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Full Length Research Paper

Hypoxia and post-aeration alter malondialdehyde content and proton efflux in root and leaf of sunflower

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Abstract

Saturated soil causes hypoxia which seriously affects the growth of plants through creating oxidative stress and lipid peroxidation. In this study, seventeen days old seedlings of four sunflower cultivars (Lacomca, Record, Progress and Hysun33) were subjected to four days of hypoxia. Plants exposed to hypoxia, re-aerated for four more days as recovery treatment. Plants were sampled daily during hypoxia and two days interval during the recovery period for measuring root and shoot dry weights, malondialdehyde and root proton efflux. Results show that hypoxia causes reduction in dry weight of all cultivars. Although, dry weight reduction in root was higher than shoot, each cultivar responded to hypoxia differently. Lacomca and Progress cultivars with less reduction in root dry weight (almost 18%) showed more tolerance to hypoxia than Record and Hysun33 cultivars (49.4% and 36.1% respectively). Higher amount of root's MDA in Record (170.6%) and Hysun33 (115.4%) compared to Progress (51.7%) and Lacomca (42.3%) cultivars was correlated to higher root dry weight reduction (49.4% and 36.1% respectively). Although root's MDA was correlated with root dry weight reduction however this trend was not the same in leafs. Re-aeration after 4 days of hypoxia showed reduction in roots and shoots dry weight, MDA and roots proton efflux.

Keywords: *Helianthus annuus* L., Hypoxia, Post-aeration, Lipid peroxidation, Malondialdehyde, Proton efflux.
Abbreviation: LP, lipid peroxidation; MDA, malondialdehyde; PUFA, polyunsaturated fatty acid; ROS, reactive oxygen species.

INTRODUCTION

Plants need oxygen for respiration and ATP production (Geigenberger, 2003). Different conditions such as over irrigation, soil compaction, poor drainage, flooding and over rainfall induce hypoxia in root zone (Drew, 1997; Garnczarska and Bednarski, 2004; Mainiero and Kazda 2005; Wang *et al*., 2009). Due to the contribution of oxygen as initial electron receptor in electron transport chain in mitochondria, oxygen concentration is an important factor for existence of organisms (Geigenberger, 2003). Hypoxia leads to electron transport chain block (Horchani *et al*., 2008) and consequently produces reactive oxygen species (ROS) and oxidative stress in cell (Geigenberger, 2003). ROS are very reactive and cause destruction of DNA, protein, lipid and carbohydrate, all resulted reduction of root and shoot dry weight and crop productivity (Bowler *et al.,* 1992; Foyer *et al.,* 1997). Drew (1997) explained that oxygen deficiency caused accumulation of toxic product of fermentation, low energy production and lack of substrate for respiration resulted root injury. Main sources of ROS (superoxide radicals, hydroxyl radicals and hydrogen peroxide) production in plants are mitochondria, chloroplast and peroxisome (Gill and Tuteja, 2010; Mittler *et al*., 2004; Møller, 2001), however mitochondria is more important than others in hypoxic condition (Blokhina, 2000). Enzymatic antioxidants such as superoxide dismutase (SOD), catalase (CAT), peroxidases (POD), gluthatione reductase (GR) (Gill and Tuteja, 2010) and non-enzymatic antioxidants such as ascorbate, glutathione (Gill and Tuteja, 2010), phenolic compounds (Hernández *et al*., 2009), and tocopherols (Holländer-Czytko, 2005) are main mechanisms of plants against ROS which defines the capability of plants to

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tolerate hypoxia.

Membrane lipid peroxidation (LP) which is induced by ROS, is a marker of oxidative stress level (Catalá, 2009). LP includes three stages: initiation, propagation and termination (Schaich, 1992). At initiation phase, ROS specifically hydrogen peroxide and singlet oxygen can attack to methylene group of polyunsaturated fatty acid (PUFA) and abstract its hydrogen atom,, then formed radical lipid ($R^{\cdot}$). Cell membrane PUFA is a suitable substrate for LP (Blokhina, 2000; Catalá, 2009) so initiation could happen under hypoxia. At propagation stage, lipid peroxy radicals can react with other PUFA (RH) and form ROOH (lipid hydroperoxides) and $R^{\cdot}$ (second radical lipid) and then produce conjugated dienes. The ROOH formed can cleavage by reduced metals, such as Fe^{2+}, producing lipid alkoxyl radical ($RO^{\cdot}$). Both alkoxyl and peroxyl radicals stimulate the chain reaction of lipid peroxidation by abstracting additional hydrogen atoms (Catalá, 2009; Frankel, 1984). At termination phase, $R^{\cdot}$ and $ROO^{\cdot}$ react together and produce non radical forms. Several aldehydes such as Malondialdehyde (MDA) are formed as results from decomposition of hydroperoxide products. The aldehyde breakdown products can form conjugates with DNA and proteins (Gill and Tuteja, 2010), Also membrane integrity and fluidity reduction is an indicator of LP so that MDA can be used as a biomarker for lipid peroxidation assay (Blokhina *et al*., 1999; Crawford and Braendle, 1996). (For more details see Gill and Tuteja, 2010 and Catalá, 2009)

pH of root media affects membrane integrity and vice versa. Although pH of inner and outer-plant (rhizosphere) is a debatable subject at physiological process in plant root, according to Bashan and Levanony (1989) pH of rhizosphere as a result of imbalance in cation and anion uptake can lead to change in electrogenic feature of soil and as a consequence causes change in nutrient uptake by plant (Berbara *et al*., 1995; França *et al*., 2006; Lew, 1998). From aspect of inner-plant, function of H^{+}-ATPase in plasma membrane with inaugurate proton efflux in plant effect on metabolism and growth such as cytoplasmatic pH regulation, maintenance of turgor, cell wall relaxation and polarity rise in growing cells (Mantelin and Touraine, 2004). Therefore proton net efflux may varied on light, aeration and source of nitrogen (NH_4^{+}, NO_3^{-}) availability (Bashan and Levanony, 1989) plant-microbial condition (Amooaghaie *et al*., 2002) as well as environmental stresses.

Previous studies have shown that different plants with different genotype have different ability to tolerate hypoxic condition even cultivars belong to a same species (Setter *et al*., 1994; Bacanamwo and Purcell, 1999, Kato-Noguchi and Morokuma, 2007). Results of different studies indicated that hypoxia leads to MDA production in leaf of *Zea mays* (Jamei *et al*., 2009) *Capsicum annum* L. (Malekahmadi *et al*., 2005) and *Malus hupehensis* and *M. toringoides* (Bai *et al*., 2010). However the times of hypoxia were different in mentioned experiments.

Sunflower (*Helianthus annuus* L.) with almost 40-50% valuable edible oil is one of the most important oilseed crops in the world. Almost one third of the oilseeds crop cultivated in Iran belongs to sunflower which mostly planted in Northern provinces. Non-uniform rainfall with high density in these regions, especially at early stage of growth, causes frequent waterlogging with shortage of oxygen in rhizosphere. Therefore, this research was conducted to evaluate the effect of short period of hypoxic condition on growth (shoot and root dry weight), membrane lipid peroxidation (MDA) and proton efflux of roots and leafs of different sunflower cultivars (Lacomca, Record, Progress and Hysun33).

MATERIAL AND METHODS

Plant Material and Treatments

Seeds of Sunflower (*Helianthus annuus* L.) cultivars (Lacomca, Record, Progress and Hysun33) were obtained from Oil Seeds Research Center of Isfahan, Iran. Sterilized sunflower seeds were germinated in dark for three days and then transferred into pots containing perlite for four days. Seven days old seedlings were moved into aerated hydroponic containers containing full strength Hoagland's solution (Hoagland and Arnon, 1950) and kept for ten days in a growth chamber (27/20 °C day/night, with a 16/8 h day/night photoperiod, irradiance: 1500 lux) for environmental adjustment. When seedlings were adapted enough to hydroponic medium (seedlings were seventeen days old), they were subjected to hypoxic condition for four days using N_2 (99.99%) bubbling procedure to remove O_2 from medium (Garnczarska and Bednarski, 2004). In contrast, the control plants were continuously aerated with regular air. Plant exposed to hypoxia for four days as well as their control plants were aerated for four more days to assay the rate of recovery. Dissolved oxygen in aerated and hypoxic solution was 5.92 and 1.94 mg L^{-1}, respectively. Plants were sampled daily during hypoxia and two days interval during the recovery time. Plant samples splited into root and shoot and then each plant part was divided into two subsamples; one subsample was weighted and oven dried and the other immediately frozen in liquid nitrogen for further analysis (MDA). However for root proton efflux analysis, intact plants from all stages of sampling were used.

Assay of Lipid Peroxidation

Malondialdehyde (MDA) was measured by the method of Heath and Packer (1968). The extent of lipid peroxidation was evaluated by the thiobarbituric acid reaction. Frozen

leaf and root tissue separately was homogenized in 0.1% trichloroacetic acid (1:10, w:v) and centrifuged at 10000 g for 15 min. One ml of the supernatant was incubated with 4 ml of 0.5% thiobarbituric acid in 20% trichloroacetic acid at 95 ℃ for 30 min in a fume hood and then cooled in ice bath. After centrifugation at 10000 g for 10 min, the absorbance of the supernatant was read at 532 nm and corrected for the non-specific absorbance recorded at 600 nm.The concentration of thiobarbituric acid reactive substances was calculated as malondialdehyde equivalent using the extinction coefficient (155 mM-1 cm-1).

Assay of Proton Efflux

Proton efflux was calculated according to Bashan *et al.* (1989). The seedling was transferred to a small glass beaker containing 200 ml of fresh Hoagland's nutrient solution. The beaker was wrapped with aluminum foil to prevent direct illumination of root and was placed in a control temperature 27±2℃ and then proton efflux was measured after 10 h. The quantity of protons released from the roots was determined by titrating the nutrient solution with 0.01 M NaOH to the initial pH value (initial pH=6.5). The amount of protons released into the nutrient solution was expressed as μmol H^+ g FW^{-1} h^{-1}.

Statistical Analysis

The experiment was conducted according to a factorial test in a completely randomized design with three replicates. Statistical analysis was carried out using Sigma Stat for ANOVA as well as Duncan's multiple-range test to compare the treatment means for this experiment.

RESULT

Shoot and Root Dry Weight of Aerated Plants

Roots and shoots dry weights of aerated sunflower cultivars (Lacomca, Record, Progress and Hysun33) were increased differently with time. The highest and the lowest root dry weights were observed in Lacomca (664.0 mg) and Progress (299.7 mg) cultivars respectively, when seedlings were 25 days old (Figure 1). Simultaneously, the highest and the lowest shoot dry weights were 2721.5 and 943.75 mg in the same cultivars respectively (Figure 1). These numbers indicated that Lacomca and Progress have higher and lower root growth rate (79.5 and 35.5 mg day^{-1} respectively), simultaneously, shoot growth rate in the same cultivars were 118.9 and 107.0 mg day^{-1} respectively.

Shoot and Root Dry Weight of Plants Subjected to Hypoxia and Afterward

Result indicated that hypoxic condition caused a significant reduction of root and shoot dry weight (Figure 2 and 3). However different cultivars responded to hypoxia differently. In comparison to their control plants (Figure 2), the magnitude of root dry weight reduction in Lacomca and Progress cultivars were lower (almost 18%) and in Record and Hysun33 cultivars were higher (49.4% and 36.1% respectively). Simultaneously, the highest and the lowest shoot dry weight reduction was found in Hysun33 (24.5%) and Lacomca (9.7%) cultivars respectively in comparison to their control plants (Figure 3).

At recovery condition, the continuous sever effect of hypoxia could be seen in aerated plants in such a way that the reduction of shoot and root dry weight has happened continuously even though plants were in aerated condition. Therefore root and shoot dry weights were reduced even in recovery condition in compared to their control plants (Figure 2 and 3). The results show that after four days of recovery, the highest reduction in dry weight of roots and shoots in comparison to their control plants were occurred in Record cultivar (45.65 and 51.9% respectively), whereas the lowest reduction of root and shoot were observed in Progress cultivar (21.8% and 36.6% respectively). It should be mentioned that small portion of the reduction is belong to hypoxic condition.

Leaf and Root Malondialdehyde (MDA) of Aerated Plants

In aerated plants, root's MDA was decreased versus time in all cultivars (Figure 4). The result shows that at 17 days old plant the amount of root's MDA were significantly different within different cultivars, however in older plants there were no differences among root's MDA in different cultivars and their amounts were lower in all cultivars.

Leaf's MDA were decreased in Lacomca and Hysun33 and increased in Record and Progress cultivars at early stage of growth (till 21^{th} day) and then were constant till the end of experiment. Lacomca cultivar also has the lowest amount of MDA as compared to others (Figure 4). In general, leaf's MDA of aerated plants were higher than the root's MDA and its variation in respect to the time was less compared to the root's MDA

Root and Leaf Malondialdehyde (MDA) of Plants Subjected to Hypoxia and Afterward

When plant exposed to hypoxic condition, additional MDA

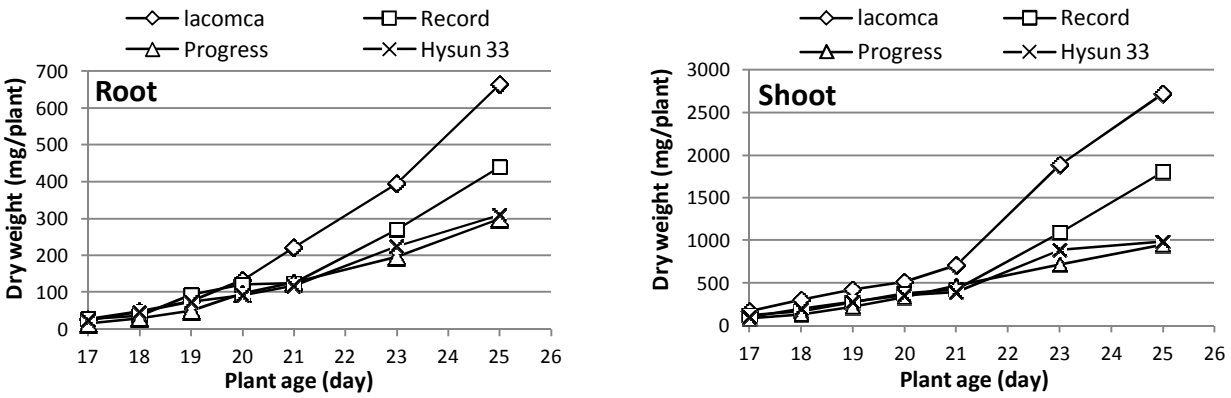

Figure 1. Roots and shoots dry weights (mg /plant) of four cultivars of sunflower under aerated condition

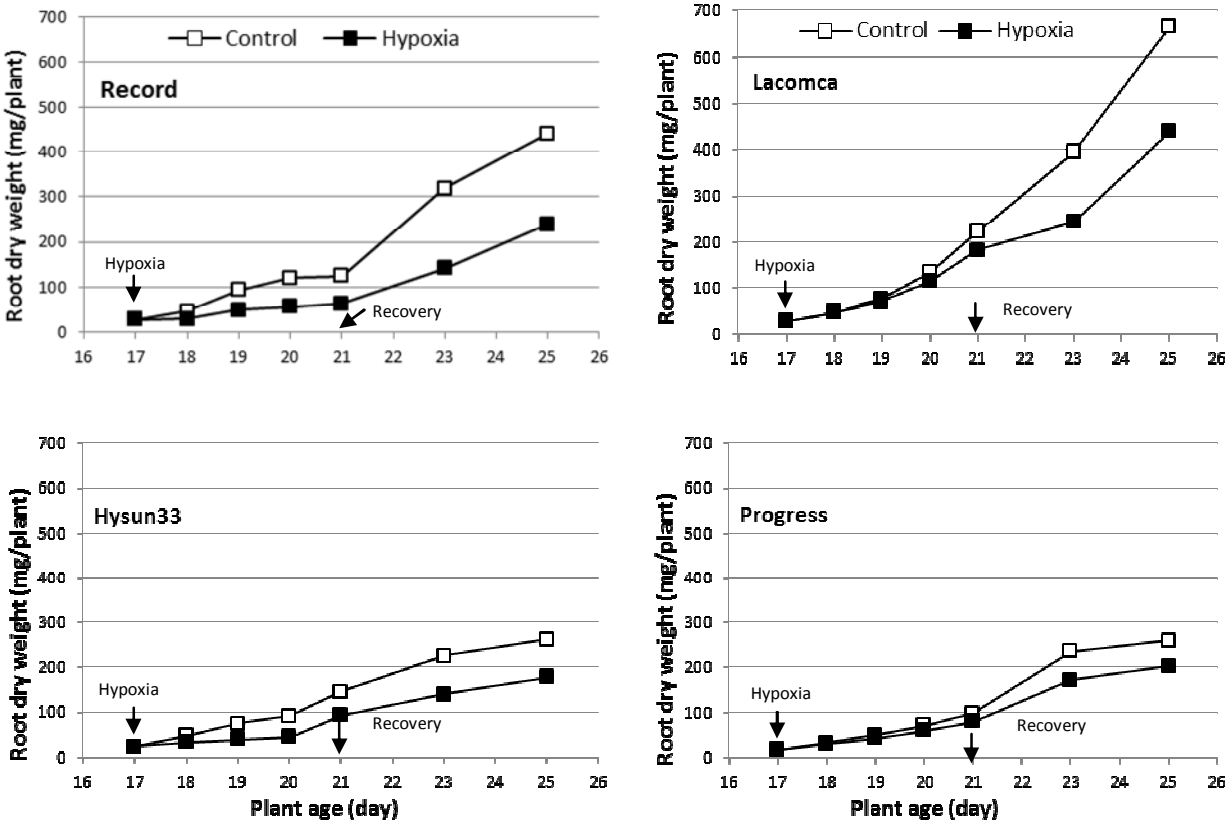

Figure 2. Effect of hypoxic condition and post-aeration on root dry weight (mg/plant) in four cultivars of sunflower

was observed in the root of four sunflower cultivars (Figure 5). The maximum amount of root's MDA was found in the first day of hypoxia and then decreased differently in different cultivars. The highest and the lowest amounts of root's MDA were 3658.0 and 1455.3 nmol gFW^{-1} in Record and Lacomca cultivars respectively in the first day of hypoxia. These are almost equals to 170.6% and 42.3% additional MDA for Record and Lacomca cultivars respectively in comparison to their control plants (Figure 5). However the amounts of root's MDA started to decrease in the second day of hypoxia and afterward. During four days of recovery, the reduction of root's MDA of four cultivars were happened continuously and approached to their control values

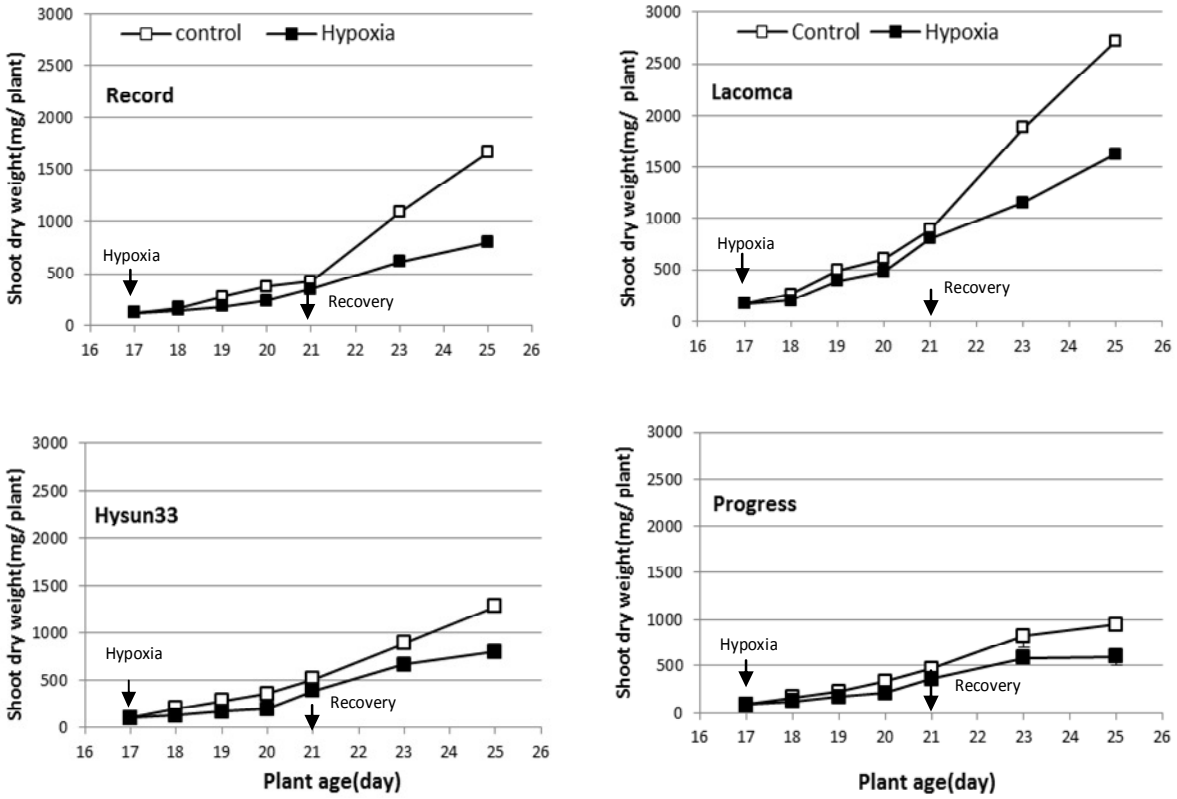

Figure 3. Effect of hypoxic condition and post-aeration on shoot dry weight (mg/plant) in four cultivars of sunflower

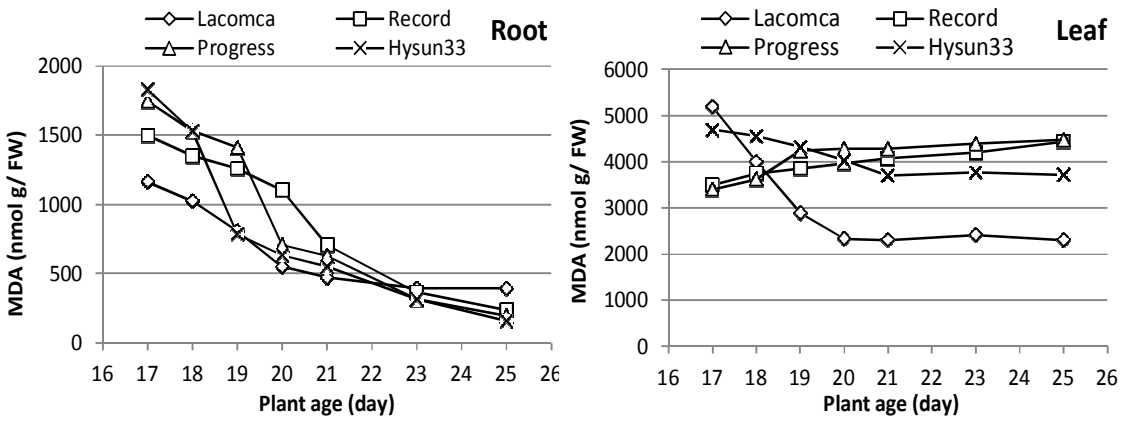

Figure 4. Root and leaf MDA (nmol/g FW) of four cultivars of sunflower under aerated condition

(Figure 5).

When plant exposed to hypoxia, the amounts of leaf's MDA were higher in all cultivars than their aerated plants but the trend of additional MDA were different in different cultivars (Figure 6). In Lacomca, the amount of leaf's MDA was almost the same during hypoxic condition but other cultivars have shown significantly higher MDA in the first day of hypoxia and then were decreased with time. The highest and the lowest MDA were observed at different days for Lacomca (151.1%) and Hysun33 (9.6%) cultivars respectively compared to their control plants.

Aeration following hypoxia causes the reduction of leaf's MDA in such a way that the amount of MDA approached to their control values in Record and Hysun33 cultivars (Figure 6). However in Lacomca its value was still far above of its control value

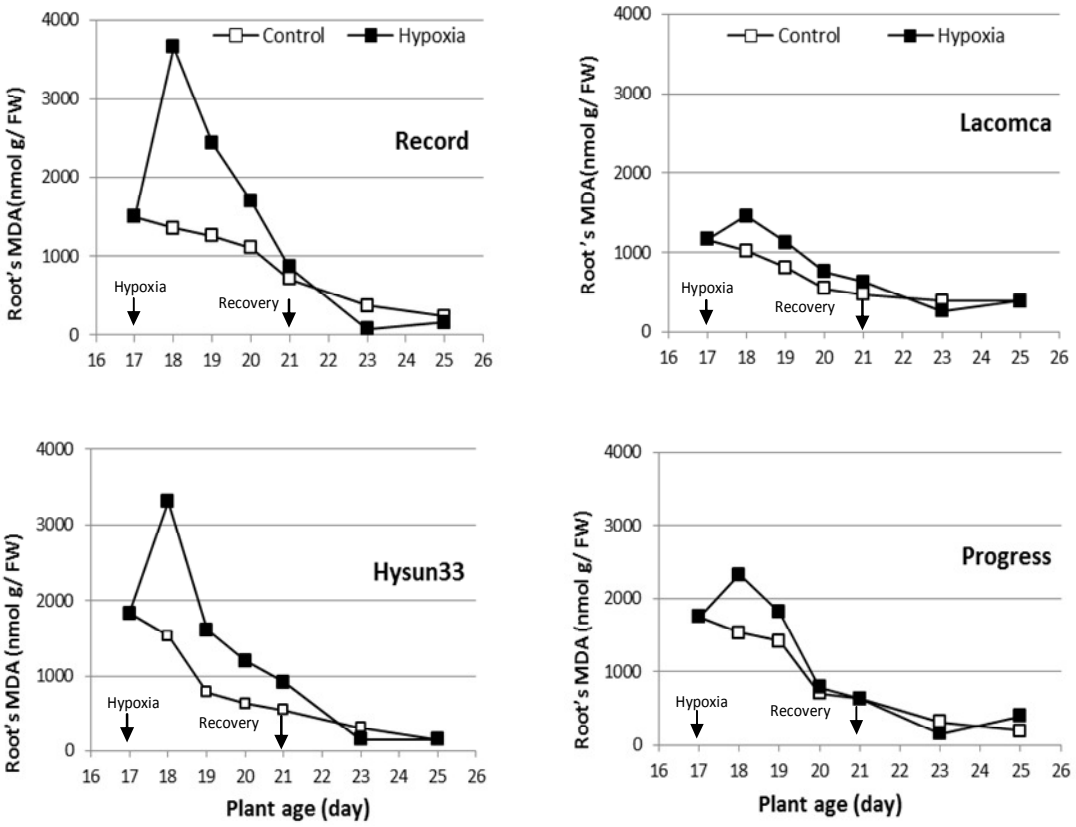

Figure 5. Effect of hypoxia and post- aeration on root's MDA (nmol/g FW) of four cultivars of sunflower

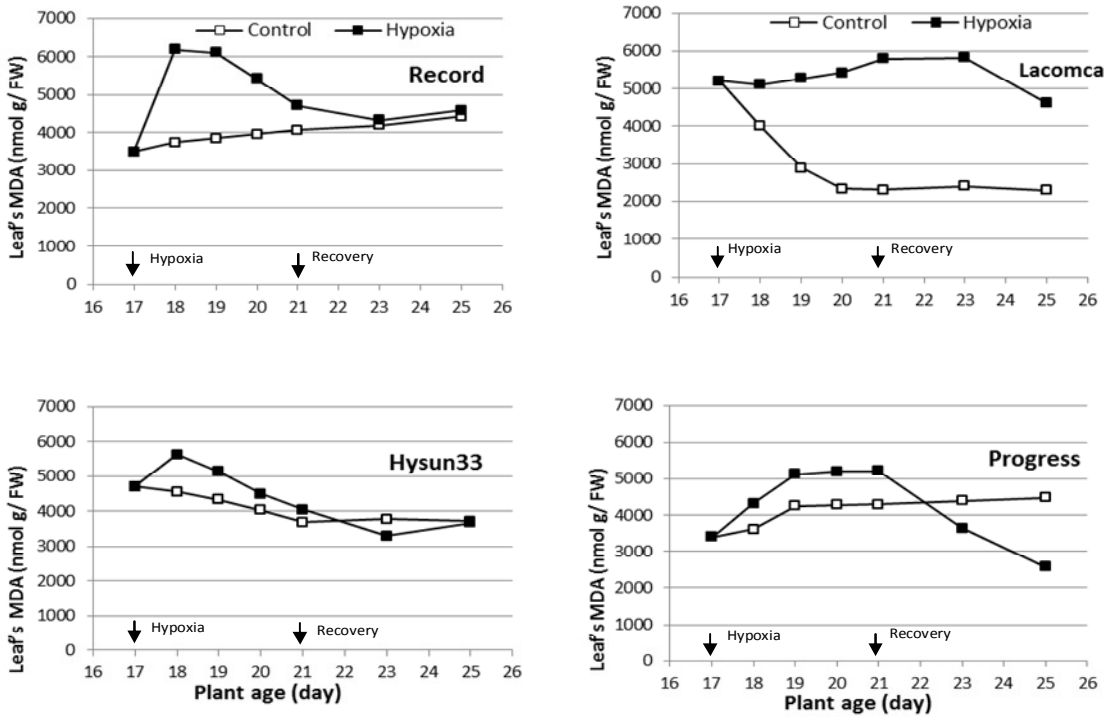

Figure 6. Effect of hypoxia and post-aeration on leaf's MDA (nmol /g FW) of four cultivars of sunflower

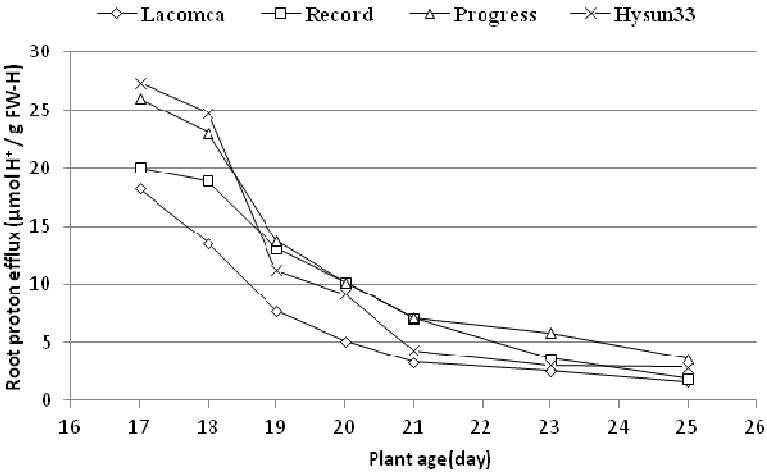

Figure 7. Root proton efflux (µmol H^+/ g FW-h) of four cultivars of sunflower under aerated condition

and in Progress was much lower than its control value.

Root Proton Efflux of Aerated Plants

Root proton efflux in aerated plants was higher at younger plants and then decreased sharply with time in all cultivars (Figure 7). Although when plants were 17 days old the highest and the lowest amounts of root proton efflux were 273.6 and 182.6 µmol H^+ g FW^{-1} h^{-1} in Hysun33 and Lacomca cultivars respectively, the proton effluxes were decreased to 35.8 and 15.7 µmol H^+ g FW^{-1} h^{-1} in Progress and Lacomca cultivars respectively at 25th growing day.

Root Proton Efflux of Plants Subjected to Hypoxia and Afterward

Under hypoxic condition, root proton efflux was higher than aerated plants. However, in younger plants the amounts were higher than older ones (Figure 8). Although root proton effluxes weren't the same in different cultivars at different stage of growth, their amounts were higher in hypoxic condition compared to their controls. The highest and the lowest root proton effluxes were observed in Lacomca (68.5%) and Record (6.2%) cultivars respectively after four days of hypoxia in comparison to their controls. The change in proton efflux of the root during recovery was almost the same as their aerated plants.

DISCUSSION

In general, roots and shoots dry weight of plants increase none-linearly with time. However the growth rate is different in different plant as well as in different condition (Poorter, 1989). In this study, the growth rates of aerated plants were none-linear as expected, however, different cultivars have different growth rate. When plants were 25 days old, the highest and the lowest root and shoot dry weighs were observed in Lacomca and Progress cultivars respectively. These values were obtained from different growth rates in different cultivars. In average, the highest and the lowest root growth rates were 79.5 and 35.5 mg day^{-1} in Lacomca and Progress respectively and for shoots were 118.9 and 107.0 mg day^{-1} for same cultivars. These variations in dry weight of different cultivars were statistically significant. Differences in growth rates of different cultivars and consequently in their dry weights could affect the rate of plant tolerance to any imposed hypoxic condition.

The results of this study show that hypoxic condition causes reduction of root and shoot dry weights differently in different cultivars (Figure 2 and 3). The amounts of root dry weight reduction in Lacomca and Progress cultivars were low (almost 18%) in comparison to their control plants and in Record and Hysun33 cultivars were high as much as 49.4% and 36.1% respectively. Simultaneously, the lowest and the highest shoot dry weights reduction were found in Lacomca (9.7%) and Hysun33 (24.5%) cultivars respectively (Figure 3). The results of other experiments show similar trend. For example, Fakhri Filsouf (2011) observed that hypoxic condition of 2 and 4 days in sunflower resulted reduction of dry weights in root (15% and 29.5% respectively) and shoots (8% and 16% respectively). These reductions were higher in root than in shoot. Grassini *et al.* (2007) show that waterlogging cause's 5-60% reduction in sunflower production. The reduction in sunflower yield is one of the consequent of reduction in vegetative growth during waterlogging. Bennicelli *et al*. (1998) observed that deficient soil aeration at 12 h causes reduction of root and shoot dry weight of KLG2210 cultivar of *Zea*. In another experiment Bai *et al*. (2010) observed that hypoxic

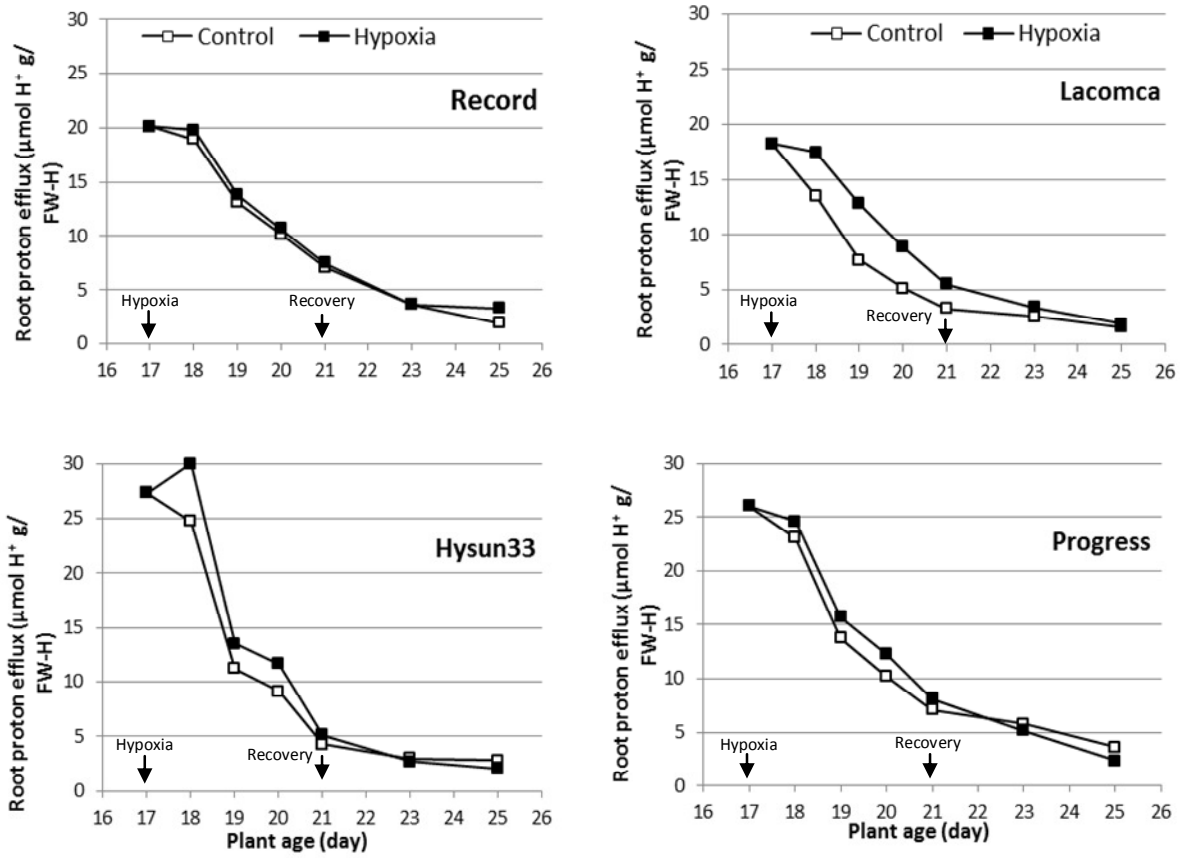

Figure 8. Effect of hypoxia and post-aeration on root proton efflux (µmol H+ /g FW-h) of four cultivars of sunflower

condition of 4 to 20 days in two *Malus* species (*M. hupehensis* and *M. toringoides*) led to inhibit the growth of both species differently. *M. hupehensis* was more tolerant to hypoxia due to less oxidative damage (root and shoot dry weight reduction, 14.2 and 22.9% respectively) compared to *M. toringoide*s (21.4 and 42.9% respectively).

In this experiment, Lacomca and Record cultivars have higher plant dry weight in aerated condition in comparison to other cultivars, whereas at hypoxic condition, Lacomca and Record cultivars showed counter partly lowest and highest root dry weight reduction respectively. In contrast, Progress and Hysun33 cultivars have shown lower plants dry weights in aerated condition in compared to other cultivars, and the amount of root dry weight reduction at hypoxic condition was low for Progress and high for Hysun33. Reductions of shoot dry weights at hypoxic condition were high for Lacomca and Record and low for Progress and Hysun33 cultivars in comparison to their control plants. This trend was due to higher growth rate of Lacomca and Record than other cultivars at aerated condition. It could be mentioning that Lacomca and Progress cultivars are more tolerant cultivars compared to others in hypoxic condition. Although these cultivars have different growth rates in aerated condition (growth rates of Lacomca and Progress were high and low respectively), root's MDA production in hypoxia was lower in both cultivars compared to others. This behavior (low root's MDA) might be the reason for higher tolerance of these cultivars to hypoxia in comparison to others. However the different behaviors of sunflower cultivars in hypoxic condition may be due to other factors such as ROS formation and lipid peroxidation which cause less vegetative growth (Blokhina, 2000; Szal *et al*., 2004).

Oxidative stress was triggers lipid peroxidation under oxygen deficiency and its activity can be measurement by MDA content. In aerated plants (Figure 4), root's MDA was decreased since seedlings were 17 days old however leaf's MDA variation was different in the beginning and then were constant up to the end of experiment (25 days old plants). The reduction of root's MDA in aerated plants may be due to adaptation of plant to the new medium (Hydroponically medium).

MDA production was inducing after exposure of plants to hypoxic condition, especially in the first day of hypoxia. The higher amounts of root's MDA in Record (170.6%) and Hysun33 (115.4%) compared to Progress (51.7%) and Lacomca (42.3%) cultivars (Figure 5) have similar trend with higher reduction of root dry weight in the same cultivars (49.4% and 36.1% respectively). In contrast, the lowest amounts of leaf's MDA (9.6%) and

the highest shoot dry weight reduction (24.5%) were observed in Hysun33 cultivar in comparison to others. Simultaneously, Lacomca has the highest leaf's MDA (151.1%) and the lowest shoot dry weight reduction (9.7%). It can be concluded that root's MDA is better index for root dry weight reduction than leaf's MDA.

Previous studies have shown that hypoxic condition led to increase MDA content in plant. For instance, Jamei *et al.* (2009) reported that 192 h hypoxia in rhizosphere of *Zea mays* led to increase in leaf's MDA (368%) in respect to aerated plants. Malekahmadi *et al*. (2005) observed that 3, 5 and 7 days of waterlogging led to a significant rise in MDA in leafs of *Capsicum annum* L.**.** Szal *et al.* (2004) observed that hypoxia for five days increased MDA in the mitochondria isolated from Barley roots and post-aeration following hypoxia for 24 h led to decrease in MDA contents.

The damage to the cell membrane due to lipid preoxidation and consequently distribution in the efflux of different components in/out of the cell is a major concern in cell biology. One of the important ions affecting different mechanisms and also uptake of essential nutrients is proton (H^+).

Root proton efflux was decreased with time sharply in aerated plant in all cultivars. The reduction of root proton efflux has happened after transferring of plant to hydroponic condition. Therefore the reduction in proton efflux might have been happening because of plant translocation to new medium and also increase in age (Figure 7). Although the highest and the lowest amounts of root proton effluxes in aerated plants belong to Hysun33 (273.6 µmol H^+ g FW^{-1} h^{-1}) and Lacomca (182.6 µmol H^+ g FW^{-1} h^{-1}) cultivars respectively (at 17 days old plant), their amounts were almost the same at the end of experiment (25^{th} day). Leakage of root protons was higher in all cultivars of sunflower at hypoxic conditions as compared to their controls (Figure 8). Magnitude of increasing root proton leakage was different in different cultivar. For example, after four days of hypoxia, the highest and the lowest root proton effluxes in comparison to their control were observed in Lacomca (68.5%) and Record (6.2%) cultivars respectively. Increase in the rate of root proton efflux might be due to injury of plasma membrane under hypoxic condition. Hypoxia triggers fermentation pathway, then lactic acid production in this pathway leads to reduction of pH in cytosol, simultaneously; lipid peroxidation caused by hypoxia leads to membrane injury and consequently reduces membrane fluidity and integrity (Jamei *et al*., 2009). This condition causes proton leakage in which we observed from plasma membrane.

Post-aeration doesn't improve the status of the plants imposed to hypoxia. The effect of hypoxia could be seen in aerated plants in such a way that the reduction of shoot and root dry weight has been continued even when the plants were settled in aerated condition (Figure 2 and 3). Same results were reported for different crops. For example Garnczarska *et al,* (2004), reported that exposure of lupine roots to increased oxygen availability after plant imposed to hypoxia caused a two fold increase in concentration of free radicals. The amount of hydrogen peroxide has slightly increased after re-aeration of roots hypoxically pretreated for 48 h and was fairly constant over the post-hypoxic period. They added that seedlings hypoxically pretreated for 72 h, the concentration of hydrogen peroxide gradually decreased during post-hypoxia. Drew (1997) suggested that perturbation of the cell structure and function during post-anoxia can be far more severe than during the period of uninterrupted anaerobiosis. In study of Skutnik and Rychter (2009) in leafs of barley indicated that production of ROS increased during post-aeration for 1 h of plant hypoxically pretreated for 4 h. At recovery period, the amount of root and leaf MDA (Figure 5 and 6) and root proton efflux (Figure 8) in all cultivars doesn't increased and finally approached to their control values.

In general, hypoxic condition causes reduction of plant dry weight in all cultivars was used in this experiment. Dry weight reduction in root was higher than shoot. However Lacomca and Progress cultivars with less reduction of dry weight showed more tolerance to hypoxia than Record and Hysun33 cultivars. The MDA production in roots was correlated with dry weight reduction however this trend was not the same in leafs. Re-aeration after 4 days of hypoxia leads to the reduction of root and shoot dry weight, MDA content and also root proton efflux.

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REFERENCES

Amooaghaie R, Mostajeran A, Emtiazi G (2002). The effect of compatible and incompatible *Azospirillum brasilense* strains on proton efflux of intact Wheat roots. *Plant Soil*. 243: 155-160.

Bacanamwo M, Purcell LC (1999). Soybean dry matter and N accumulation responses to flooding stress, N sources and hypoxia. *J Exp Bot*. 50: 689-696

Bai T, Li C, Ma F, Feng F, Shu H (2010). Responses of growth and antioxidant system to root-zone hypoxia stress in two *Malus* species. *Plant Soil.* 327: 95-105.

Bashan Y, Levanony H (1989). Effect of root environment on proton efflux in Wheat roots. *Plant Soil*. 119: 191-197.

Bashan Y, Levanony H, Mitiku G (1989). Changes in proton efflux of intact wheat roots induced by *Azospirillum brasilense* Cd. *Can. J. Microbiol*. 35: 691-697.

Bennicelli RP, Stepniewski W, Zakrzhevsky DA, Balakhnina TI, Stepniewska Z, Lipiec J (1998). The effect of soil aeration on superoxide dismutase activity, malondialdehyde level, pigment content and stomatal diffusive resistance in Maize seedlings. *Environ. Exper. Bot*. 39: 203-211.

Berbara RLL, Morris BM, Fonseca H, Reid B, Gow NAR, Daft MJ (1995). Electrical currents associated with arbuscular mycorrhizal

interactions. *New Phytol*. 129: 433-438.
Blokhina O (2000). Anoxia and oxidative stress: lipid peroxidation, antioxidant status and mitochondrial functions in plants. *Sci. (Biol.) Dissertation*, Finland: Helsinki, Univ.
Blokhina OB, Fagerstedt, KV, Chirkova, TV (1999). Relationships between lipid peroxidation and anoxia tolerance in a range of species during post-anoxic reaeration. *Physiol. Plant*. 105: 625-632.
Bowler C, Montagu M, Inze D (1992). Superoxide dismutase and stress tolerance. *Annu Rev Plant Biol*. 43: 83-116.
Catalá A (2009). Lipid peroxidation of membrane phospholipids generates hydroxy-alkenals and oxidized phospholipids active in physiological and/or pathological conditions. *Chem. Phys. Lipids*. 157: 1-11.
Crawford RMM, Braendle R (1996). Oxygen deprivation stress in a changing environment, *J. Exp. Bot*. 47: 145-159.
Drew MC (1997). Oxygen deficiency and root metabolism: injury and acclimation under hypoxia and anoxia. *Annu. Rev. Plant Biol*. 48: 223-250.
Fakhri Filsouf F (2011). Effect of hypoxia on Prolin and carbohydrate content in adventitious root in sunflower. *MS thesis, Dept. Biol.* University of Isfahan, Iran. (In Persian).
Foyer CH, Lopez-Delgado H, Dat JF, Scott IM (1997). Hydrogen peroxide- and glutathione-associated mechanisms of acclimatory stress tolerance and signaling. *Physiol Plant*. 100: 241-254.
França MGC, Rossiello ROP, Ramos FT, Zonta E (2006). Root growth and proton efflux rates of rice seedlings in a greenhouse environment. *Acta Bot. Bras.* 20: 25-30.
Frankel EN (1984). Chemistry of free radical and singlet oxidation of lipids. *Prog Lipid Res*. 23: 197-221.
Garnczarska M, Bednarski W (2004). Effect of a short-term hypoxic treatment followed by re-aeration on free radicals level and antioxidative enzymes in *Lupine* roots. *Plant Physiol. Biochem.* 42: 233-240.
Garnczarska M, Bednarski W, Morkunas I (2004). Re-aeration-induced oxidative stress and antioxidative defenses in hypoxically pretreated *Lupine* roots. *J. Plant Physiol.*161: 415-422.
Geigenberger P (2003). Response of plant metabolism to too little oxygen. *Curr. Opin. Plant Biol.* 6: 247-256.
Gill SS, Tuteja N (2010). Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. *Plant Physiol. Biochem*. 48: 909-930.
Grassini P, Indaco GV, Pereira ML, Hall AJ, Trápani N (2007). Responses to short-term waterlogging during grain filling in Sunflower. *Field Crop. Res*. 101: 352-363.
Heath RL, Packer L (1968). Photoperoxidation in isolated chloroplasts: I. kinetics and stoichiometry of fatty acid peroxidation. *Arch. Biochem. Biophys.* 125: 189-198.
Hernández I, Chacón O, Rodriguez R, Portieles R, López Y, Pujol M, Borrás-Hidalgo O (2009). Black shank resistant Tobacco by silencing of glutathione s-transferase. *Biochem. Biophys. Res. Commun.* 387: 300-304.
Hoagland DR, Arnon DI (1950). The water-culture method for growing plants without soil. *Calif. Agric. Exp. Stn. Circ*. 347 (2nd edit).
Holländer-Czytko H, Grabowski J, Sandorf I, Weckermann K, Weiler EW (2005). Tocopherol content and activities of tyrosine aminotransferase and cystine lyase in *Arabidopsis* under stress conditions. *J. Plant Physiol.* 162: 767-770.
Horchani F, Aloui A, Brouquisse R, Aschi-Smiti S (2008). Physiological responses of tomato plants (*Solanum lycopersicum*) as affected by root hypoxia. *J. Agron. Crop. Sci.* 194: 297-303.
Jamei R, Heidari R, Khara J, Zare S (2009). Hypoxia induced changes in the lipid peroxidation, membrane permeability, reactive oxygen species generation, and antioxidative response systems in *Zea mays* leaves. *Turk. J. Biol*. 33: 45-52.
Kato-Noguchi H, Morokuma M (2007). Ethanolic fermentation and anoxia tolerance in four rice cultivars. *J. Plant Physiol*. 164: 168-173.
Lew RR (1998). Immediate and steady state extracellular ionic fluxes of growing *Arabidopsis thaliana* root hairs under hyperosmotic and hypoosmotic conditions. *Physiol Plant*. 104: 397-404.
Mainiero R, Kazda M (2005). Effects of Carex rostrata on soil oxygen in relation to soil moisture. *Plant Soil*. 270: 311-320.
Malekahmadi F, kalantari KM, Torkzadeh M (2005). The effect of flooding stress on induction of oxidative stress and concentration of mineral element in pepper (*Capsicum annum*) plants. *Iranian J. Biol.* (In Persian). 18: 110-119.
Mantelin S, Touraine B (2004). Plant growth-promoting bacteria and nitrate availability: impacts on root development and nitrate uptake. *J. Exp. Bot*. 55: 27-34.
Mittler R, Vanderauwera S, Gollery M, Van Breusegem F (2004). Reactive oxygen gene network of plants. *Trends Plant Sci*. 9: 490-498.
Møller IM (2001). Plant mitochondria and oxidative stress: electron transport, NADPH turnover, and metabolism of reactive oxygen species. *Annu. Rev. Plant Biol*. 52, pp. 561-591.
Poorter H (1989). Plant growth analysis: towards a synthesis of the classical and the functional approach. *Physiol. Plant*. 75: 237-244.
Schaich KM (1992). Metals and lipid oxidation. *Lipids*. 27: 209-218.
Setter TL, Ella ES, Valdez AP (1994). Relationship between coleoptile elongation and alcoholic fermentation in Rice exposed to anoxia. II. Cultivar differences. *Ann. Botany*. 74: 273-279.
Skutnik M, Rychter AM (2009). Differential response of antioxidant systems in leaves and roots of barley subjected to anoxia and post-anoxia. *J. Plant Physiol.* 166: 926-937.
Szal B, Drozd M, Rychter AM (2004). Factors affecting determination of superoxide anion generated by mitochondria from *Barley* roots after anaerobiosis. *J. Plant Physiol.* 161: 1339-1346.
Wang K, Bian S, Jiang Y (2009). Anaerobic metabolism in roots of *Kentucky bluegrass* in response to short-term waterlogging alone and in combination with high temperatures. *Plant Soil.* 314: 221-229.