692.7	0.000**
Cultivar (C)	3.8	18.2	0.111	3.7	124.2	0.004**	7.3	955.8	0.000**
S × C	1.6	2.5	0.911	0.4	4.1	0.967	4.7	204.6	0.002**
Accelerated aging (AA) test									
	germination			shoot length			root length		
Salt stress level (S)	87.8	342.7	0.000**	98.0	4623.2	0.000**	95.3	9712.7	0.000**
Cultivar (C)	6.9	40.1	0.032	0.4	31.8	0.119	2.9	450.7	0.000**
S × C	5.3	10.4	0.432	1.6	38.0	0.033	1.8	92.7	0.007**

SS – sum of squares, MS – mean squares; ** – $P < 0.01$

The results of this experiment show that the increasing of concentrations of NaCl in germination media significantly decrease seed germination of oilseed rape in both (SLM and AA) tests (Fig. 7). In all cultivars, significant differences in germination were not observed between the controls and the lowest salt concentration (100 mmol dm⁻³ NaCl), but a significant decrease occurred only at higher salt concentrations (150–200 mmol dm⁻³ NaCl). In cultivar 'Banacanka' there was noticed the biggest difference between the control and the highest concentration (14.6% in SLM, 16.8% in AA test).

However, after the accelerated aging the highest (9.8–13.1%) seed decay was observed in cultivar 'Jasna' at all NaCl concentrations, compared to the control. These results confirm the classification in which oilseed rape is considered moderately tolerant to salinity. Tavakkoli et al. (2010) suggest that very often it is not known whether the reduction in seed germination at high salt levels is caused primarily by disturbances in osmotic regulation, which leads to difficulties in the absorption of water in saline conditions, or is the result of toxic effects of Na⁺ and Cl⁻ ions which also can be manifested.



Explanations under Figure 1

Figure 7. Seed germination at the standard laboratory method (SLM) and the accelerated aging (AA) test in oilseed rape cultivars after the treatment with different NaCl concentrations

Similar relations between the cultivars, but lower germination were observed at the AA test. Performing this test seeds absorb water from the humid environment and thereby the moisture content in seeds increases, while the high temperature leads to accelerated aging and deterioration of seeds simultaneously (ISTA, 2015).

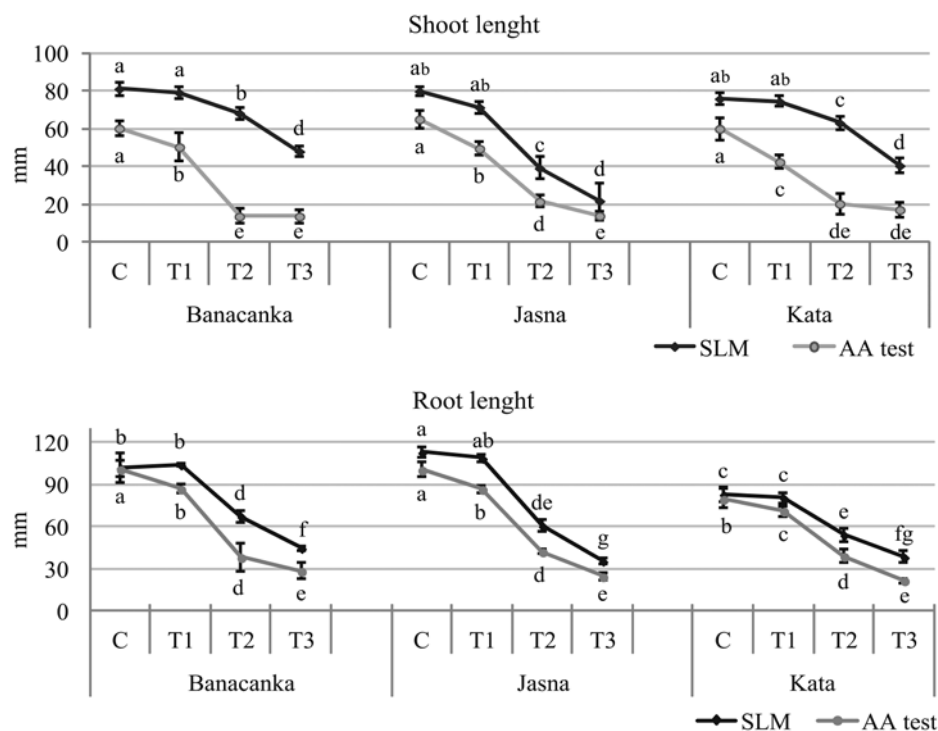
In all the cultivars, there was a significant correlation between seed germination and all parameters

of antioxidative status, except GPX activity, confirming the activation of the antioxidant defence mechanism in the saline conditions during germination process. The largest decrease in germination in stressed conditions in both tests was observed in cultivar 'Jasna'.

Seedling growth. In order to examine the influence of different NaCl concentrations on morphological changes of seedlings, growth parameters

were determined. The most important source of variation for shoot length was salt stress level while effect of cultivar was statistically significant only at the SLM and salt stress level \times cultivar interaction at AA test (Table 2).

The results of seedling length show that at lower NaCl concentrations both shoots and roots still have the capacity to cope with salt stress, whereas at higher concentrations, there is a significant occurrence of damage and necrosis of tissue (Fig. 8).



Explanations under Figure 1

Figure 8. Shoot and root length at the standard laboratory method (SLM, above) and the accelerated aging (AA) test (below) in oilseed rape cultivars after the treatment with different NaCl concentrations

Cultivar 'Jasna' had the lowest values of shoot growth at SLM and the highest value at AA test in control. Also, in this cultivar, there was the largest reduction at the concentration of 150 mmol dm⁻³ NaCl (25.1% at SLM, 67.3% at AA test) and at the concentration of 200 mmol dm⁻³ NaCl (55.3% at SLM, 78.7% at AA test). In cultivar 'Kata', root length was lowest at all concentrations of NaCl in both tests, while in 'Jasna' there were observed the highest values of this parameter only in SLM. The obtained results in the AA test were significantly lower than the values obtained at SLM in the control and all salt treatments. A significant reduction in growth at all NaCl concentrations indicates that the seeds which have been exposed to the accelerated aging treatment are significantly less able to resist even the lowest level of salt stress.

Salinity leads to the reduction in cell division and cell elongation, primarily because of the difficulty to absorb nutrients mostly due to the accumulation of large amounts of reactive oxygen species, inhibition of cellular enzymes, loss of turgor and hormone imbalance which affects plant growth, and later has a negative effect on the production of biomass and yields (Ashraf et al., 2010). Abdel Haleem et al. (2012) have suggested that salinity causes growth reduction in *Vigna unguiculata* at various NaCl concentrations (50, 100 and 200 mmol dm⁻³).

Analysing the differences between the shoot and length, depending on the concentration of NaCl, at both tests it was observed that the values were the most similar at the control. These results may suggest that

high concentrations of NaCl were the dominant factor in compared to the other negative factors covered AA test, such as high temperature and humidity.

Plant species and cultivars that have better seed germination and seedling growth under salt stress will be more tolerant to stressful conditions in the later stages (Ashraf, McNeilly, 2004). There was noted a positive correlation between salinity tolerance at seedlings stages and adult plants of *Brassica* genus (Ashraf, Ali, 2008).

Conclusions

1. Salt stress level, cultivar, and salt stress level \times cultivar interaction were significant sources of variation for all the analysed parameters: superoxide dismutase (SOD), guaiacol peroxidase (GPX), reduced glutathione (GSH) quantity, lipid peroxidation intensity (LP), radical-scavenging determination (DPPH-RSC), hydroxyl radical scavenging (\cdot OH radicals) and ferric reducing antioxidant power (FRAP) except for germination and shoot length.

2. Different salt (NaCl) concentrations led to the induction of both enzymatic and non-enzymatic antioxidants in the seedling shoots of oilseed rape. The mechanisms that enable tolerance to salt stress in oilseed rape include increase of LP, GSH and SOD activity while GPX activity was reduced. The antioxidant capacity measured by FRAP, DPPH and \cdot OH radicals was also activated.

3. Although there were clear differences between cultivars, considering the parameters of the antioxidant status under salt stress, NaCl concentration was a

predominant source of variation causing the activation of defence mechanisms in seedlings.

4. Salt stress level at the concentrations of 150 and 200 mmol dm⁻³ NaCl significantly reduced seed germination rate and seedling growth limiting normal function at the cellular level. 'Jasna' was clearly proved to be the most tolerant cultivar because of the highest SOD activity and accumulation of antioxidants.

5. Testing seed viability complemented with the determination of the antioxidant parameters were shown to be useful indicator for the selection of oilseed rape tolerant cultivars under the conditions of salinity. This fact is especially important considering that the problem of salinity is very complex and that a large number of parameters are responsible for tolerance.

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Druskos poveikis rapsų daigų gyvybingumui ir antioksidaciniam aktyvumui

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Santrauka

Tyrimo tikslas – nustatyti druskos (NaCl) sukkelto streso įtaką trijų veislių – ‘Banacanka’, ‘Jasna’ ir ‘Kata’, rapsams (atitinkamo – sėjamojam rapsui, *Brassica napus* L.). Atsakas į druskos poveikį vertintas pagal antioksidacinius fermentus superoksido dismutazę (SOD) ir 2-metoksifenolio gvajakolo peroksidazę (GPX), sumažėjusią glutationo (GSH) koncentraciją, lipidų peroksidacijos (LP) intensyvumą, radikalų surišimo difenil-1-pikrilhidrazilo hidratu (DPPH-RSC) gebą, hidroksilo radikalų surišimą ir Fe³⁺ redukavimo į Fe²⁺ testą (FRAP).

Rapsų sėklos septynias dienas daigintos trijų koncentracijų – 0, 100, 150 ir 200 mmol dm⁻³ NaCl tirpale. Jų gyvybingumas buvo vertintas laboratorijoje pagal daigumą, daigų ir šaknų ilgį, taikant sėklų pagreitinto senėjimo testą.

Dėl druskos poveikio visų trijų veislių rapsų daigų padidėjo lipidų peroksidacija ir sumažėjo GPX aktyvumas. Nustatyta, kad veikiant didžiausios koncentracijos NaCl tirpalu, pažeidžiamiausi buvo veislės ‘Banacanka’ augalai, kurių lipidų peroksidacija padidėjo 237 %, lyginant su kontroliniais. Veikiant skirtingos koncentracijos NaCl tirpalu buvo indukuotas visų trijų veislių rapsų augalų SOD aktyvumas. Taip pat buvo indukuota antioksidacinė geba, įvertinta pagal FRAP, DPPH-RSC ir ‘OH radikalus; esant didžiausios koncentracijos NaCl tirpalui, ypač sustiprėjo veislės ‘Jasna’ rapsų FRAP (282 %). Dėl druskos poveikio sumažėjo visų tirtų veislių rapsų sėklų daigumas, daigų ir šaknų ilgis.

Pagal didžiausią SOD aktyvumą ir daugiausia sukauptų antioksidantų veislės ‘Jasna’ rapsai buvo labiausiai prisitaikę prie druskos sukkelto streso. Be to, šis tyrimas rodo, kad kai kurie antioksidacinės gebos rodikliai gali būti naudingi biocheminiai žymekliai atrenkant druskingumui atsparias rapsų veisles.

Reikšminiai žodžiai: antioksidaciniai fermentai, *Brassica napus*, daigumas, druskos sukeltas stresas, lipidų peroksidacija, pagreitinamas senėjimas.