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Effects of drought stress on growth, solute accumulation and membrane stability of leafy vegetable, huckleberry (*Solanum scabrum* Mill.)

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Abstract

The present study sought to investigate the factors implicated in growth impairment of huckleberry (a leafy vegetable) under water stress conditions. To achieve this, seedlings of plant were subjected to control, mild stress and severe stress conditions for 30 days. Plant growth, plant water relation, gas exchange, oxidative stress damage, electrolyte leakage rate, mineral content and osmolyte accumulation were measured. Water deficit markedly decreased leaf, stem and root growth. Leaf photosynthetic rate was tremendously reduced by decrease in stomatal conductance under stress conditions. Malondialdehyde (MDA) content markedly increased under mild (82%) and severe (131%) stress conditions, while electrolyte leakage rate (ELR) increased by 59% under mild stress and 3-fold under severe stress. Mineral content in leaf was high in stressed plants, while proline content markedly increased under mild stress (12-fold) and severe stress (15-fold), with corresponding decrease in osmotic potential at full turgor and an increase in osmotic adjustment. These results suggest that maintenance of high mineral content and osmotic adjustment constitute important adaptations in huckleberry under water deficit conditions and that growth depression under drought stress would be mainly caused by increased electrolyte leakage resulting from membrane damage induced by oxidative stress.

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Electrolyte leakage rate, Lipid peroxidation, Mineral content, Osmotic adjustment, Oxidative stress

Introduction

Huckleberry (*Solanum scabrum* Mill.) is one of the most important indigenous leafy vegetables in West, East and Central Africa, where it is cultivated and marketed (Zhou *et al*., 2006). The plant is cultivated mainly for its leaves and soft stems and is very rich in protein, vitamin A, iron and calcium. It is also used medicinally to overcome stomach aches, duodenal ulcers, swollen glands and tooth problems (Mwai *et al*., 2007). It is mostly cultivated during the rainy season when it is abundant and less expensive. However, during dry periods when its cultivation is restricted to wet areas or river banks, it becomes scarce and highly priced on the market. Muthomi and Musyimi (2009) reported that huckleberry is sensitive to drought with 97% reduction in leaf area and 84% reduction in shoot height. Although the adverse effect of drought stress on plant growth is associated with the hydraulic status of plants, in huckleberry, physiological factors underlying reduction in growth by water stress have not been examined. Understanding physiological changes of this vegetable to drought stress is important as it should provide clues on how to improve its production.

The detrimental effects of drought stress on plants are a consequence of osmotic strain on cytoplasm. In many plants, drought stress decreases stomatal conductance and transpiration rate (Yokota *et al*., 2006). Under drought stress condition, stomatal closure helps to maintain higher leaf

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water potential and hence, high leaf water content; however this often leads to decreased leaf photosynthesis (Chaves *et al*., 2002). The limitation of CO_2 assimilation imposed by stomatal closure reduces the internal CO_2 concentration in leaves and induces an imbalance in photosystem II (PSII) photochemical activity, thereby altering the electron requirements for photosynthesis and consequently leading to increased susceptibility of PS II to photo-damage (Murata *et al*., 2007). The Fv/Fm ratio, which represents the maximum quantum yield of primary photochemical reaction of PSII, is widely used to estimate the degree of photo-inhibition of PSII (Maxwell and Johnson, 2000). This parameter has been shown to respond to drought stress in various plants (Souza *et al*., 2004; Slot *et al*., 2008; Hajiboland and Amirazad, 2010).

Additionally, drought stress causes oxidative damage through an increase in reactive oxygen species (ROS) such as superoxide radical, hydrogen peroxide, and hydroxyl radical, which may cause various cellular damages including, protein oxidation, nucleic acid damage and peroxidation of membrane lipids (Gill and Tetuja, 2010; Sharma *et al*., 2012). Peroxidation of membrane lipids compromises membrane stability and integrity, resulting in leakage of electrolytes, water and soluble materials out of cell into the intercellular space of plants. Malondialdehyde (MDA) is a product of lipid peroxidation that has been used to assess the degree of oxidative damage under stressful conditions (Gill and Tetuja, 2010). High MDA content correlates with oxidative damage, whereas decrease in MDA content correlate with low oxidative damage. Sánchez-Rodríguez *et al*. (2010a) reported that susceptible cultivars showed higher degree of lipid peroxidation than the tolerant plants.

Furthermore, drought stress often brings about mineral deficiency resulting from reduced uptake and transport in plant. This reduced uptake and transportation is brought about firstly by decreased mineralization of the nutrients in soil under low soil water content, and secondly by reduced transpiration rate, which is responsible for carrying the elements to aerial parts in the transpiration stream (Hu and Schmidhalter, 2005). In response to drought stress, plants may accumulate low molecular weight osmolytes such as sugars and specific amino acids. Accumulation of these compatible solutes helps plants to maintain turgidity and hence normal metabolic processes, and this solute accumulation has been suggested as a major mechanism underlying adaptation and/or tolerance of plants to drought stress through osmotic adjustment (Munns, 2002; Yokota *et al*., 2006).

The objective of the present study was to determine the effects of drought stress on growth, leaf water relations, photosynthesis, solute accumulation, MDA content and electrolyte leakage in huckleberry, as well as, to ascertain physiological factors responsible for its growth reduction under drought stress.

Materials and Methods

Plant material and growth conditions: Seeds of huckleberry (line BIR/S0246) were obtained from the Institute of Vegetable and Tea Science, National Agriculture and Food Research Organization of Japan. The experiment was conducted in pots in greenhouse of the Faculty of Applied Biological Sciences, Hiroshima University under following conditions: 65% humidity, 20º-25º C day/15-18°C night temperature and natural sunlight. Pots (20 cm at the top and 15.5 cm at the bottom in diameter, and 22.5 cm in depth) were used and filled with 3.1 kg of mixture of regosol, perlite and peat moss (2:1:1, v/v). Basal fertilizer of 2.96 g pot^{-1} of N as ammonium sulfate, 2.69 g of P_2O_5 pot^{-1} as super-phosphate and 1.15 g pot^{-1} of K_2O as potassium sulfate was added to soil mixture at the time of mixing with dolomitic calcium carbonate (6.28 g pot^{-1}) to adjust soil pH (H_2O) to 6.0. After 11 days of germination, seedlings were transplanted into the pots and watered every day with about 200 ml of tap water in order to minimize nutrient loss through leakage. When plants were well established in pots (10 days after transplanting), they were arranged in a randomized complete block design with 4 replicates and subjected to drought treatments for 30 days. Treatments consisted of control plants (irrigated regularly) mild-stressed plants (irrigated twice a week) and severe-stressed plants (irrigated once a week).

Growth measurement: At the end of experiment, plants were separated into root, stem and leaf and fresh weights recorded. Portion of these samples were them frozen in liquid nitrogen and stored at -80°C until further were use for physiological analyses, while the rest were oven-dried and their dry weights were obtained.

Leaf water relation: Relative water content (RWC) was determined following to the method of Rhodes and Matsuda (1976). Leaf water potential (LWP) was measured using model 600 pressure chamber instrument (PMS Instrument Company, Oregon). Osmotic potential was determined with cell sap extracted as described by Saneoka *et al*. (2005) using a vapor pressure osmometer (model 5500 Wescor, Logan, Utah). OP at full turgor was then calculated according to Sinclair and Venables (1983). Osmotic adjustment was further determined as difference between OP at full turgor of control and stressed plants.

Gas exchange and chlorophyll fluorescence: The 4th or 5th leaf from top of each plant was selected for estimation of gas exchange. Photosynthetic rate, stomatal conductance and transpiration rates were measured using a portable photosynthesis meter (LI-6400P, Li-cor Lincoln, NE).

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Photosynthetic flux density was set at 1,000 μ mol $m^{-2} s^{-1}$, the ambient CO_2 concentration was set at 380 μ $molmol^{-1}$ and the leaf temperature was 25 °C.Chlorophyll fluorescence was measured according to the method of Bilger *et al*. (1995), using an Imaging-PAM M-Series Chlorophyll Fluorometer (Heinz Walz, Germany).

Mineral contents: Samples for analysis of K^+, $Ca^{2+,}$ $Mg^{2+,}$ and P were prepared according to Novozamski *et al*. (1983). K^+ was measured using flame photometer (ANA 135, Tokyo Photoelectric, Tokyo, Japan), while Ca^{2+} and Mg^{2+} with atomic absorption spectrophotometer (Hitachi, Tokyo, Japan). P was measured with UV-spectrophotometer (U-3310, Hitachi Co. Ltd. Tokyo, Japan), using molybdenum reaction solution method of Chen *et al*. (1956). Total nitrogen was determined by Kjeldahl method after digestion with concentrated H_2SO_4 and H_2O_2 (5:1, v/v).

Proline content and total soluble sugar content: Proline was extracted from freeze-dried powdered samples with 3% sulfosalicylic acid and measured following the method of Bates *et al*. (1973).Total soluble sugar was extracted with 80% ethanol (v/v) at 80°C and measured using anthrone-sulfuric acid method (Spiro, 1966).

Electrolyte leakage: Electrolyte leakage was determined according to the method of Murray *et al.* (1989) with minor modifications. Leaf samples were introduced into some distilled water and electrical conductivity (EC) was measured after 12 hr (A) using conductivity meter. Samples were later autoclaved to determine total EC (B). Electrolyte leakage rate (ELR) was then calculated by the following formula :

$$ELR\ (\%) = A/B \times 100.$$

Lipid peroxidation: Malondialdehyde (MDA) content was determined by thiobarbituric acid (TBA) reaction as described by Heath and Packer (1968) with some modifications. Fresh leaf samples were homogenized in an extraction buffer (10 mM HEPES pH 7, 15% tricarboxylic acid, 0.375% thiobarbituric acid, 0.25 N HCl, 0.04% butylated hydroxytoluene and 2% ethanol). Absorbance of the reaction was read at 535 nm and 600 nm, and MDA contentwas calculated using extinction coefficient of $1.57 \times 10^{-5} mMcm^{-1}$.

Statistical analysis: All the collected data were subjected to analysis of variance using SPSS statistics package, Student Version 18, and means (n = 4) were separated using Duncan Multiple Range Test at p = 0.05.

Results and Discussion

Leaf, stem and root dry weights decreased under drought stress condition (Fig. 1). Under mild stress, dry weight reduction in dry weight was lesser in leaf (43%), than stem (58%) and root (50%). Under severe water stress, root dry weight was least affected (57% reduction) as compared to stem (65%) and leaf (59%). Overall, among all the organs, stem was most affected under both the stress treatments. This marked reduction in dry weight under two water stress levels is similar to the observations of Muthomi and Musyimi (2009) on *Solanum scabrum* Mill., subjected to 35 day of water deficit. Growth reduction under water deficit conditions is due to number of factors acting singly or synergistically to influence adaptation of a plant to stress.

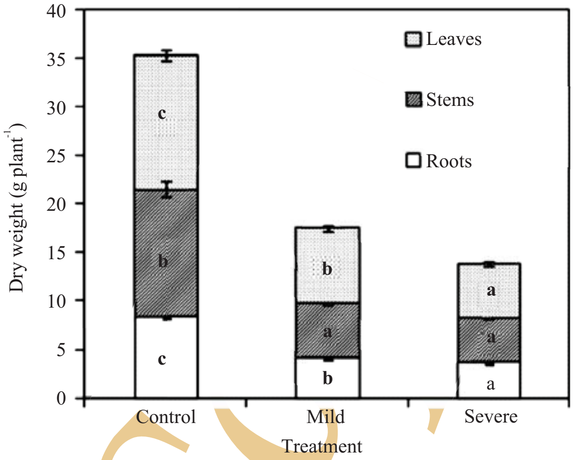

Fig. 1: Effects of drought stress on plant dry weight. Control, regularly watered plants; Mild, plants watered twice a week; and Severe, plants watered once a week.Values are means ± SE of 4 replicates. The same letter for each organ indicates no significant difference ($p \leq 0.05$)

Decrease in photo synthetic activity is one of the factors affecting plant growth. To evaluate the effect of water deficit on photosynthetic machinery and gas exchange parameters, photosynthetic rate (*Pn*), stomatal conductance (*gs*), transpiration (*Tr*) and quantum yield of PS II activity were measured. Water stress markedly decreased *Pn, gs* and *Tr.* Reduction in *Pn, gs* and *Tr* under mild stress treatment as compared to control was 80%, 93% and 88%, respectively (Fig. 2). However, there was no significant change in these parameters with increasing water deficit. Although, *Pn* was reduced by drought stress, the quantum yield of PS II remained unaltered under both stress treatments. Generally, stomatal closure is one of the first response of plants to water deficit or osmotic stress, since plants need to minimize evaporation and maintain cell turgor necessary for normal metabolic processes (Munns, 2002) However, although this adaptation often leads to maintenance of high water content in plant, it poses a serious threat to plant's survival as it reduces CO_2 diffusion and consequently reduced internal CO_2 concentration, necessary for normal photosynthetic processes. Reductions in *gs, Pn* and *Tr* (Fig. 2) is

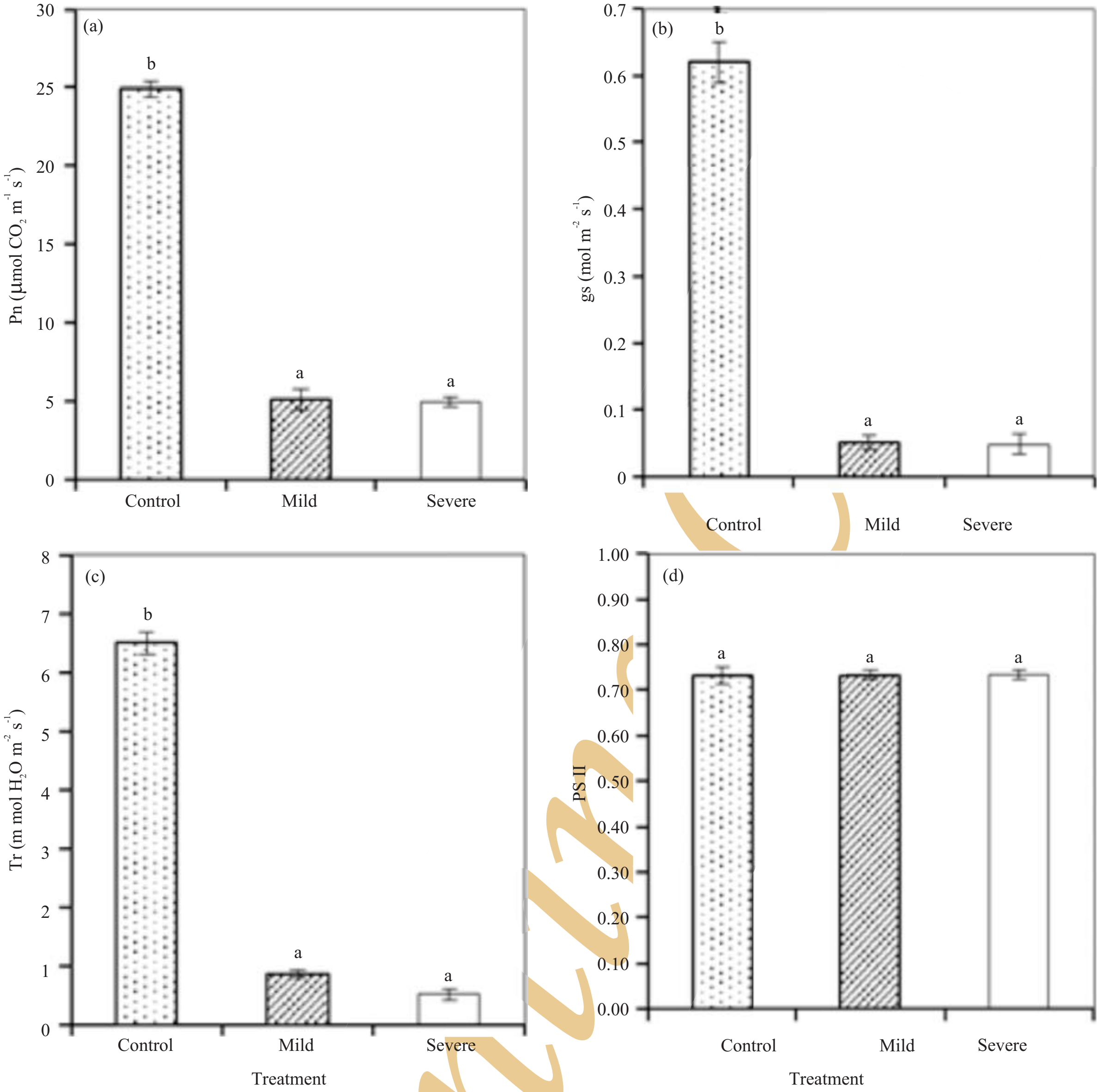

Fig. 2 : Effects of drought stress on (a) photosynthetic rate (**Pn**), (b) stomatal conductance (**gs**), (c) transpiration (**Tr**) and (d) quantum yield of PSII. Values are means ± SE of 4 replicates. The same letter indicates no significant difference ($p \leq 0.05$)

characteristic of many plants under drought stress conditions (Ashraf and Iram, 2005; Hamidou *et al*., 2007; Bolla *et al*., 2010). This indicates that reduced *gs* may account for reduction in *Pn*. However, aside reduced *gs* damage to photosynthetic machinery, especially PSII, which is most vulnerable component, is another cause for decrease in photosynthesis under drought stress (Maxwell and Johnson, 2000). However, there was no significant alteration in maximal quantum yield of PSII between stressed and unstressed plants (Fig. 2). These request are similar to the findings of Martinez *et al*. (2003), Slot *et al*. (2008), Ohashi *et al*. (2006). Therefore, low *gs*-induced reduction in Pn could be a potential factor limiting the growth of huckleberry under water deficit conditions.

Another factor contributing to growth reduction under water stress is nutrient deficiency. Since mineral deficiency is a characteristic of drought-stressed plants, concentrations of

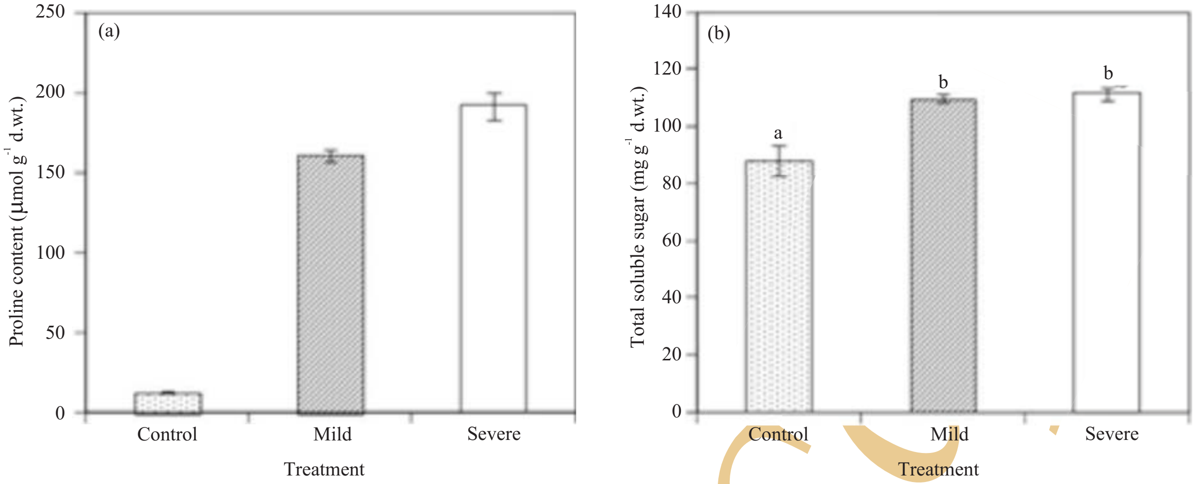

Fig. 3 : Effects of drought stress on (a) proline and (b) total soluble sugar content. Values are means ± SE of 4 replicates. The same letter indicates no significant difference ($p \leq 0.05$)

Table 1: Effects of drought stress on relative water content (RWC), leaf water potential (LWP), osmotic potential (OP) at full turgor and osmotic adjustment (OA). Values are means ± SE of 4 replicates. The same letter indicates no significant difference ($p \leq 0.05$).

Treatments	RWC (%)	LWP (MPa)	OP at full turgor (MPa)	OA (MPa)
Control	78.2±1.73^c	-0.08±0.01^a	-0.74	0
Mild	64.3±1.17^b	-0.18±0.02^b	-1.03	0.29
Severe	59.4±2.11^a	-0.33±0.02^c	-1.22	0.48

N, P, K^+, Ca^{2+} and Mg^{2+} were determined. Under mild water deficit condition, the concentration of N, K^+ and Mg^{2+} significantly increased (24, 10 and 28% increase, respectively) while concentration of P and Ca^{2+} were not altered significantly. With increasing stress, while the concentrations of N, P, K^+ and Mg^{2+} further increased (29, 25, 13 and 42% respectively) that of Ca^{2+} did not increase significantly (Table 2). Mineral elements are essential for plant growth and development. However, during drought stress these minerals become deficient in plants due to reduced soil moisture and reduced mobilization of root uptake, and reduced transport in plant owing to reduced transpiration rates (Hu and Schmidhalter, 2005; Eichert and Fernandez, 2012). Therefore, tolerant plants like cherry tomato cultivar 'zarina' (Sánchez-Rodríguez *et al*., 2010 b) and grape vine (Patakas *et al*., 2002) have been found to maintain high level of these minerals. Thus, high mineral content in the present study indicate that growth reduction in huckleberry under water deficit conditions is not associated with mineral deficiency. However, maintenance of high mineral content under conditions of reduced *Tr* indicates that huckleberry osmotically adjusts to low water potentials by accumulating compatible solutes, which enables them to absorb water and solutes.

In order to determine whether huckleberry adjusts to low water potential induced by water deficit, relative water content, leaf water potential, osmotic potential, osmotic adjustment and proline and soluble sugar contents were estimated. RWC and LWP were significantly reduced under both stress treatments (Table 1). LWP decreased by about 2-fold under mild stress and by 4-fold under severe stress, followed by decreased RWC by 18% and 24% under mild and severe stress conditions. Proline content showed 12-fold increase under mild stress and 15-fold increase under severe stress (Fig. 3). Soluble sugar content also increased under both stress treatments, although it did not differ with increasing stress. Osmotic potential at full turgor significantly reduced under mild stress (28% reduction) and severe stress (39%), while osmotic adjustment (OA) was enhanced under two stress treatments (Table 1).Proline and soluble sugars have been implicated in osmotic adjustment as an adaptive response to drought stress (Mafakheri *et al*., 2010). Particularly, proline accumulation during stress has been considered an adaptive character used in screening for stress tolerance: not only does it participate in osmotic adjustment, but it is also important in growth enhancement (Ueda *et al*., 2008). Significant increase in osmotic potential at full turgor and consequently increased osmotic adjustment in stressed plants (Table 1), owes certainly to significant increase in proline and total soluble sugars. This osmotic adjustment certainly is responsible for minimizing reduction of RWC to below 25% (Table 1) under both stress levels and keeping plants alive till the end of the study. Unaltered soluble sugar contents between stress treatments may be associated with reduction of *Pn* to same rate and/or utilization of photosynthate to synthesize amino acids (Lawlor and Cornic, 2002; Flexas *et al*., 2006). This result indicated that huckleberry maintained high water content under water

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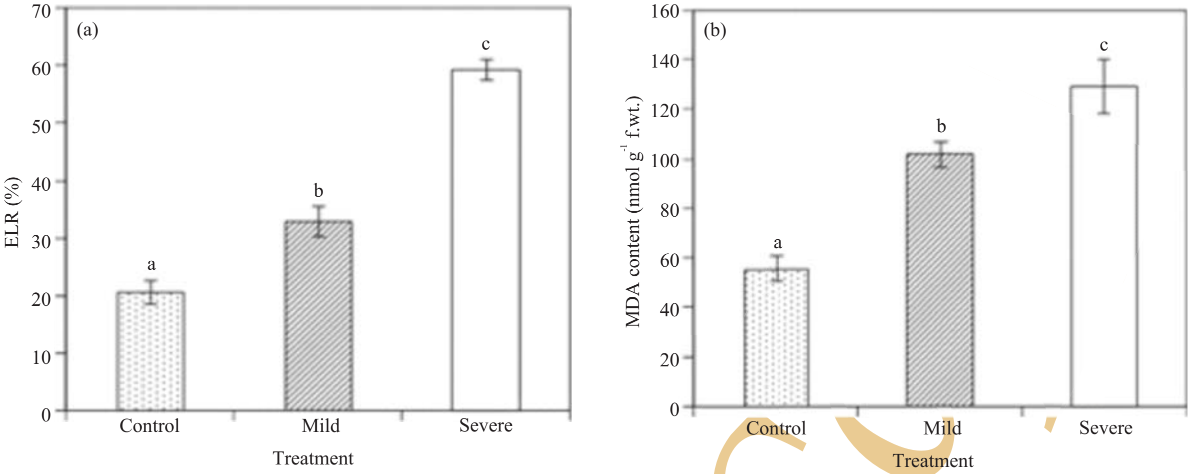

Fig. 4 : Effects of drought stress on (a) electrolyte leakage rate (ELR) and (b) malondialdehyde (MDA) content of huckleberry plants. Values are means ± SE of 4 replicates. The same letter indicates no significant difference ($p \leq 0.05$)

Table 2: Effects of drought stress on N, P, K$^+$, Ca^{2+} and Mg^{2+} content in leaves

Treatments	**N**	**P**	**K$^+$**	**Ca^{2+}**	**Mg^{2+}**
			(mg g^{-1})		
Control	40.9 ± 0.7a	4.46 ± 0.14a	58.40± 2.4a	8.045± 0.26a	2.47± 0.15a
Mild	50.6 ± 0.8b	4.67 ± 0.03a	64.5± 2.3b	8.19± 0.09a	3.71± 0.11b
Severe	52.6± 0.2b	5.56 ± 0.16b	66.1± 1.2b	8.830± 0.42a	3.50 ± 0.11b

Values are mean of four replecates ± SE; significant difference ($p \leq 0.05$)

deficit conditions through osmotic adjustment achieved by accumulating essentially proline.

To determine whether growth reduction in huckleberry is associated with drought stress-induced oxidative damage, MDA content and ELR were measured. The ELR showed significant increase in plants under mild stress (59%) and about 3-fold in plants under severe stress as compared to control (Fig 4). This marked increase in ELR coincided with significant increase in MDA content which was higher under severe stress (131% increase) than under mild stress (82% increase). Oxidative damage is one of the main causes of growth reduction under stress conditions (Gill and Tetuja, 2010). One of the main damages inflicted by oxidative stress is lipid peroxidation, which is often used to assess oxidative stress damage in plants (Mittler, 2002; Gill and Tetuja, 2010; Sharma *et al.*, 2012). Peroxidation of membrane lipids usually weakens the membranes causing minerals and other important cellular metabolites to leak out of the cells, consequently leading to the death of cell (França *et al.*, 2000; Bajji *et al.*, 2002; Gill and Tetuja, 2010). Thus, significant increase in ELR, especially in plants under severe water stress, where the rate was about 3-fold relative to that of control plants could be the main cause of growth impairment in huckleberry under water deficit conditions.However, sometimes, plants tend to avoid ROS building up to toxic levels in cells through strategies including suppression of photosynthesis,and photosystem and antenna modulation (Mittler, 2002). This would imply in the context of the present research that, low *Pn* under stress condition could be a way of avoiding oxidative stress, although at the same time compromising growth of plant through reduced photosynthate production and transport. However, oxidative stress under severe water stress is associated with metabolic impairment (Flexas *et al.*, 2006) which is reason to believe that growth reduction in this plant could be due to oxidative damage leading to electrolyte leakage.

It can be concluded from the present study that under water deficit condition huckleberry maintains high water content though osmotic adjustment, as well as, high mineral content. However, these were seemingly not enough to protect the plants, as oxidative stress damage caused increase in electrolyte leakage which is believed to be the main cause of growth retardation in huckleberry.

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