

ANTIOXIDANT ACTIVITIES IN SELECTED VARIETIES OF RED LETTUCE (*Lactuca sativa* L.) EXPOSED TO WATER DEFICIT STRESS

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ABSTRACT

The influence of water deficit stress on the leaf characteristic and antioxidant profile of three red lettuce varieties (*Lactuca sativa* L.), a commonly grown salad vegetable in the Cordillera region, was studied. The leaf area, leaf length and leaf width of the lettuce plants were reduced by medium water stress (MW) and low water stress (LW). However, significant increases in total phenolics (TP) and ascorbic acid (AA) were observed in LW-treated plants. This resulted in a 13% increase in total antioxidant capacity in Red Rapids and 8% increase for both Lollo Rosa and Falbala. Total anthocyanins, however, was not influenced by water stress imposition. Moreover, the enzymatic antioxidants (i.e., superoxide dismutase, ascorbate peroxidase and catalase) increased significantly in lettuce treated with MW and LW. Red Rapids showed the highest increase in enzymatic activity while the deep red varieties Lollo Rosa and Falbala exhibited higher increases in terms of TP and AA. This research indicates that controlled water deficit can increase TP, AA and enzymatic contents in red lettuce resulting in a higher valued crop with higher antioxidant content.

Key words: anthocyanins, antioxidant enzymes, DPPH radical scavenging, *Lactuca sativa*, phenolics

INTRODUCTION

Water deficit is an abiotic stress that elicits a wide range of plant responses, including altered gene expression and cellular metabolism to changes in growth rate and plant productivity (Shao et al. 2008). During water deficit stress, changes in cellular chemistry to adjust to the reduced turgor are well documented. A common initial response is the accumulation of osmolytes such as prolines and betaines to maintain turgor and to stabilize proteins (Boo and Jung 1999; Sperdouli and Moustakas 2012). Moreover, plant response to water stress involves the activation of complex antioxidant pathways to scavenge reactive oxygen species (ROS) that are generated within the cells (Gill and Tuteja 2010). In addition to their role as agents of plant defense, these metabolites are well-known medicinally bioactive compounds with beneficial health-related properties. Thus, a field of research attracting interest is the regulation of environmental stress that enhances phytochemical production for the purpose of growing more healthy horticultural crops. Water deficit stress has been used for inducing various phytochemicals in crops. Specifically, it has been found that there is correlation between antioxidant capacity and stress tolerance (Hameed et al. 2011; Sperdouli and Moustakas 2012). Mild water stress have been recommended in certain crops in order to increase health-promoting phytochemicals (Cisneros-Zevallos 2003). For instance, less irrigation doubled the glucosinolate levels in broccoli (Schreiner 2005). Glucosinolates are known to help prevent the occurrence of certain cancers in humans (Shapiro et al. 1998). In *Zingiber montanum*, a 120-day water deficit resulted in high volatile oil content (Manochai et al. 2010). In the study of Luna et al. (2012), the higher content of phenolic compounds was shown in lettuce cultivated with the lower irrigation regime as compared with higher irrigation. All these examples show that it is possible to regulate water stress to provide an opportunity to increase the

levels of these phytochemicals. Enhancing the phytochemical content of these crops not only increases their market value but also promotes health benefits to consumers.

Lettuce (*Lactuca sativa* L.) is one of the most commonly consumed fresh leafy vegetable (Coria-Cayupan et al. 2009). Currently, red lettuce is popular in salad mixes due to its anthocyanin content that contributes to the higher value it fetches compared to green lettuce (Mulabagal et al. 2010). The current interest of the populace on healthy food puts focus on the search for better and healthier options. Lettuce is already established as rich sources of several health promoting nutrients (Liu et al. 2007; Llorach et al. 2008). However, increasing the health potentials of this plant using abiotic stresses has been scarcely investigated. Also, specific studies addressing the impact of controlled irrigation (deficit and excess levels) on lettuce enzymatic activities are limited (Luna et al. 2012).

Antioxidant content is becoming a progressively more important parameter with respect to fruit and vegetable quality such that researches on its induction are significant undertakings. This research therefore sought to investigate the effect of water deficit stress on the antioxidative profile of red lettuce with the purpose of documenting whether such treatments actually result in antioxidant-enriched lettuce plant. Specifically, this study focused on red lettuce varieties exposed to regulated water deficit stress to determine the effect of water stress on non-enzymatic and enzymatic antioxidants.

MATERIALS AND METHODS

Plant materials and experimental design. Three red lettuce (*L. sativa* L.) varieties were used: Lollo Rosa and Falbala represent the deep red leaf varieties while Red Rapids represent the light red type. The seeds of these varieties were germinated and grown in seed beds. Seedlings grown in each seed bed (fifty holes) were thinned to two when they have developed two true (expanded) leaves. After 21 days, seedlings of same heights were transplanted into plastic pots (20 cm diameter and 25 cm height) containing three kilograms of sandy-loam soil (20% and 80%, respectively). One week after transplanting, the seedlings were thinned to one per pot and allowed to grow for another two weeks. All plants were watered daily to field capacity prior to assignment of water deficit treatments. Lettuce seedlings and plants were grown and maintained in greenhouses with full sunlight in Beckel, La Trinidad, Benguet (1,639 meters above sea level). Fertilizer was not used throughout the experiment.

Field capacity of the soil was determined using the gravimetric method of measurement. Water deficit treatments were based on the method of Alvarez et al. (2009) and Sanchez-Rodriguez et al. (2011) with some modifications. Briefly, at five to six weeks after planting (at vegetative stage), 45 healthy plants of similar sizes, for each red lettuce variety, were randomly assigned into three groups based on water treatment: Control (WW) – Well watered to field capacity; Medium watered (MW) – Watered to 60% of field capacity; Low watered (LW) – Watered to 30% of field capacity.

Imposition of the treatments was done every three days for two weeks. Soil water potential was measured every three days using an equitensiometer (EQ2xD-05 Delta-T Devices Ltd. United Kingdom). Three pots from each treatment were used to determine the mean water potential three days after the start of treatment (prior to rewatering). Average water potential was as follows: WW was -0.03 MPa; MW was at -0.18 MPa and for LW was at -0.55 MPa. After imposition of these treatments, leaf characteristics and antioxidant components were measured.

The study was conducted using a Randomized Complete Block Design (RCBD), with three replications. The main effect of water deficit stress levels (MW and LW) was investigated. In all the assays, there were also three replications and one replicate consisted of five plants. These assays were conducted at the Natural Sciences Research Unit, Saint Louis University.

Leaf characteristics. Leaf characteristics included leaf length, leaf width and total leaf area. This was done by tracing the edges of all the harvested leaves on clean bond papers. These were then cut and

weighed in an analytical balance. A one-by-one cm paper was also weighed. The recorded weight was transformed into leaf area (cm²) using ratio and proportion calculation.

Determination of total phenolics (TP) - Extraction and assay procedure followed the method employed by Volden et al. (2009) with some modifications. Freshly harvested leaf samples were washed with water, tap dried and 500 mg of cut leaves was prepared and placed in test tubes. Each tube was added with 20 mL of 1% hydrochloric acid in methanol and covered to prevent evaporation. Incubation was done in an ice bath for 24 hours. An aliquot (100 mL) of extracts or standard solutions of gallic acid (20, 40, 60, 80 and 100 mg/L) was added to small tubes containing 5 mL of distilled deionized water. Then, 500 mL of Folin-Ciocalteu's reagent (diluted 1:10 with distilled water) was added to the mixture and shaken. After 10 min, 1.5 mL of 7 % Na₂CO₃ solution was added to the mixture. After incubation for 25 min at room temperature, the absorbance against the prepared blank, methanol was read at 765 nm (Shimadzu UV mini 1240 UV-VIS, Japan). TP content was expressed as mg gallic acid equivalents (GAE)/100 g fresh weight.

Determination of total anthocyanin (TA) - TA content of lettuce was measured using the pH differential method of Sun et al. (2009) and You et al. (2011). Briefly, a 0.2 mL aliquot of acidic methanolic extract was diluted to a final volume of 4 mL using hydrochloric acid (pH 1.0) and in another tube using sodium acetate (pH 4.5) solution. After an equilibration period (15 min), the absorbance of each solution was measured immediately at 700 nm and 510 nm with methanol as the blank. The anthocyanin content was calculated using the formula of Sun et al. (2009) and was expressed as mg/100 g fresh weight.

Determination of total ascorbic acid (AA) - AA was determined following the method described by Barros et al. (2007). The dried methanolic extract (100 mg) was extracted with 10 ml of 3% metaphosphoric acid for 45 min using an orbital shaker at room temperature and filtered through Whatman No. 4 filter paper. One mL of filtrate was then mixed with 8 mL of 2,6-dichlorophenolindophenol, incubated for 3 min and the absorbance was measured at 515 nm against the solvent blank, methanol, within 30 min. Analytical grade L-ascorbic acid (0.020–0.12 mg/mL) was used for the standard curve construction and was expressed as mg/100 g fresh weight.

Determination of antioxidant activity - The antioxidant activity of the extracts was evaluated by the method as described by Duan et al. (2007). Leaf samples (0.5 g) were macerated and extracted using 15 mL analytical grade methanol. Then, 0.1 mL of the centrifuged methanolic extract was mixed with 2.9 mL of 0.1 mM diphenylpicrylhydrazine (DPPH)-methanol solution. DPPH assay measures hydrogen atom donating activity and hence provides a measure of free-radical scavenging antioxidant activity. Tubes were then incubated in the dark for 30 min at room temperature and the decrease in the absorbance at 517 nm was measured. The control and blank tubes contained methanol instead of the antioxidant solution and DPPH solution, respectively. Calculation of total antioxidant activity used the following equation: DPPH-scavenging activity (%) = [1-(absorbance of sample – absorbance of blank)/absorbance of control] x 100.

Assays for enzymatic antioxidants - Each 0.5 g of leaf sample was homogenized and proteins extracted according to Posmyk et al. (2009). Leaf homogenization was done using mortar and pestle in cold 10 mL 25 mM phosphate buffer (pH 7.8) containing 0.2 mM EDTA, 2 mM ascorbate and 2% (w/v) PVP (polyvinylpyrrolidone). The homogenate was strained then centrifuged at 4°C for 20 min at 13,000 x g, and the supernatant obtained was used for protein and enzyme analysis. Total protein was determined using the Bradford method (Bradford 1976).

Superoxide dismutase activity - Superoxide dismutase (SOD) activity was measured following the procedure of Giannopolitis and Ries (1977) with some modifications by Duan et al. (2007). The reaction mixture contained 1.3 mM riboflavin, 13 mM methionine, 63 mM nitrogen blue tetrazolium (NBT) in 0.1 M phosphate buffer (pH 7.8), and 50 mL of the enzyme extract in a final volume of 3 mL. SOD

activity was assayed by measuring the ability of the enzyme extract to inhibit the photochemical reduction of NBT. Glass cuvettes containing the mixture were illuminated under a fluorescent lamp for 15 min. Identical tubes that were not illuminated served as blanks. After illumination, absorbance was measured at 560 nm (Shimadzu UV mini 1240 UV-VIS, Japan). One unit of SOD was defined as the enzyme activity which was able to inhibit the photoreduction of NBT to blue formazan by 50%.

Ascorbate peroxidase activity - Ascorbate peroxidase (APX) levels was determined according to Jiang and Zhang (2002). One mL of the reaction mixture contained 50 mM potassium phosphate buffer (pH 7.0), 0.5 mM ascorbic acid, 0.1 mM H₂O₂ and 200 mcL enzyme extract. The reaction is monitored spectrophotometrically by following the decrease in A₂₉₀ for three min as ascorbate is oxidized.

Catalase activity - Catalase (CAT) activity was measured using the methods of Posmyk et al. (2009). The assay mixture contained 3.125 mM H₂O₂ in 50 mM phosphate buffer (pH 7.0) and 200 mcL of enzyme extract in a total volume of 3 mL. The activity was determined by monitoring the decrease in A₂₄₀ due to H₂O₂ consumption.

Statistical analysis. Experiments were carried out in triplicates and the data on antioxidant activity and content were reported as mean values ± standard error. Two-way analysis of variance (ANOVA) was performed on data to determine the difference between mean values using SPSS. The mean comparison was performed using Student Neumann-Keuls (SNK) at 5% level of significance.

RESULTS AND DISCUSSION

Growth and antioxidant synthesis are fundamental metabolic processes that may be altered by water deficit. As expected, the results showed that decrease in water availability limited leaf expansion in lettuce as indicated by decreased leaf length, width and area (Table 1). Greater growth in terms of the three leaf parameters (area, length and width) were observed in lettuce cultivated at WW regime compared to those grown under MW and LW deficit regimes. MW condition significantly decreased leaf area by 21% in Red Rapids and Lollo Rosa and by 15% in Falbala. These were accompanied by an almost similar percentage reduction in leaf length and leaf width. Moreover, LW plants showed the lowest growth among the three varieties with the greatest decrease in leaf area observed in Lollo Rosa, exhibiting a 38% reduction in leaf area as compared to the control (WW).

Table 1. Leaf characteristics of three lettuce varieties subjected to WW–well watered, MW–medium watered, LW–low watered conditions.

Water Regime	Varieties	Parameters		
		Leaf Area (cm ²)	Leaf Length (cm)	Leaf Width (cm)
WW	Red Rapids	138.9 ^a	19.7 ^a	15.2 ^a
	Lollo Rosa	141.3 ^a	17.5 ^b	14.4 ^a
	Falbala	129.4 ^a	18.0 ^b	12.7 ^{bc}
MW	Red Rapids	110.2 ^{bc}	15.5 ^c	11.6 ^{cd}
	Lollo Rosa	111.1 ^{bc}	15.0 ^c	13.5 ^{ab}
	Falbala	110.6 ^{bc}	15.5 ^c	11.9 ^{cd}
LW	Red Rapids	92.5 ^{cd}	15.6 ^c	10.0 ^e
	Lollo Rosa	87.1 ^d	15.7 ^c	11.1 ^{de}
	Falbala	90.9 ^{cd}	14.8 ^c	10.4 ^{de}

In each column, means marked with different letters are significantly different (p < 0.05)

During water deficit stress, compromised plant growth is attributed to reduction in turgor pressure and cell expansion (Bacelar et al. 2006; Liu et al. 2011). Despite this, the treatments used had a striking effect on the antioxidant quality of lettuce specifically on leaf non-enzymatic antioxidants and total antioxidant capacity indicating a shift in metabolic allocation within the plant. It was found that there was an increasing trend in total phenolics in relation to water deficit (Fig. 1). Among the three varieties, Red Rapids exhibited the lowest content in TP under all the water regimes while Lollo Rosa and Falbala had higher and comparable contents in both MW and LW conditions. However, when treated with LW regime, the highest increase was observed in Red Rapids, exhibiting an 18% increase in comparison to the control (WW), followed by Lollo Rosa and Falbala with an 8% and 6% increase, respectively.

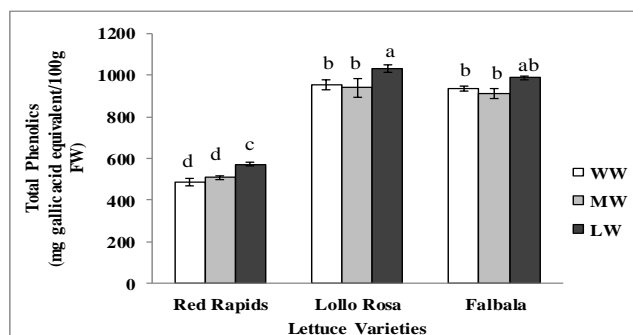


Fig. 1. Total phenolic contents in leaves of the lettuce varieties subjected to varying water regimes (WW–well watered, MW–medium watered, LW–low watered conditions). Vertical bars represent the standard error. Means marked with different letters are significantly different ($p < 0.05$).

This increase seems to be a general response of plants as this was also observed in other species including grapes and olives (Bacelar et al. 2006; Weidner et al. 2011). Increase in total phenolics could be attributed to the response of plants toward oxidative stress (André et al. 2009). Phenolic compounds that comprise a broad group of antioxidant phytochemicals act by inactivating lipid free radicals preventing decomposition of hydroperoxides into free radicals that may decrease the fluidity of membranes (Bacelar et al. 2006; Ksouri et al. 2007). Those assayed by the Folin-Ciocalteu method includes primarily the caffeic acid and chlorogenic acid derivatives (Kim et al. 2008; Mahmoudi et al. 2012). It is most likely, therefore, that these compounds increased in the lettuce varieties in this study.

In addition, it was found in literature that some phenolics are associated with slightly bitter taste of lettuce and can be the substrates for enzymatic browning (Vidal et al. 2018). In the present study, this was not particularly investigated. Nonetheless, it was observed that the appearance, texture and color of the lettuce plants did not noticeably change after the imposition of MW and LW treatments, despite the general decrease in leaf size or fresh mass. Quantitative investigation of these marketable characteristics, which influence buyer decisions or consumer preferences, may be included in future studies. A good direction was provided by Malejane et al. (2018) in a similar study. Lettuce growers should balance the benefits in terms of improved phytochemical profile and the drawbacks, i.e. reduced fresh weight.

Water deficit treatments did not significantly alter TA levels (Fig. 2). Previous studies revealed variable results as to the impact of water deficit on anthocyanin synthesis. No change in anthocyanin levels were observed in drought-stressed wheat and soybean (Alexieva et al. 2001; Shen et al. 2010). However, other plants showed increase in TA after exposure to drought stress and a combination of drought and UV-stress (Efeoglu et al. 2009; Yang et al. 2005). These seemingly discordant plant responses in terms of TA content to water deficit may be study-specific. Close and Beadle (2003) forwarded the theory that photoinhibition might have occurred in these studies, as the plants were also

exposed to light stress and subsequently tested for their response for water deficit stress. The anthocyanins may have been required more for photoprotection rather than for osmotic adjustment. In the present study, TP increased (Fig. 1) while TA remained unaffected (Fig. 2) under LW condition in all the varieties. This may indicate that the phenylpropanoid pathway was induced but the products synthesized were likely more of the simple phenolic acids (caffeic acid and chlorogenic acid) rather than the anthocyanins.

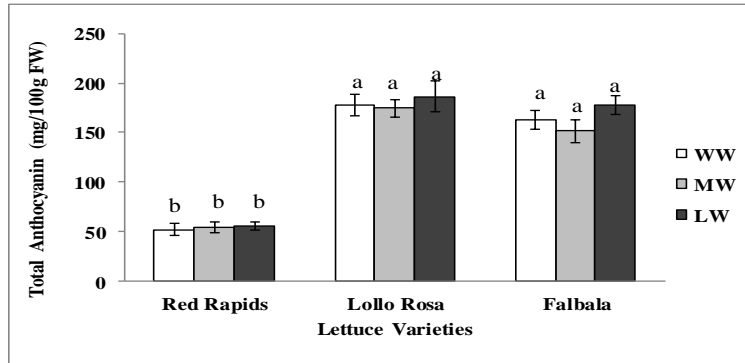


Fig. 2. Total anthocyanin content in leaves of the lettuce varieties subjected to varying water regimes (WW–well watered, MW–medium watered, LW–low watered conditions). Vertical bars represent the standard error. Means marked with different letters are significantly different ($p < 0.05$).

Another non-enzymatic antioxidant response related to water stress in plants is that of ascorbic acid synthesis. Together with TP, AA also increased with increasing severity of water stress (Fig. 3). The highest increase was observed in Falbala (55.4%) while the lowest was seen in Red Rapids (28.4%). Previous studies also demonstrated an increase in the AA content in different plant species subjected to water deficit, such as wheat and maize (Khanna-Chopra and Selote 2007). Ascorbate and its metabolism products during this type of stress are utilized to prevent the formation of ROS (Sanchez-Rodriguez et al. 2010). Ascorbic acid is a versatile compound that participates in plant stress response in multiple mechanisms. First, AA is needed in the Halliwell-Asada cycle or ascorbate-glutathione cycle. In this reaction, the enzyme APX scavenges H_2O_2 by utilizing ascorbate as the electron donor to reduce the radical to H_2O and O_2 (Leyva et al. 2011). Second, AA can directly eliminate several ROS, i.e. singlet oxygen, O_2^- and H_2O_2 , in a non-enzymatic way (Conklin 2001; Gill and Tuteja 2010).

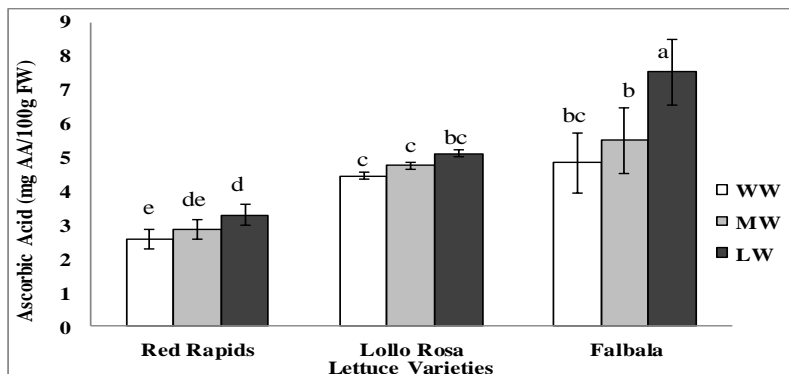


Fig. 3. Ascorbic acid content in leaves of the lettuce varieties subjected to varying water regimes (WW–well watered, MW–medium watered, LW–low watered conditions). Vertical bars represent the standard error. Means marked with different letters are significantly different ($p < 0.05$).

The increase in both phenolic compounds and ascorbic acid at LW was observed concurrently with increase in DPPH radical scavenging activity (Fig. 4). It is most likely then that these phytochemicals contributed to the significant increase in DPPH radical scavenging activity. Kang and Saltveit (2002) and Rebey et al. (2012) observed a correlation between the phenolic content and total antioxidant activity under drought, since phenolic compounds contribute directly to antioxidant activity. The same assumption was made for ascorbic acid by previous studies (Llorach et al. 2008; Boo et al. 2011).

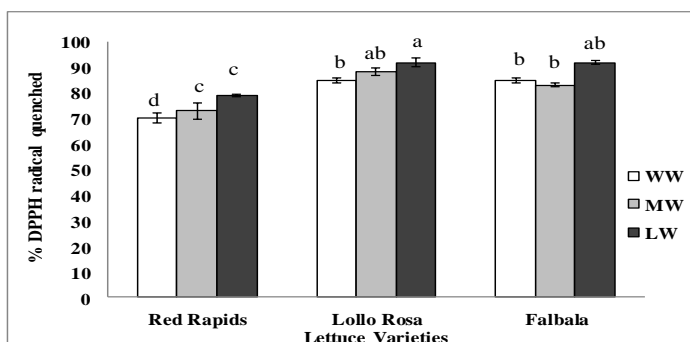


Fig. 4. Antioxidant activity of phenolic extracts of the lettuce varieties subjected to varying water regimes (WW–well watered, MW–medium watered, LW–low watered conditions). Vertical bars represent the standard error. Means marked with different letters are significantly different ($p < 0.05$).

Plant responses to water stress involves the activation of complex anti-oxidative pathways to scavenge reactive oxygen species (ROS) within cells by several means (Gill and Tuteja 2010). Since crops have different mechanisms to react to water deficit, improvements brought about by this stress will vary between crops. Enhanced DPPH scavenging activity was associated by Kang and Saltveit (2002) and Ksouri et al. (2007) with increased tolerance to abiotic stress. The results of this study therefore suggest that exposure of lettuce to water deficit elicited antioxidant metabolic pathways, which possibly were able to detoxify harmful ROS substrates thus affording the test plants protection from water deficit stress. Based on these observations, it can be said that antioxidant responses were dependent upon the severity or magnitude of the imposed stress as well as with the genotype. It was also noteworthy that in comparison to the non-enzymatic antioxidants, enzymatic antioxidants were generally increased at both MW and LW conditions (Fig. 5, 6 and 7).

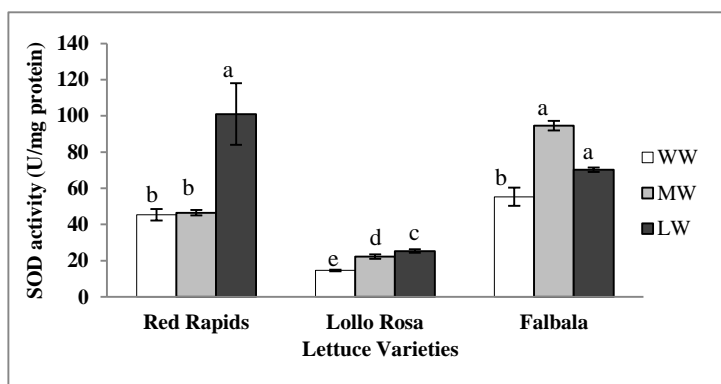


Fig. 5. Superoxide dismutase (SOD) activity in leaves of the lettuce varieties subjected to varying water regimes (WW–well watered, MW–medium watered, LW–low watered conditions). Vertical bars represent the standard error. Means marked with different letters are significantly different ($p < 0.05$).

Three enzymatic antioxidants were measured: SOD, CAT and APX. Increase in SOD activity was observed as the magnitude of water stress increased except in the case of Falbala (Fig. 5). MW condition increased SOD activity in the deep red varieties and not in Red Rapids ($p \leq 0.05$). Under LW condition, on the other hand, SOD activity increased significantly in all the lettuce varieties although the percentage of increase differed. SOD activity doubled in Red Rapids and rose by 73% and 27% in Lollo Rosa and Falbala, respectively.

An increasing trend in CAT activity was also observed in all the three varieties with increasing severity of water stress, although the magnitude of increase varied among them (Fig. 6). Red Rapids showed the highest increase in CAT activity exhibiting a twofold rise under MW condition and a 2.4 fold increase in enzyme activity under LW regime. On the other hand, Falbala exhibited the lowest percentage increase at 33% in terms of CAT activity.

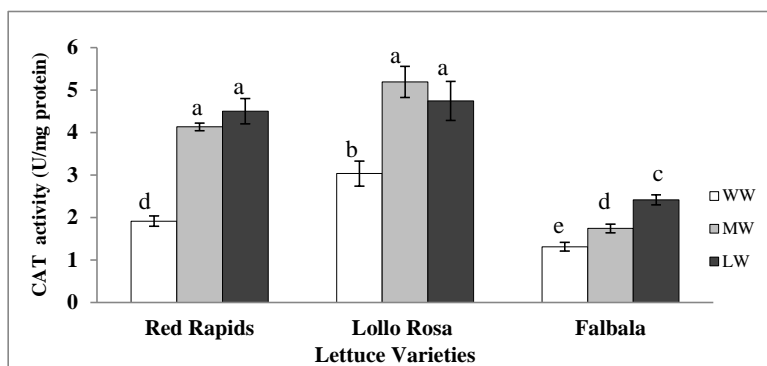


Fig. 6. Catalase (CAT) activity in leaves of the lettuce varieties subjected to varying water regimes (WW–well watered, MW–medium watered, LW–low watered conditions). Vertical bars represent the standard error. Means marked with different letters are significantly different ($p < 0.05$).

Similar to the results in CAT, Red Rapids had the highest rise in APX activity, exhibiting a two-fold increase under both MW and LW conditions (Fig. 7). Under MW condition, APX levels increased by 65% in Falbala and remained unaffected in Lollo Rosa. On the other hand, LW always caused significant increases in APX activities in all the varieties, that is, it doubled in Red Rapids and rose 78% and 94% in Lollo Rosa and Falbala, respectively.

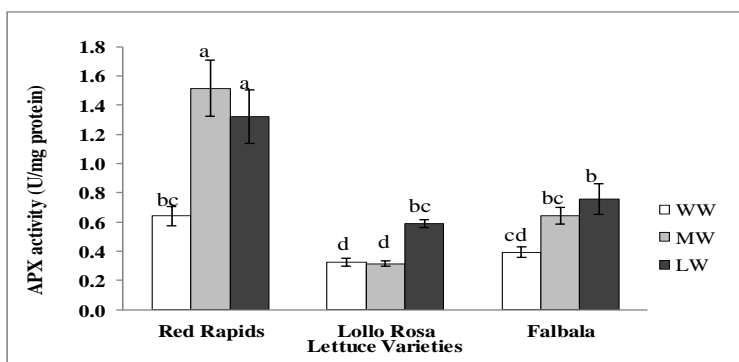


Fig. 7. Ascorbate peroxidase (APX) activity in leaves of the lettuce varieties subjected to varying water regimes (WW–well watered, MW–medium watered, LW–low watered conditions). Vertical bars represent the standard error. Means marked with different letters are significantly different ($p < 0.05$).

The above observations on enzymes may be attributed to the hypothesis that antioxidant enzymes are the first line of defense against ROS generated in the chloroplasts (Nakano and Asada 1981). Several antioxidant enzymes participate in the detoxification of ROS including SOD, APX and CAT. An increasing trend in these enzymatic antioxidants was generally observed with increased severity of water deficit. This severity-dependent increase in antioxidants is consistent with previous reports in many other plant species (Bian and Jiang 2009; Hameed et al. 2011).

Since the activities of antioxidant enzymes reflect the need for detoxification of ROS, the observations in the present study is likely the result of enhanced synthesis of the enzymes and/or of build-up of protective mechanism to ameliorate possible oxidative damage triggered by LW. Similarly, Bian and Jiang (2009) mentioned that maintaining a high level of antioxidative enzyme activities may contribute to increasing the capacity for stress tolerance during ROS generation. On the contrary, there are literature showing that severe drought stress decreased the activities of antioxidant enzymes in some plant species indicating that the scavenging function of the antioxidant enzymes may be impaired by the imposed stress (Liu et al. 2011). Such was not the case in this study. The high activities of SOD, APX and CAT therefore suggest that these enzymes may have provided sufficient protection against oxidative stress in red lettuce and that the imposed water deficit were not stressful enough as to depress the activities of these antioxidant enzymes.

Differences in antioxidant protection were observed between the light-red variety (Red Rapids) and the deep-red varieties (Lollo Rosa and Falbala). Non-enzymatic antioxidants were evidently higher in the deep-red varieties in all instances of water treatments as compared the light red variety. This translated to higher total antioxidant capacity. These dark red varieties may be said to have more health benefits. The high TP and AA in Lollo Rosa and Falbala may be interpreted as these genotypes' primary defense against ROS generation in water deficit. In comparison, Red Rapids had the highest increase in SOD activity in response to water deficit (LW) and was consistently the highest among the three varieties in APX activity in all the treatments. Red Rapids exhibited significant increases in TP and AA but was still lesser as compared to Lollo Rosa and Falbala. These results do not necessarily mean that the Red Rapids are less protected than the red varieties during water deficit. Instead, it may be that this genotype was protected mainly by the increase in enzymatic antioxidants than by the non-enzymatic antioxidants. Hence, characteristic induction of specific antioxidative mechanisms was observed by employing different antioxidant pathways as protection against possible oxidative damage.

CONCLUSIONS

Overall, water stress induced the synthesis of both enzymatic and non-enzymatic antioxidants in lettuce. The results indicate that plants have used these antioxidants possibly to offset the oxidative damage resulting from water stress. The antioxidant responses were variable depending on the severity of the water stress as well as on the genotype. Lollo Rosa and Falbala generally exhibited higher increases in TP and AA while Red Rapids showed higher enzymatic antioxidants after exposure to water deficit stress. The induction of the biosynthesis of antioxidant compounds translated to an increase in the total antioxidant capacity in lettuce. Since lettuce is a commonly consumed salad vegetable, these results suggest that water deficit stress may be utilized as a feasible approach for the production of a higher valued crop because of its higher antioxidant content. Hence, future work on valuation of the healthy benefits of red lettuce may be done to possibly offset the reduction in fresh yield brought about by water stress deficit. Future studies should determine the specific antioxidant compounds and the other phytochemicals that could be induced by water deficit stress.

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