Studying the effect of drought stress on yield and physiological characteristics in genotypes of canola (Brassica napus L.)

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Abstract
In order to study the effect of deficit irrigation on some physiological traits of genotypes of canola, a pot experiment was conducted in 2015-2016 in Fars province (Rostam). The pot experiment was examined in a factorial experiment form in a completely randomized design with three replications. The factors to be studied include 20 canola genotypes and drought stress in three levels (normal irrigation after 80 mm evaporation from pan class A, irrigation stop in flowering stage, irrigation stop at seed filling stage). Drought stress increases the activity of catalase and peroxidase enzymes, malondialdehyde, Cell membrane leakage, proline, and carbohydrates, and reduces RWC and chlorophyll a, b, and carotenoids.

Key Words: Canola, deficit irrigation, drought stress, genotypes

Introduction
Drought stress, as the most important factor limiting growth and yield of crops, affects about 40 to 60 percent of global agricultural lands (Shahoo et al., 2006; Rashidi, 2013). The average annual yield loss of crops due to drought in the world is about 17 percent that can increase to more than 70 percent a year. Currently, approximately 7% of the world's population lives in areas that suffer from water shortage and this could increase to 67 percent by 2050 (Wallach, 2006). Iran is considered a dry and a semi-dry country: average annual rainfall of Iran is equivalent to one-third of the world average rainfall, rainfall patterns in much of the country is irregular and unpredictable, and the lack of available water resources is evident (Peter, 2000; Ikaani and Khosh Akhagh, 2003). Due to limited water resources, it is recommended that deficit irrigation be used as an efficient option for increasing water productivity in projects. Another strategy to cope with this situation is using plant genetic engineering and breeding in projects to produce and introduce drought-tolerant genotypes (Ehsani and Khaledi et al, 2004).

After cereals, oilseed crops are the world’s second food supply and in addition to having rich fatty acids, they contain protein. Unfortunately, in spite of good potential for the cultivation of oilseeds in Iran, our country is still one of the major importers of oilseeds, and only about 10% of the need of the country for vegetable oil is provided from domestic sources. Due to the increasing population, statistics indicate an increase in imports of vegetable oils to Iran (Ministry of Commerce statistics). Increase in the price of oilseeds in global markets requires that efforts for agronomic and breeding to develop crops of oilseed production in Iran as a strategic objective for food security. This is because annually a major part of foreign exchange is spent on imports of vegetable oils (Aliari et al., 2000). Hence, the need for a coherent and long-term planning with the aim of achieving self-sufficiency in edible oils production is undeniable.

Materials and methods
In order to study the effect of deficit irrigation on some physiological traits of genotypes of canola, a pot experiment was done in Fars province (in Rostam) in 2015-2016. The pot experiment was examined in a factorial experiment form in a completely randomized design with three replications. The factors to be studied include drought stress in three levels (normal irrigation (N), stress in flowering stage (SF), and stress in seed filling (SF)) and canola genotypes in 20 levels (promising lines of canola) . At the time of planting in each pot, seven seeds of each genotype in planted. The pots with a diameter of 20 cm and a height of 30 cm are used and before planting, the pots are irrigated once. After three-leave stage of seedlings and additional thinned plants, a seedling is retained in each pot. Rostam is one of the cities of Fars province, which is located in the northwest of the province. The monthly average temperature and the average monthly rainfall of Rostam according to ten-year statistics of meteorology and soil analysis results are listed in the following table (Table 2). The names of the genotypes tested are given in Table 2 (Table 3).
Table 1: Average monthly temperature and rainfall in Rostam

<table>
<thead>
<tr>
<th>Month</th>
<th>Dec</th>
<th>Nov</th>
<th>Dec</th>
<th>Jan</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rain (mm)</td>
<td>56.3</td>
<td>126.1</td>
<td>18.9</td>
<td>0.1</td>
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<tr>
<td>Temp (°c)</td>
<td>12.4</td>
<td>16.6</td>
<td>24.7</td>
<td>31.2</td>
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Table 2: Results of soil test

<table>
<thead>
<tr>
<th>EC (mmol.cm⁻³)</th>
<th>PH</th>
<th>OC (%)</th>
<th>P (ppm)</th>
<th>K (%)</th>
<th>Clay (%)</th>
<th>Silt (%)</th>
<th>Sand (%)</th>
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<tbody>
<tr>
<td>0.97</td>
<td>7.61</td>
<td>0.71</td>
<td>12.05</td>
<td>117</td>
<td>52.32</td>
<td>42.3</td>
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Table 3: The names of genotypes

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<tr>
<th>Genotype</th>
<th>KRS18</th>
<th>KRS28</th>
<th>SS.29</th>
<th>SS.18</th>
<th>RS.17</th>
<th>KRS 33</th>
<th>KRS 68</th>
<th>KRS 18</th>
<th>AS 01</th>
<th>AS 02</th>
<th>AS 03</th>
<th>AS 05</th>
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<td>9</td>
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<tr>
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<td>AS 12</td>
<td>AS 04</td>
<td>AS 22</td>
<td>AS 41</td>
<td>AS 32</td>
<td>AS 02</td>
<td>AS 25</td>
<td>AS 68</td>
<td>AS 29</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Number</td>
<td>11</td>
<td>12</td>
<td>13</td>
<td>14</td>
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<td>19</td>
<td>20</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Measurements of Experimental traits: Oil content and oil yield: Oil content was measured with the help of NAR device. In order to assess oil yield, grain yield was multiplied by oil percentage (Shannon, 1984). Measuring the relative water content (RWC) of the leaves: to measure RWC, Firth and Lowell method (1999) was used.

\[
RWC(\%) = \left( \frac{FW - DW}{TW - DW} \right) \times 100
\]

FW: fresh weight; DW: dry weight; TW: timidity weight

Activity of catalase and peroxidase antioxidant enzymes: To investigate the activity of these enzymes, first leaf protein was extracted by Agrawal et al. (2005) method. MDA concentration of leaf: MDA concentration was measured according to Valentovic et al. (2006) method. Cell membrane leakage: Valentovic et al. (2006) method was used for measuring Cell membrane leakage.

Proline and soluble carbohydrates concentration of the leaf: proline concentration was measured using Bates et al. (1973) method. For measuring soluble carbohydrates, Dubois et al. (1956) was used.

Data analysis: After measuring and calculating traits listed in the test, the results were analyzed. To variance analysis and mean comparison, SAS software version 9.1 was used. Mean comparisons of traits measured were done using the least significant difference (LSD) at 5 percent. Charting was performed using Excel software.

Results and discussion:

Seed yield: Canola yield was significantly affected by drought conditions, and genotype (Table 4). With stopping irrigation at flowering and grain filling stages, canola grain yield significantly reduced 35 and 18 percent, respectively (Figure 1). These results suggest that the lack of moisture during the flowering is much more damaging than in grain filling stage. Flowering time, time of formation of physiological tanks, and capacity determining time is for storing photosynthetic supply. As the lack of moisture during the flowering stage would reduce these tanks, canola yield significantly decreased. On the other hand, it is clear that irrigation stop during grain filling stage leads to the dependence of the plant on storage compounds and any type with higher reserves in the leaves, stems, and other organs would easily overcome the problems of irrigation stop at filling time. Similar to the experiments, Gan et al. (2004) reported that irrigation stop at flowering stage reduces grain yield 58 percent. According to previous reports (Mendham and Salisbury, 1995), we can say that the drought stress and its effects on yield depend on the occurrence time in plants, and it is reported that the reproductive phase of the plant is more susceptible to drought stress (Hall 1992; Poulsen, 1994). By applying drought stress at different growth stages of canola, Dean et al. (2011) reported that the highest performance loss happens the flowering stage that is consistent with these experimental results. In a general view, it was found that genotypes 4 and 6 have the highest seed yield and genotypes 17 and 18 had the least (Figure 2).

Oil content: Genotype and drought stress have a significant impact on canola seed oil content (Table 4). The results showed that irrigation stop at flowering and grain filling stages decreased 7.5% and 3% of canola oil seed yield, respectively (Figure 3). The affectability of oil biosynthesis from drought is similar to biosynthesis affectability of photosynthetic products. The present results are consistent with the results of Champolivier (1996),
who showed that drought stress during reproductive stage corresponded to the decrease of oil. Reduction in the oil content in the event of drought stress during flowering stage in canola has been reported by Tsfamaryam et al. (2010) and Nilsson et al. (1997). These results are consistent with the findings of the Sinki et al. (2007). The highest oil percent (41.93%) was achieved in genotype 10, although it was not statistically significantly different from genotypes 1, 5, 6, 8, 9, 11, 14, 16 and 20 (Figure 4). The lowest seed oil percent (40.25%) was observed in genotype 12 (Figure 4).

Oil yield: Genotype and drought stress had a significant effect on the yield of canola seed oil (Table 4). Drought stress caused a significant reduction on seed oil yield and compared to irrigation stop in flowering and grain filling, respectively 40 and 21% oil yield decreased (Figure 5). Decrease in oil yield was the reason of decrease in oil percent yield in drought conditions. Oil yield loss caused by drought stress has been reported by many researchers (Ganskara et al., 2006; Szumigalski, and Van Acker, 2006; Tesfamariam et al., 2010). The results showed that genotype six has the highest oil yield (2.35 g plant$^{-1}$) and the lowest oil yield (1.67 g plant$^{-1}$) was in genotype 18 (Figure 6).

Relative Water Content (RWC): RWC was significantly affected by the interaction between genotype and drought stress (Table 4). In Figure 7, it is clearly seen that, leaf RWC has distinguishable levels. This means that drought stress has clearly influenced leaf water status. In other words, drought stress has caused tension in different levels of canola. The RWC status of leaf in control samples is high, while with irrigation stop at flowering stage, RWC has dropped, and finally with irrigation stop at grain filling stage, it has reached its lowest level (Figure 7). Although drought stress led to relative RWC of leaf, this reduction was different among different genotypes for genetic diversity. In this regard, Kaj et al. (2004) connected difference of relative RWC of the leaf content in canola varieties to differences in their root systems and therefore, we can say that genotypes with higher root power have more power in water uptake from the soil and maintaining more moisture in their leaves. Other researchers as well (Foshing et al., 2006) have reported RWC loss due to drought stress. The results of this test are consistent with the test of Baji (2001).
Catalase activity (CAT): Catalase is involved in destroying free radicals, which are damaging the structure and composition of cells. Catalase activity was affected by the interaction between genotype and drought stress levels (Table 5). Catalase activity in normal irrigation (control) was lower than the levels of stress and maximum catalase activity was observed in plants that stress was applied at flowering stage (Figure 8). The highest catalase activity was in genotype 4 and during irrigation stop at flowering stage. Catalase activity is a defense mechanism to plant stress and this could be due to the high stress tolerance index in genotype 4. In the event that plants face stress, too much hydrogen peroxide is produced in the plant, which is toxic to cells and its compounds. In such circumstances, catalase enzyme starts to work and analyzes Hydrogen Peroxide (Bakke and Scanners, 2003). In other words, catalase production is in response to stress. It seems that such a process has happened in this experiment because catalase activity in stressed genotypes is much higher than the control (Figure 8). It was observed that genotypes that have higher yields under drought stress have higher levels of enzyme activity.

Peroxidase activity (GPX): Catalase is of antioxidant enzymes of the plants that by removing
free radicals in plant help stability (Bowen et al., 2007). The interaction between genotypes and drought stress on peroxidase activity is significant (Table 5). Further investigations showed that peroxidase activity in normal conditions is very low and after applying stress to the plants, it significantly increased (Figure 9). The highest activity of peroxidase was reached when irrigation stop was carried out in the flowering stage. In this regard, genotype six had the maximum peroxidase activity that might account for the high stress tolerance index is in this genotype, as peroxidase stops damage from free radicals to membranes and vital component of the cell. Today, researchers believe that the increase in antioxidant enzymes increases plant tolerance to environmental stresses (Guo et al., 2005; Esfandiari, et al., 2008).

Malondialdehyde (MDA): One of the reactions accelerated in the presence of active oxygen species is membrane lipid peroxidation that leads to production of aldehydes such as malondialdehyde (MDA) and products such as ethylene (Liu and Huang, 2000). In fact, the concentration of MDA is a measure of the activity of free radicals. In this experiment, it was found that the concentration of MDA in the canola leaf extract is affected by the interaction between genotype and drought stress (Table 5). The highest concentration of MDA was reached when irrigation stop was applied at flowering stage and the lowest value was observed in control samples (Figure 10). Among the genotypes whose irrigation was stopped in flowering stage, the highest concentration of MDA was in the genotypes 18 and 11 (Figure 10) and both of these genotypes were in the least drought stress ranks. This states that the low drought tolerance of these genotypes could be due to damage caused by free radicals, and the product of this kind of damage is high concentrations of malondialdehyde concentration and in both the figure is much higher than other genotypes. Studying the sensitivity of canola genotypes to drought stress confirms that genotypes 18 and 11 were most sensitive genotypes to drought stress. It is also stressed that the vulnerability of these genotypes from drought could be due to damage of free species of oxygen, which leads to more production of malondialdehyde in cell extracts of these genotypes. Jiang and Huang (2001) consider increase in the concentration of MDA as reflecting the increase in lipid peroxidation reactions and oxidation of fatty acids. The lowest concentration of MDA in the genotypes whose irrigation was cut in flowering stage was found in genotype 6 that is probably the reason of these genotypes drought stress resistance. Parvanova et al. (2004) considered MDA the final product of peroxidation of lipid as a sign of free radical generation and membrane damage in stress. The result of a study on Rose hybrideah showed that MDA concentration increased during drought stress (Jin et al., 2006).

Cell membrane leakage (CML): Cytoplasmic membrane is the first place affected by the damaging effects of environmental stresses (Kocheva and Georgiev, 2003). Maintenance of membrane stability due to its role in controlling the entry and exit of ions, molecules and maintaining turgor (Clark et al., 1991) is of great importance. Therefore, it can be used as a measure to evaluate drought tolerance. Since the change in the health of cell membranes is associated with electrolyte leakage (Kocheva and Georgiev, 2003), evaluation of electrolyte leakage from leaf tissue has been suggested as a test method for cell membrane stability (Kocheva and Georgiev, 2003). The amount of Cell membrane leakage in canola was significantly affected by drought stress (Table 5). As is seen in Figure 11, the permeability of the cell membrane because of drought stress has increased. This amount was much higher than irrigation stop at flowering stage than when it was stopped during grain filling. It was observed that irrigation stop at flowering and grain filling stages led the leakage of the cell membrane to reach 2.1 and 2.6 times the amount of control (Figure 11). These results indicate that drought stress has further damaged cell membranes.

Jiang and Huang (2002) while studying the reaction of two varieties of grasses to drought stress and ABA observed that under drought stress, in both species, electrolyte leakage increased and this situation was consistent with the results of this study. Increase in lipid peroxidation and a decrease in cell membrane stability index in beans (Turkan et al., 2005) have been reported.

The concentration of soluble carbohydrates in the leaf: Test results of the test showed that the concentration of leaf carbohydrates significantly affected by the interaction between genotype and drought (Table 5). When stress was applied, leaf carbohydrates concentration significantly increased. The highest concentration of carbohydrates in the leaves was obtained when irrigation was cut during flowering of canola (Figure 12). The highest concentration of carbohydrates in the leaves was in genotypes 6 and when irrigation cut was carried out in the flowering stage. Carbohydrates increase in drought stress is a kind of physiological response to drought that is mainly used in osmotic regulation mechanism and to retain water in the cells. The ability to produce more carbohydrates and subsequently osmotic adjustment allows the plant to retain more water and does biological and physiological reaction under limited water conditions, and finally has high efficiency (Chimenti et al., 1996). It seems this is true in the case of genotype 6, because leaves have high concentrations...
of carbohydrates and has high production yield, drought tolerance, and insensitivity to drought stress. The relationship between concentrations of carbohydrates of leaves with long drought tolerance becomes more apparent by examining the changes in leaf carbohydrates concentration in genotypes 11 and 17 because they had the lowest concentration of leaf carbohydrates. One of the important mechanisms of plant adaptation in response to osmotic stresses such as drought and salinity stress is active or inactive accumulation of soluble organic and inorganic ingredients such as sugars and amino acids (Martin et al., 2002). A number of cellular organelles are responsible for making these materials such that soluble compounds cause the water to returns to the cell or preserve the cells from damage caused by dehydration (Bloom Valed, 2000). By reviewing the responses of maize to drought stress, Valentovic, et al. (2006) stated that drought stress increased the leaf carbohydrate concentration of maize. The increasing concentration of carbohydrates in the leaves of maize resistant to drought was more.

Proline concentration in the leaf: The results showed that interaction of genotype and drought stress on proline content in leaves of canola was significant (Table 5). Proline concentration was higher than that of the control when drought stress was applied (Figure 13). When the irrigation stop was in at the time of flowering, more proline was produced compared to other levels of stress (Fig. 13). In general, the concentration of proline increased under stress conditions. Proline acts as a nitrogen storage tank or soluble substance that reduces the cytoplasmic osmotic potential and helps plant in stress tolerance (Eide et al., 2004). Proline acts as a source of energy to adjust the redox potential as an energy source, and eliminates the active oxygen species and provides the conditions required for continued absorption of water from root, but this combination is costly for plants and plants produces these materials by reducing the function. Proline concentration in drought stress conditions is due to oxidation-reduction of proline or stimulating its synthesis from glutamate or increasing the activity of protease (Sharma, 2006). Moreover, proline has the duty to protect cytosolic enzymes and cell structure, so proline increases under stress conditions that was consistent with the results of this study. In general, increase in the amount of proline is seen as a stress adaptation mechanism in plant, through which the plant is trying to change the amount of water to maintain osmotic potential (Haar and Cross, 1997). Before this, proline increase in response to drought stress had been reported (Dean et al., 2011, Omidi et al., 2010).

**Table 5: Analysis of variance of enzyme activity and concentrations of carbohydrate and proline of canola leaf in pot experiment**

<table>
<thead>
<tr>
<th>genotype/drought</th>
<th>CAT (mg protein)</th>
<th>GPO (mg protein)</th>
<th>MDA (μmol.g^{-1} FW)</th>
<th>CML (mg.g^{-1} DW)</th>
<th>Carbohydrates (μmol.g^{-1} FW)</th>
<th>Proline (μmol.g^{-1} FW)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotype</td>
<td>19</td>
<td>15</td>
<td>3.7**</td>
<td>0.01**</td>
<td>31</td>
<td>199**</td>
</tr>
<tr>
<td>Drought</td>
<td>2</td>
<td>203**</td>
<td>1407**</td>
<td>0.5**</td>
<td>4541**</td>
<td>4835**</td>
</tr>
<tr>
<td>Error</td>
<td>120</td>
<td>0.08</td>
<td>0.47</td>
<td>0.00096**</td>
<td>248</td>
<td>26**</td>
</tr>
<tr>
<td>CV (%)</td>
<td>-</td>
<td>2.2</td>
<td>2.3</td>
<td>1.3</td>
<td>207</td>
<td>2.4</td>
</tr>
<tr>
<td>Coefficient of determination(R^2)</td>
<td>-</td>
<td>0.99</td>
<td>0.99</td>
<td>0.99</td>
<td>0.98</td>
<td>0.99</td>
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** and *, significant at 0.01 and 0.05, respectively.

**Figure 8: Interaction between genotype and drought stress on the activity of catalase**

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Figure 9: The interaction of genotype and drought stress in peroxidase activity

Figure 10: The interaction of genotypes and drought stress in malondialdehyde

Figure 11: Cell membrane leakage of canola in levels of drought stress

Figure 12: The interaction of genotype and levels of drought stress in carbohydrates

Figure 13: The interaction of genotypes and drought stress levels in proline

Conclusion:
Irrigation stop at flowering and grain filling stages of canola led to 35 and 18 percent yield decrease. Genotypes 4 and 6 had the highest seed yield and genotypes 17 and 18 had the least. Irrigation stop at flowering and grain filling stages decreased 7.5% and 3% of canola oil seed yield, respectively. The results showed that the highest seed oil percent (41.93%) was obtained in genotype 10. The lowest seed oil percent (40.25 percent) was observed in genotype 12. Drought stress caused a significant reduction in seed oil yield and compared to control (normal irrigation), irrigation stop at flowering and grain filling stages reduced seed oil yield to 40 and 21 percent. The decline in oil yield was due to decrease in oil yield reduction of the percent of seed oil in drought stress conditions. Drought stress increased the activity of antioxidant enzymes catalase and peroxidase in canola genotypes, but the increase in enzyme activity among genotypes was not the same for genetic diversity, and it was observed that the genotypes that have higher yields under drought stress had higher levels of enzyme activity. Among the genotypes whose irrigation was stopped in flowering stage, the highest concentration of MDA was in the genotypes 18 and 11 and both of these genotypes were in the least drought stress ranks. The highest concentration of carbohydrates in the leaves was obtained when irrigation was cut during flowering stage of canola.

References