EFFECT OF DROUGHT STRESS ON ANTIOXIDANT STATUS OF WHEAT SEEDLINGS

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Abstract

The aim of this experiment was to study antioxidant status of two wheat varieties under drought stress. Investigated parameters were: relative water content (RWC), lipid peroxidation, proline content, and activity of antioxidant enzymes (SOD, CAT, and POD). The plants were grown for 3 weeks before drought was imposed by completed withheld of watering for 6 days. The results showed that ability of some wheat varieties to enhance enzymatic antioxidant activities might be an important attribute linked to drought tolerance. This could limit cellular damage caused by active oxygen species during water deficit.

Introduction

Wheat is one of the most important food staples in Serbia with an average annual production of 2.5 million tons. In the EU, wheat accounts for 47% of cereals with an annual production of over 140 million tons. Apart from the economic importance, wheat have significant place in human diets because of its high amount of carbohydrates, minerals, proteins, vitamins, and phenolic compounds [1].

Drought is the most important limiting factor for plant growth and productivity and it is becoming an increasingly severe problem in many regions of the world. It is estimated that drought causes an annual loss of 20% in wheat yield [2]. Under unfavorable conditions, such as drought, plants suffer from oxidative stress that affects numerous metabolic process and cause damages to DNA, proteins, and lipids. Accumulation of reactive oxygen species (ROS) and activation of antioxidant system in plants is observed by many authors [3, 4]. Major ROS-scavenging enzymes are superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), and glutathione-reductase (GR). Antioxidants of low molecular weight such as ascorbic acid, tocopherol, and glutathione are also present in wheat. Active together they contribute to maintaining redox balance in cells.

There is a much variation in drought tolerance among wheat genotypes. Comparative investigations of Paul et al. [5] have shown that observed wheat cultivar –dependent differences can be related to plants antioxidant capacity.

The aim of this work was to study the variation in antioxidative enzymes activity (SOD, CAT, and POD), level of lipid peroxidation, proline content and electrolyte leakage in wheat leaves imposed to drought stress.

Experimental

Wheat seeds (*Triticum aestivum* L. cv. ‘NS 40S’, cv. ‘Renesansa’) were obtained from Institute of Field and Vegetable Crops, Novi Sad, Serbia and stored in refrigerator. Seeds were surface sterilized with 70% ethanol for 5 min and then with 5% NaOCl for 15 min and then rinsed with tap water several times. Seeds were sawn in pots with mixture of soil and send (7:2). Drought stress was imposed on 20 day-old wheat seedlings by stop watering pots for 5 days. The seedlings (green part) were excised, rapidly weight (1 g) and ground with pestle in an ice

cold mortar with 10 ml of 100 mM phosphate buffer, pH 7. One part of plant material was frozen and stored at -70°C until use for determination of free proline content.

For determination of relative electrolyte leakage, ten pieces of fresh leaf samples were washed in deionized water and then were soaked in tubes with 20 ml of deionized water at 25°C. Electrical conductivity of bathing solution was measured after 24 h (L_1) using conductivity meter (model EL30, Mettler Toledo, USA). The tubes with samples were then incubated in a boiling water bath (100°C) for 25 min and a final conductivity reading (L_2) was taken after cooling to room temperature. Relative electrolyte leakage was calculated according to formula: REL (%) = (L_1/L_2) • 100% [6]. Lipid peroxidation (LP) was estimated by measuring the concentration of malodialdehyde (MDA) [7]. Absorbance was read at 532 and 600 nm and MDA content was calculated using an extinction coefficient 155 mM^{-1} cm^{-1} by subtracting the absorbance at 532 nm from that at 600 nm. The concentration of protein was measured according to Bradford [8]. The content of free proline was determined as describe earlier by Bates [9]. The superoxide dismutase activity was determined according to the method described by Giannopolitis and Ries [10] by measuring the ability of the enzyme extract to inhibit the photochemical reduction of nitro-blue tetrazolium. One unit of SOD activity was defined as the amount of enzyme required to produce a 50% inhibition of reduction of NBT at 560 nm. Catalase (CAT) activity was assayed according to Aebi [11]. The decomposition of H_2O_2 was followed spectrophotometrically by the decrease in absorbance at 240 nm. One unit of catalase activity corresponded to the amount of enzyme that decomposes 1 μmol of H_2O_2 per minute. The activity of POD was determined using oxidation of guaiacol, as described by Diaz et al. [12].

Results of the biochemical parameters represent data expressed as means of determinations made in triplicates and tested by ANOVA followed by comparisons of means by the Duncan's test ($p < 0.05$). Data were analyzed using software STATISTICA version 13.2 (StatSoft, Inc., USA).

Results and discussion

The results obtained in this study are presented in one figure and one table. The relative electrolyte leakage and lipid peroxidation are presented in Figure 1. Under drought stress relative electrolyte leakage and lipid peroxidation significantly increase, when compared to the controls (Fig.1). In general, lipid peroxidation, assayed as malondialdehide (MDA) formation, was higher in control leaves of cultivar 'Renesansa' (42.8 nmol MDA/g FW) than in control cultivar 'NS 40S' (29.9 nmol MDA/g FW). An increase in relative electrolyte leakage indicates deterioration in cellular membrane systems. Also, observed higher levels of MDA under drought conditions indicate oxidative damage of membrane lipids, resulting from uncontrolled free radical production. This result is in agreement with those of Popovic et al. [3], who investigated the responses of poplar clones to drought and reported significant increase of reactive oxygen and nitrogen species and lipid peroxidation.

Drought stress is associated with increased oxidative stress and induction of antioxidant enzymes is a general mechanism of adaptation strategy which plant use to overcome oxidative stresses [13]. In this study, activities of SOD, CAT, and POD were analyzed and presented in Table 1. The activity of SOD enzyme decreased under the drought stress conditions in the cultivar 'Renesansa', which is in agreement with findings of Tian and Lei [14] who found that SOD under severe drought stress decreased a lot. In the cultivar 'NS 40S' the values of SOD activity under stress were significantly above the control levels. The activity of CAT stayed at control level. The activity of POD had similar trends at both cultivars and did not significantly change under drought stress.

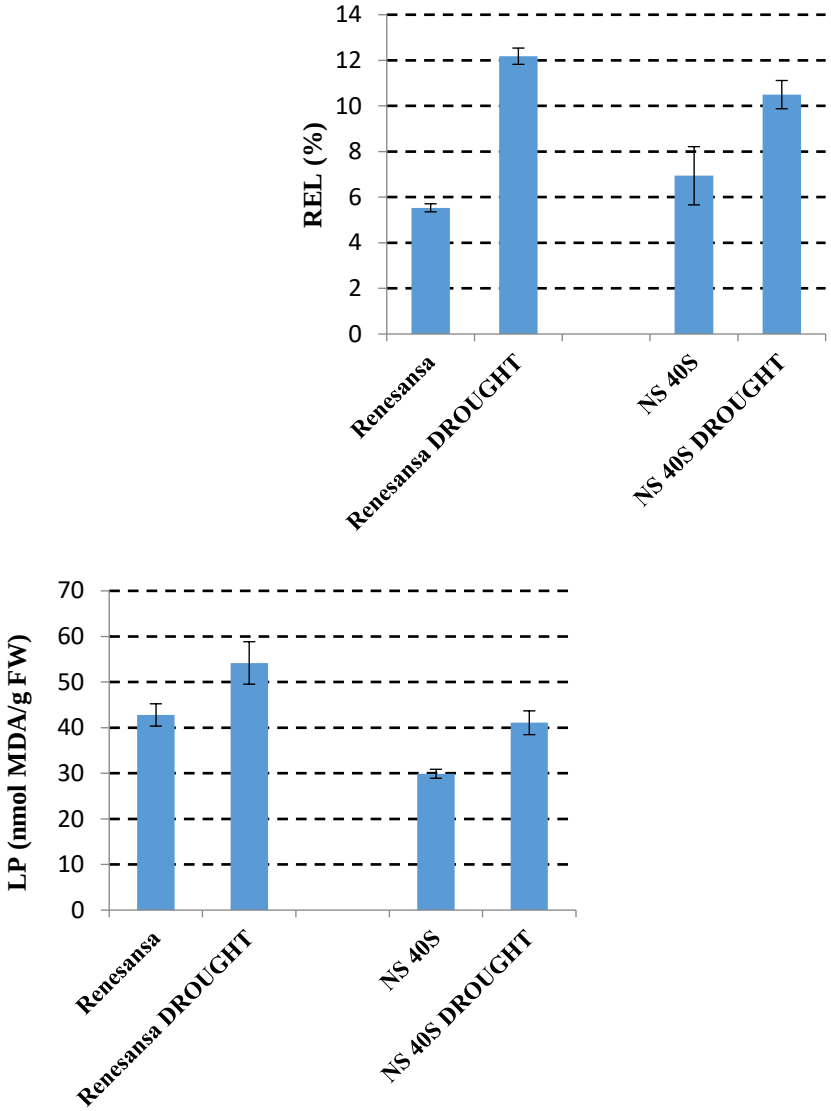

Figure 1. Effect of drought stress on relative electrolyte leakage (REL) (A) and lipid peroxidation (LP) (B) in wheat seedlings. Values are the means of the three different experiments. Error bars represent SD with $n = 3$. The bars with different letters indicate significance of difference at $p < 0.05$ level by Duncan's test.

Table 1. Antioxidant enzyme activities and PRO levels in wheat seedlings leaves of control and stressed plants

	cv.	control	drought
SOD (U mg^{-1} protein)	Renesansa	46.97^{a}	29.05^{b}
	NS 40S	29.95^{b}	47.49^{a}
CAT (nmol H_2O_2 mg^{-1} protein min^{-1})	Renesansa	2.92^{a}	2.97^{a}
	NS 40S	3.94^{a}	3.45^{a}
POD (µmol guaiacol mg^{-1} protein min^{-1})	Renesansa	5.48^{b}	5.81^{b}
	NS 40S	7.46^{a}	7.33^{a}
PRO (mg g^{-1})	Renesansa	10.07^{d}	60.72^{a}
	NS 40S	31.11^{c}	50.86^{b}

*Values marked with same letter do not differ significantly at $p < 0.05$ (Duncan's test)

Table 1 also shows the results concerning free proline accumulation in wheat seedlings. The highest free proline quantity was detected in drought stressed seedlings of cultivar 'Renesansa'. The proline content in our study is in consistency with results obtained by Paul et al. [5]. It is interesting to observe that proline induction was smaller in cultivar 'NS 40S' which showed the higher SOD activity under drought treatment. This indicate that cultivar 'NS 40S' was effectively preventing the formation of ROS by antioxidant systems without the need for a large extent of proline production. This is in agreement with previous investigations of Babić et al. [15] who indicated cultivar 'NS 40S' as drought tolerant cultivar. In contrast, cultivar 'Renesansa' suffered large electrolyte leakage under drought conditions also showed very high level of proline production, indicating the inefficiency of other antioxidant protective mechanisms.

Conclusion

Water stress is one of the most important environmental factors that regulate plant growth and development, and limit plant production. In our study, obtained data showed that drought stress caused oxidative damage to wheat seedlings through excessive generation of ROS. In both investigated cultivars drought stress increased electrolyte leakage and lipid peroxidation, as well as proline accumulation. In cultivar 'NS 40S' was observed higher activation of antioxidant system, so the use of this wheat cultivar in dry land conditions is supported by these results.

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