EFFECTS OF WATER STRESS ON CHLOROPHYLL FLUORESCENCE, SOLUBLE PROTEIN, PEROXIDASE AND MALONDEALDEHYDE CONTENT IN SOUR CHERRY CV. MIGREZ SEEDLINGS

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ABSTRACT

Lack of available water is one of the most important factors limiting plant distribution in the world. Water stress causes changes in morphological, physiological and biochemical of plants. The present study was designed to investigate the effects of drought on sour cherry cv. migrez seedlings. For this purpose, cherry seedlings in a randomized complete block design with six replications was used. Chlorophyll fluorescence parameters (Fm, Fv, Fv / Fm and Fo), soluble protein, peroxidase and malondialdehyde content were evaluated. Three irrigation treatments were applied: a well-watered control treatment, -5 and -10 bar water stress treatment. For the -0.5 and -10 bar treatment, water applied when soil water potential reached -5 and -10 bar, respectively. The results of the experiment showed that drought stress caused a significant increase in the value of the other parameters of Fo and Fm, Fv and Fv / Fm values were also significantly decreased with increasing salinity in that order. The results of the analysis of variance table showed a significant effect stress on soluble protein content and peroxidase malondialdehyde content is very significant. Significant mean difference between treatments in terms of lipid peroxidation and protein peroxides show. In terms of production malondialdehyde content control with 86/240 minimum display other treatments had significant differences, however, between the other treatments the difference was not significant and re-watering improvements in the level of lipid peroxidation in both treated with 5 - and 10 - bar found. The lowest protein control (98/3 mg g fresh leaves) and most of the severe drought treatment (10/5 mg g fresh leaves) are mild and dry, but the difference between treatment and control there was no significant difference. Reduces the amount of protein in the treatment of severe drought and re-watering is moderate. Amount of POX enzyme in the control treatment (83/12) and the most severe drought treatment (18/23) but the difference was not significantly different between the treatment and control of mild stress and re-watering reduces the amount of peroxidase the treatment is gentle and severe drought.

KEYWORDS:

Weather and climate factors essential role in plant growth and development and the impact on agriculture and uncontrollable variables are considered. Tolerant plants for each of the meteorological factors is limited and any abnormalities in these factors may directly or indirectly have a significant impact on agricultural production (MousaviBayeghi, 1390). The lack of available water, climatic factors that determine the distribution and dispersal of plants throughout the world, and may alter the morphological, physiological and biochemical of plants (HashemiDezfoli et al, 1374).

Water deficit (water stress or drought) caused by exposure to plants exposed to low water potential. except environment with high humidity, plants during the life cycle itself of different levels of water deficit suffered (Karafyllidis et al., 1996).

Stress are the main limiting factors of photosynthesis in plants (Bradford and Hsiao, 1982). In recent years, the technique of Chlorophyll fluorescence has become ubiquitous in plant ecophysiology studies (Maxwell and Johnson, 2000). Chlorophyll fluorescence excitation energy in photosynthesis is one of the ways that photosynthesis has been studied extensively in the literature. The physiology of chlorophyll fluorescence to determine the status of the plant and the amount of damage to the photosynthetic apparatus has been used in drought conditions (Hakam et al, 2000). Represents the fluorescence properties of fluidity, stability and structure of the membrane. When photosystem I, sees the damage by a variety of stresses, characteristic fluorescence changes. Changes in the chlorophyll fluorescence system for the identification and evaluation of graded stress tolerance to plants (Yamada et al, 1996; Greaves and Wilson, 1987). Typically chlorophyll fluorescence to assess the response of plant species to stresses such as freezing, drought and salinity are used. Chlorophyll fluorescence in primary health herb...
can also reduce the symptoms before declining to be identified clearly. So chlorophyll fluorescence can be used as a rapid method for the detection and identification of plant tolerance to environmental stresses applied (Percival and Henderson, 2003). High correlation between the cessation of photosynthesis (which is caused by excessive stimulation) and the ratio of variable fluorescence to maximum there (Roosta and Sadjadi, 1389).

The firstelectronreceptor(QA),theoxidation stateisdarkconditions. Sointhissituation, as reflectedfluorescence the field fluoresce nceorZero(FO) is considered. When some of QA reduction of lightis called fluorescence quenching of variable fluorescence (FV) is called. If QA isfullyrestored, the maximum fluorescence (Fm) will be seen (Klughammer and Schreiber, 2008).This effect occurs when etulation sample sareirradiated with lightly Flashs. This information can be used to calculate the efficiency of the photo chemical photosystemII(Amirjani, 1389).

Chlorophyll fluorescence and leaf photo syntheticsystem constructsan index of energy spurringrapid and non-destructive determination of the resistance of plantsto environmental stresses(Stribet and Strosser, 1998).Some factorsthat areobtainedin a short time, this method is veryusefulinformaton onthestatus and health ofplantmetabolic processesarepresented (Maxwell and Johnson, 2000).

One of the major biochemical changes caused by changes in soil moisture and reduce the impact on crop yields in the decomposition of plant proteins or prevent the Center of proteins for some of them are also making a small band tension. This is an important modification in gene expression has created a number of enzymes that are activated or deactivated, followed by permission of the change in the specific structure of tissues plants (Heikkilaet al., 1984).

Incidence of water stress changes Status Polyraybozomeffectiven tissue proteinsynthesis. Polyraybozom of dehydration under educditrate depends on the different species and in different parts of a plantare different. Whatisthe capacityof a plantto producemore Polyraybozom better able to toleratedroughtconditions(Scott et al., 1979).

The maincategoriesofstress-specific proteinsare appearlateembryogenesisproteins(LEAs),aproteinthat respondstoABA(RABs),Dhydrynsandvegetativestorage protein. MostLEAsproteinsprotectcellsagainst cell structurecomponentswithered stateon the basis of the issue arising fromlackof waterandDhydryns, Moyansthcoagulation andfoldingofmanylarget molecules, prevent andmaintain the integrity of the cell structure(Close, 1997).

oxidative stress is caused by Dehydration (Turkanet al., 2005). Lack of water causes the stomata to close, resulting in a lack of inhibition CO₂ and photosynthesis in the chloroplast, leading to the formation of reactive oxygen species that cause lipid peroxidation of the cell membrane is damaged (Mascheret al., 2005). Plants, especially those in high-stress environments enzymatic and non-enzymatic antioxidant defense systems are equipped to grow so they can take control of reactive oxygen species produced by activated oxygen species formed, however, may and cellular antioxidant defenses happen all the time oxidative damage disabling if (Hernandez et al., 2004). So over the course of Dhydrationcoordination mechanisms may be protective against oxidative damage to macromolecules and membranes remain structure (Hoekstra et al., 2001). Under mild stress conditions, radicals are effectively digested by the antioxidant defense system in periods of severe stress, increase system speed Digestivesaturated radical production resulting in cell damage (Mundreeet al., 2003). Failure of antioxidant defense system may cause oxidative damage to cellular components and membrane lipids (Pinheiroet al., 2004).

Peroxidationof membranelipidscanoccurburyreactive oxygen species, resulting in reduced permeability of the cellmembraneisselective(DolatAbadianet al., 1388). Thesereactive oxygen speciescan betoxicandextremelyhighbiodiversity, with Biomoleculesuch aslipids,proteins, nucleic acids, etc., and theprocess ofoxidative degradationreactions such aslipid peroxidation, chlorophyll degradation, proteinabnormalities, DNA damage andmutationsinnucleic acids(Demirevskaa et al., 2008).

Plantscope withincreased levels of antioxidant systemsemploy active oxygenwhich consists of non-enzymaticdefense such asalpha-tocopherol, ascorbic acidand vitaminA (Upadhyayaet al., 2008)andenzyme systemsincluding catalase(CAT),superoxidedismutase(SOD), ascorbateperoxi dase(APX),glutathione reductase(Gr)andpolyphenoloxida se,(PPO)(Hamrahiet al., 1389).
Given that drought characteristic geographic our country is no escape from this natural and unchangeable, and due to the lack of water and irrigation systems, incorrect use of local varieties and cultivars planted in some of the boundary Low efficiency the reasons for the decline are inappropriate operate in the country. Therefore methods such as proper utilization of the water with the use of proper agronomic practices such as crop plants, resistant, examining the reactions morphological, physiological and metabolic relationships of the plants coping with stress successfully transfer, it and susceptible cultivars, of high-yielding Drought Resistance attributable and planting drought resistant plants in climates where dehydration and development of cultivated plants in arid regions to provide more fruitfulness this regard will be useful.

MATERIALS AND METHODS

Sour cherry cv. migrez seedlings (rootstock Mahaleb) in March 1389 of the city of Marivan purchased and was accredited from nurseries university in Kurdistan outdoors intervals were 50 and 100 cm. After full deployment seedling in the soil, gypsum blocks to measure soil moisture and determine when watering the soil around the seedlings were placed at a depth of 20 cm. Tested in a randomized complete block design with six replications and three drought treatments in this experiment are:

Control: the treated plants were watered every other day.
The second treatment: when soil water potential in the treatment of 5 - bar the plants were irrigated.
The third treatment: when soil water potential in the treatment of 10 - once the plants were irrigated.

Sampling to measured different parameters in addition to the peak stress 24 hours after irrigation water treatments, 5 and 10 bar were taken.

In order to assess the strength migrez cherry Cultivar to drought stress effects on plant physiological traits including chlorophyll fluorescence figure, malondialdehyde content, protein and peroxidase were studied.

2100 PAM chlorophyll fluorescence measuring device was used to measure the fluorescence of German control plants under stress, choosing a leaf from each replicate And clips device that has a special shape and it is attached to the leaves divid device to connect it valve pack leaf adaptation to darkness after 20 minutes diode connected device to clip after clip by pressing on the valve opening device upon application of saturating flash of light (8000 mol m sec for the 1 s) fluorescence parameters chlorophyll fluorescence as a minimum (F0), maximal fluorescence (Fm) and variable fluorescence (Fv), the fluorescence maximum value minus the minimum fluorescence (Fv = Fm - F0) is obtained as the ratio of variable fluorescence to maximum fluorescence (Fv / Fm) appear on the display device.

To measure lipid peroxidation level small onedaldehyde content(MDA)in thereaction with thiobarbituric acid method(Heat and Packer, 1969) as areaction product of fatty acid oxidation was measured. To this end, 1/0 g fresh weight of leaves in 4 ml Trichloroacetic acid(TCA).0.1 was completely ground was centrifuged at 10,000 rpm for 5 min, then 1 ml of Supernatant extract in 4 ml Trichloroacetic acid 20 percent containing of thiobarbituric acid 0.5 percent, and in 95°C water bath for 30 min, was used. After cooling in ice-water bath, absorbance was read at wavelengths of 532 and 600 nm. To prepare extracts for the determination of protein and peroxidase 0.5 g of leaf tissue using liquid nitrogen and the leaves are powdered then added to 50 mg of PVP with the 5/1 ml potassium phosphate buffer (PH=7) containing Sodium Metabysol f the leaves in 100 ml buffer to crush the leaf tissues. Samples with 15,000 rpm and 4 °C for 20 min and then placed in a centrifuge to extract the micro-tubes 500 Micro USB and Micro 175 was mixed with 50% glycerol. Then, instantly, the extract containing micro-tubes at 50 ml and sent to concentration of total protein (Bradford, 1979) were used. Peroxidase enzyme activity method (Hemeda and Khein., 1990) were measured. In order to measure peroxidase reagent following were used in 780 ml potassium phosphate buffer 50 mM (PH=6/6) 90 microliters goulicol 1% , 90 ml hydrogen peroxide 0.3 % materials in a bed of ice in a Kueit (1 ml) , pooled and immediately 40 micro liters of extract protein extracted from the leaves, strawberry containing enzymes, plant is a Kueit added . curve shifts uptake and peroxidase activity at a wavelength of 470 nm and the number of micromoles H2O2 decomposed min mg protein were reported.

The data were analyzed using SAS software. Comparison of 1 and 5% level using Duncan's multiple range test was performed.

RESULTS
The results of the analysis of variance table showed that the effect of drought on chlorophyll fluorescence parameters such as Fo, Fm, Fv and Fv / Fm was significant (Table 4-4). Comparison shows a significant difference in stiffness between the different treatments and the control so that the change was an increase in Fo.0.274 the least severe dryness and treated with 0.421 had the highest effects. So that there was no significant difference between the mean difference was not significant between control and drought. Fo has not improved much rewatering. The comparison shows that the Fm, Fv, Fv / Fm was reduced to about three parameters control the amount of 1.378, 1.104 and 0.803 to the highest allocated. Associated with the trait Fm between control and drought treatments, moderate and severe are significant, but the treatment of moderate drought and severe drought not statistically significant in both land surface irrigation reuse improves Fm to a level that none of the treatments was not statistically significant, are average water level so that it can increase irrigation extreme drought re-treatment with any of the other treatments, but not significantly different from control. Re-treatment of the severe drought in the irrigation amount was increased to the extent that the treatment did not significantly moderate drought and re-watering (Table 4-5).

The results of the analysis variance table shows that drought has had a significant effect on the amount of soluble protein. Based on the results of the analysis of variance table land on malondealdehyde content and peroxidase highly significant effect (Table 4-7). Significant mean difference between treatments in terms of lipid peroxidation and production malondealdehydes shows. Lipid peroxidation levels increased with increasing severity of drought control so the 240.86 indicating the lowest value and the other treatments had no significant differences between the other treatments, but the difference was not significant. So with this improvement in lipid peroxidation in both irrigation treatments -5 and -10 bar were observed (Figure 4-10). Comparison shows that the protein is an increase with increasing salinity stress. So that the least amount of control (3.98 mg / g fresh leaves) and most of the severe drought treatment (5.10 mg / g fresh leaves), but the difference was significant difference between the treatment and control of mild drought did not exist. Retermining reduces the amount of protein in the treatment of severe and moderate drought (Figure 4-11). there was no significant difference between the control and further reduce the amount of irrigated land in the treatment of severe and moderate peroxidase (Figure 4-12).

### Table 4-4: Variance analysis of the impact drought on physiological parameters of sour cherry cv. migrez

<table>
<thead>
<tr>
<th>Mean Square</th>
<th>df</th>
<th>source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fv/Fm</td>
<td>Fv</td>
<td>Fm</td>
</tr>
<tr>
<td>0.004ns</td>
<td>0.01ns</td>
<td>0.022ns</td>
</tr>
<tr>
<td>0.029**</td>
<td>0.174**</td>
<td>0.097*</td>
</tr>
<tr>
<td>0.001</td>
<td>0.014</td>
<td>0.022</td>
</tr>
<tr>
<td>4.81</td>
<td>14.56</td>
<td>12.46</td>
</tr>
</tbody>
</table>

ns, *and**, respectively, non-significant, significant at 5% and 1%

### Table 4-5: Mean comparison treatment effect drought on chlorophyll fluorescence parameters

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Fv/Fm</th>
<th>Fv</th>
<th>Fm</th>
<th>F0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.803a</td>
<td>1.104a</td>
<td>1.378a</td>
<td>0.274b</td>
</tr>
<tr>
<td>Drought-5 bar</td>
<td>0.681c</td>
<td>0.721bc</td>
<td>1.055b</td>
<td>0.334b</td>
</tr>
<tr>
<td>Raining-5 bar</td>
<td>0.73b</td>
<td>0.904ab</td>
<td>1.225ab</td>
<td>0.321b</td>
</tr>
<tr>
<td>Drought-10 bar</td>
<td>0.615d</td>
<td>0.678c</td>
<td>1.099b</td>
<td>0.421a</td>
</tr>
<tr>
<td>Raining-10 bar</td>
<td>0.693bc</td>
<td>0.795bc</td>
<td>1.145ab</td>
<td>0.349ab</td>
</tr>
</tbody>
</table>

Heterologous with the letters There are significantly different between treatments.
Table 4-7: Variance analysis of the impact of drought on malondialdehyde content, soluble protein and peroxidase enzymes of sour cherry cv. Migrez

<table>
<thead>
<tr>
<th>Source</th>
<th>Mean Square</th>
<th>df</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>replication</td>
<td>4225.21**</td>
<td>5</td>
<td>ns</td>
</tr>
<tr>
<td>drought</td>
<td>6458.11**</td>
<td>4</td>
<td>*</td>
</tr>
<tr>
<td>Error</td>
<td>7208.97</td>
<td>20</td>
<td>**</td>
</tr>
<tr>
<td>CoeffVar(CV)</td>
<td>20.23</td>
<td></td>
<td>13.54</td>
</tr>
</tbody>
</table>

ns, * and ** respectively, non-significant, significant at 5% and 1%

Figure 4-12: Effect of drought treatment on malondialdehyde content of sour cherry cv. Migrez

Figure 4-12: Effect of drought treatment on protein content of sour cherry cv. Migrez

Figure 4-12: Effect of drought treatment on peroxidase enzyme of sour cherry cv. Migrez
DISCUSSION

In this experiment, drought stress increased and decreased F0Fm, Fv and Fv / Fm was. Changes in the yield of Chlorophyll fluorescence were first observed as early as 1960 by Kautsky and co-workers. Fm decreased under drought stress may result in damage to water decomposition cycle or electron transport is photosystem II (Retuerto et al., 2006). The results of the various tests campylotropis polyantha(Fang-Lan et al, 2011), wheat (Sayah et al, 1390), sunflower ( Taherabadi et al 1390) and the Persian Oak ( Zolfaghari et al., 1390) is consistent. Another drought impacts in other environmental stresses, including oxidative damage by oxygen free radicals, superoxide radicals, hydrogen peroxide and hydroxyl radicals occurs (Zhu, 2001). Production of free radicals, resulting in lipid peroxidation induced decomposition of unsaturated fatty acids (Salehi et al., 1386). This led to the formation of a toxic product called malondealdehyde content and decreases lipid peroxidation and loss of membrane selective permeability of the cell membrane stability (Basaga, 1989). Changes in plasma membrane permeability, leading to leakage of potassium ions and other soluble compounds present in the cell, oxidation of amino acids and eventually leads to cell death (Gratao et al., 2005). Increase in lipid peroxidation induced by decreasing CO₂ is closing stomata (Pinheiro et al., 2004). Plant transpiration and water loss in water to prevent your pores are closed and thus prevents the absorption CO₂. Oxygen free radicals and superoxide anions to unsaturated fatty acids attack and alter the structure and function of membranes and the production of synthetic compounds are aldehydes (Tohidi et al., 2009). Aldehydes are very harmful and inhibitors of cell metabolism enzyme activity, synthesis Tubulins are required to form a spindle and mitosis process (Peixoto et al., 1999). One reason for the slowdown occurs by mitotic cell membrane damage and aldehydes are produced (Allen and Ort, 2001). Malondealdehyde content increased in the test results based on Gisela5 (Sivritepe, 2008) and Bean (Turkan, 2005) is consistent.

Molecular analysis of drought tolerance in plants under drought stress in plant metabolism is more complete understanding. Many respondents in dryness induced genes have been identified by researchers. Expression of these genes causes osmotic synthetic preservatives such as amino acids, proteins, sugars and betaine are. In this study, an increase in protein content was observed under water stress. Hoekstra and co-workers in 2001 observation the protein synthesis under water stress due to the new defense enzymes and proteins (Hoekstra et al, 2001). Observed that many LEAs are induced by drought stress in Arabidopsis that are accumulated in the cytoplasm and nucleus, leading to accumulation of LEAs acquired resistance to water loss (Caruso et al., 2002). Water deficit in soybean also lead to changes in metabolism, cell wall proteins results in 82, 72, 57 and 35 kDa, which are structural components of cell wall assembly. These proteins are involved in regulating the growth and adaptation of plants under stress (Tausz et al., 2002). Increase in protein content have been reported in many experiments (Ahmdimousavi et al. 1389).

To neutralize the toxic effects of reactive oxygen species very effective antioxidant systems, which require two non-enzymatic and enzymatic systems in plant cells. Increase the production of antioxidant enzymes to turn off...
the water deficit is observed in the active radicals (Lascano et al, 2003). Enzymatic mechanisms to deal with stress, including peroxidase, ascorbatperoxidase, catalase and superoxide dismutase is. These enzymes are indicators of drought resistance in plants and the role of active oxygen compounds are metabolized by oxidative stress and prevent damage to the accommodations . In the experiment, observed that the increased peroxidase activity (Sivritepe et al., 2008) in Giesla5 and (Fazeli et al, 2006) corresponded to the sesame plant.

References


