Changes in physiological traits and fatty acid composition in sesame (Sesamum indicum L.) cultivars under various foliar application and drought stress conditions


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Abstract. Sesame is a source of excellent vegetable oil (35-63%). The present study aimed to evaluate the effect of phosphorus nano-chelate and chitosan application under end-season drought stress conditions based on split plot-factorial in three replications in the research field of Shahed University in Tehran, Iran during 2015-16 on the cultivation of sesame during summer. Main plots included drought stress in three levels including full irrigation as control (non-stress), irrigation cut-off at 50% seed ripening (75 BBCH), and 50% flowering (65 BBCH) as mild and severe stress levels, respectively. Subplots consisted of spraying treatments at four levels including non-spraying (control), phosphorus nano-chelate (2 ppt), chitosan (3 g.L⁻¹), and a combination of phosphorus nano-chelate + chitosan, as well as two cultivars such as sesame Oltan and Naz. Based on the results, the effects of drought stress, foliar application, and cultivar were significant on seed yield, oil and protein contents, peroxidase activity, and palmitic acid content. The highest seed yield was achieved by using phosphorus nano-chelate in Naz cultivar under full-irrigation condition (2114.7 kg.ha⁻¹). Severe drought stress caused a decrease in more than 60%, compared to the control treatment. Regarding foliar treatments, the highest percentage of oil was observed in using phosphorus nano-chelate, as well as the integrated use of phosphorus nano-chelate and chitosan, which increased by 12.39 and 14.97%, respectively, compared to the control treatment. Oil content in the seed of Naz cultivar was 7.22% higher than that of Oltan cultivar seed. In addition, the severe drought stress and Naz cultivar had the highest seed protein percentage (23.43 and 22.49%, respectively). The activities of catalase and superoxide dismutase antioxidant enzymes were directly related to an increasing drought stress. Further, Oltan cultivar had the highest activities of SOD and POX enzymes. The highest saturated fatty acids (palmitic and stearic) content were achieved in the non-foliar application (control treatment) in Oltan cultivar. Regarding the interaction effects of foliar treatment and cultivar, the lowest linoleic percentage was achieved in Oltan cultivar under non-foliar treatment and the highest percentage of the acid was observed in other treatments. In conclusion, the foliar application of phosphorus nano-chelate and chitosan improved the quantitative and qualitative yield of sesame and increased the growth and quality under drought stress condition.

Keywords: enzyme activity, linoleic, oil, palmitic, protein, seed yield, nano fertilizer

Abbreviations: CAT: catalase, SOD: superoxide dismutase, ROS: reactive oxygen species, POX: peroxidase, ppt: part per thousand, BBCH: Biologische Bundesanstalt, Bundessortenamt, and Chemische Industrie

Introduction

Drought is a major environmental stress affecting plant morphology, physiology, and biochemistry (Shao et al., 2008). Plants can avoid the harmful effects of drought in several ways such as stomata closure, leaf rolling, osmotic adjustments, a reduction in
the cellular expansion, and alterations of various essential physiological and biochemical processes which can affect growth, productivity, and quality (Farouk and Amany, 2012). Traits are correlated with drought tolerance such as yield components and physiological traits, which are suitable indicators for selecting drought tolerant genotypes in breeding programs in order to reduce the impact of water deficit on crop yield (Almeida et al., 2008). Biochemically, water deficit was observed to stimulate the accumulation of the ROS (Dossa et al., 2017). In order to prevent or alleviate injuries from ROS, plants have evolved an antioxidant defense system including non-enzymatic compounds like ascorbate, glutathione, tocopherol, carotenoids, flavonoids, and enzymes such as SOD, CAT, POX, ascorbate peroxidase, glutathione reductase, and polyphenol oxidase (Agrwal and Pandey, 2004). Yousefzadeh Najafabadi and Ehsanzadeh (2017) reported that the drought stress in the sesame plant led to an increase in carotenoid concentration, CAT, SOD, and POX activities. A large number of studies have focused on the effects of drought stress on sesame yield, yield components, and oil content (Kadkhodaie et al., 2014). However, few studies emphasized the available information regarding the effects of drought stress on the fatty acid compositions of sesame genotypes. For example, Kim et al. (2006) planted 18 cultivars withholding irrigation for only 15 days at flowering and maturity stages and determined the seed oil yield and composition, compared with the well-watered plants as a control. Based on the results, the seed oil content was not significantly affected by drought stress while the oleic acid content increased. However, the linoleic acid content decreased under drought conditions. In another study, Abedi and Pakniyat (2010) reported that drought stress results in increased protein content, phosphorus content, CAT activity, and SOD activity. Jouyban and Moosavi (2012) concluded that an increase in the irrigation interval led to an increase in the protein content of sesame.

Chitosan, known as elicitors, is considered as a natural biopolymer modified from chitin, which is the main structural component of squid pens, cell walls of some fungi and crab shells. They were introduced as a material to improve grain yield under unfavorable conditions due to their bioactivities to plants such as inducing the plant resistance against a wide range of diseases through antifungal, antibacterial, antivirus activities (Wang et al., 2006), stimulating the growth of plants and seed germination (Chandrrkrachang, 2002), improving soil fertility and enhancing the mineral nutrient uptake of plant (Dzung, 2007), increasing the content of chlorophylls, photosynthesis, and chloroplast enlargement (Limpanavech et al., 2008), escalating nitrogen fixing nodes related to the species of leguminous plants and reducing the effects of abiotic stress on plants (Dzung et al., 2011).

Nanotechnology provides new interdisciplinary venture into agriculture and food sciences by converging science and engineering. In addition, nanoparticles have potential applications in agriculture system, viz., the detection of pollutants, plant diseases, pests, and pathogens, controlled delivery of pesticide, fertilizers, nutrients, and genetic material, and can act as nano architects in forming and binding soil structure (Ghormade et al., 2011). The excess use of chemical fertilizers causes an irreparable damage to the soil structure, mineral cycles, soil microbial flora, plants, and even more on the food chains across ecosystems leading to heritable mutations in future generations of consumers. Considering the abovementioned issues, the use of nano-fertilizers in agriculture is considered as one of the solutions to the problems (Solanki et al., 2015). Jan et al. (2014) reported that the application of phosphorus plays a vital role...
in forming and translocating carbohydrates, root development, crop maturation, and resistance to disease pathogens.

Sesame (*Sesamum indicum* L.) is an ancient oilseed crop mainly grown in drought-prone environments, which is highly valued due to its high oil yield (~55%), as well as quality and stability (Pathak et al., 2014). Although sesame is considered as drought tolerant crop, the productivity is heavily affected by severe drought stress mainly when it occurs during anthesis (Dossa et al., 2017). Resistance to water stress in sesame is important in many countries with low rainfall (Golestani and Pakniyat, 2015). The fatty acid composition of sesame seed oil determines its commercial value, and drought stress and genotype may affect both the quality and quantity of oil which is extractable by sesame seed processors (Kadkhodaie et al., 2014).

Restricted water resources are considered as a limiting factor for irrigation applications around the world. Therefore, the present study aimed to evaluate the changes in the quality and biochemical traits of two sesame genotypes under drought stress and foliar application of chitosan and phosphorus nano-chelate in second cultivation.

Materials and methods

**Plant material and growth conditions**

The present study was conducted to investigate the changes in the quality and biochemical traits related to two sesame genotypes under drought stress and foliar application in chitosan and phosphorus nano-chelate in the second cultivation at research field of Shahed University in Tehran, Iran, during 2016. The experiment was conducted during a one-year factorial split plot experiment based on completely randomized block design with three replications. The research farm is located at geographical characteristics of latitude 31° and 36’ and longitude 48° and 53’ and the height of this area from sea level is equal to 1050 m. The place soil was tested and recognized as silty loam clay soil with pH about 8.10 (*Table 1*).

*Table 1. Some physical and chemical properties of soil in the experimental area (depth of 0-30 cm)*

<table>
<thead>
<tr>
<th>Texture</th>
<th>pH</th>
<th>Electrical conductivity (dS.m⁻¹)</th>
<th>Organic matter (%)</th>
<th>Total nitrogen (mg.kg⁻¹)</th>
<th>Phosphorus (mg.kg⁻¹)</th>
<th>Potassium (mg.kg⁻¹)</th>
<th>Iron (mg.kg⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experiment farm</td>
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<td>2.00</td>
<td>1.11</td>
<td>1100</td>
<td>16.6</td>
<td>303.6</td>
<td>7.0</td>
</tr>
<tr>
<td>Optimum range</td>
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<td>1.51</td>
<td>2.00</td>
<td>2000</td>
<td>16.0</td>
<td>350.0</td>
<td>6.6</td>
</tr>
</tbody>
</table>

The experimental factors including drought stress at full irrigation as control (non-stress), mild stress (irrigation cut-off at 50% seed ripping equivalent 75 BBCH) and severe stress (irrigation cut-off at 50% flowering equivalent 65 BBCH) were in the main plots and spraying treatments including non-spraying (control), phosphorus nano-chelate (2 ppt), chitosan (3 g.L⁻¹), and the combination of phosphorus nano-chelate + chitosan and Oltan, and Naz single branch sesame cultivars were in the subplots. Based on the available documents, 75 BBCH is equivalent to seed about 50% of the final size.
and 65 BBCH equals to 50% of flower open (Attibayeba et al., 2010). The experimental site and planting farm are presented in Figure 1 and the climatic and meteorological conditions in Table 2.

**Figure 1.** Experimental site and planting farm

**Table 2.** Climate and meteorological data during the experiment period

<table>
<thead>
<tr>
<th></th>
<th>Jan</th>
<th>Feb</th>
<th>Mar</th>
<th>Apr</th>
<th>May</th>
<th>Jun</th>
<th>Jul</th>
<th>Aug</th>
<th>Sep</th>
<th>Oct</th>
<th>Nov</th>
<th>Dec</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maximum temperature</td>
<td></td>
<td></td>
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<td>(°C)</td>
<td>17</td>
<td>18.9</td>
<td>24.2</td>
<td>31.4</td>
<td>35.4</td>
<td>14.4</td>
<td>44</td>
<td>43</td>
<td>37.8</td>
<td>34.4</td>
<td>18.8</td>
<td>16.8</td>
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<tr>
<td>Minimum temperature</td>
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<tr>
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<td>-11</td>
<td>0.5</td>
<td>0</td>
<td>13.8</td>
<td>15.4</td>
<td>19</td>
<td>19</td>
<td>15.8</td>
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<td>0.2</td>
<td>2.9</td>
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<tr>
<td>Average temperature</td>
<td></td>
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<tr>
<td>(°C)</td>
<td>4.6</td>
<td>4.6</td>
<td>12.2</td>
<td>18.3</td>
<td>24.6</td>
<td>29.2</td>
<td>32</td>
<td>31</td>
<td>27</td>
<td>17.3</td>
<td>8.9</td>
<td>6.2</td>
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<tr>
<td>precipitation (mm)</td>
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<td>10.5</td>
<td>14.8</td>
<td>6</td>
<td>9.1</td>
<td>2.3</td>
<td>0.8</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>8.2</td>
<td>8.4</td>
</tr>
<tr>
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<tr>
<td>(m.s(^{-1}))</td>
<td>3.6</td>
<td>3.8</td>
<td>4.5</td>
<td>4.6</td>
<td>4.5</td>
<td>5.5</td>
<td>5.5</td>
<td>4.1</td>
<td>4</td>
<td>4.6</td>
<td>3.7</td>
<td>3.3</td>
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<tr>
<td>Maximum wind speed</td>
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<tr>
<td>(m.s(^{-1}))</td>
<td>18</td>
<td>13</td>
<td>20</td>
<td>15</td>
<td>26</td>
<td>25</td>
<td>32</td>
<td>14</td>
<td>18</td>
<td>13</td>
<td>14</td>
<td>13</td>
</tr>
</tbody>
</table>
Regarding each experimental plot, six 3.5-m lines with 50 × 10 cm spacing, and main and subplots were 2 and 1 m, respectively. The seed was planted in late May after harvesting the wheat. Field preparation operations such as plowing, disc and the like were conducted before planting. Then, the first irrigation was given after sowing and the second was performed after 3 days. Accordingly, the irrigation time was determined by using Class A evaporation pan (in 70 mm evapotranspiration) (Shakeri et al., 2015). In the next procedure, irrigation treatments such as irrigation up to 50% flowering and seed ripening were performed after foliar use of nutrients. As for chitosan, chitosan-poly (D-glucosamine) prepared from SIGMA-ALDRICH was 3 g.L⁻¹, and a combination of the Khazra nano fertilizer (17% of phosphorus) was used at 2 ppt concentration for phosphorus nano-chelate. Then, spraying with these compounds was conducted at the beginning of flowering and seed ripping. In addition, drought stress treatments were conducted by irrigation at 65 BBCH (severe stress) or 75 BBCH (mild stress).

The extended Biologische Bundesantalt and Chemische (BBCH) scale and its associated decimal code were used to describe the different growth stages of *Sesamum indicum* L.

**Seed yield**

Seed harvest was done after physiological maturity, which occurred at the beginning of November by considering the browning about 75% of the capsules on the main stem in 50% of the plants. First, the grain yield was calculated based on the unit area and harvesting one square meter of each experimental plot and measuring grain weight (Mehrabi and Ehsanzadeh, 2011).

**Seed oil and protein contents**

Grain protein measurements were performed using the Bradford method (1976). In addition, the grain oil percentage was conducted by the method proposed by Leiboritz et al. (1987). Oil and protein percentages were multiplied in the seed yield in order to calculate the oil and protein yield.

**Seed phosphorous contents**

In order to measure the seed, phosphorus percentage was used based on the method proposed by Badigannavar et al. (2015). Finally, the obtained number was included in the standard equation and calculated as the concentration in the plant dry matter.

**Protein and antioxidant enzyme assay**

Before the leaves became yellowish, ten leaves of each plot were sampled for biochemical traits at the end of the seed ripening. In order to determine the total protein content and enzyme activity, 1 g of plant material was homogenized in 1 M Tris-HCl buffer (pH 6.8). The homogenate was centrifuged in a refrigerated centrifuge at 13000 g for 20 min (SIGMA 3-30K), and the obtained supernatant was used for determining protein assaying and enzyme. All the steps were conducted at 4 °C. Then, the protein content of the extracts was determined according to Bradford’s (1976) method by using bovine serum albumin as the standard (Fazeli et al., 2007).
Catalase assay

The total CAT (EC 1.11.1.6) activity was measured according to the method proposed by Beers and Sizer (1952). The reaction solution consisted of 50 μL of the enzyme extract, 100 mM phosphate buffer (pH 7.0), 0.1 μM EDTA, and 20 mM H$_2$O$_2$ in a total volume of 1.5 mL. Then, the enzyme activity was estimated by decreasing the absorbance of H$_2$O$_2$ at 240 nm due to H$_2$O$_2$ consumption. In addition, the decrease in H$_2$O$_2$ was monitored by reading the absorbance at the latter wavelength through using a UV-visible spectrophotometer (model Lambda 25, PerkinElmer, USA), and quantified by its molar extinction coefficient (36 mM$^{-1}$ cm$^{-1}$). Finally, the enzyme activity was expressed as μmol H$_2$O$_2$ min$^{-1}$ g$^{-1}$ FW.

Peroxidase assay

Peroxidase (POX; EC 1.11.1.7) activity was assayed as described by Aghighi Shahverdi et al. (2018). The reaction mixture consisted of 4 cm$^3$ of 0.2 M acetate buffer (pH 4.8), 0.4 cm$^3$ of H$_2$O$_2$ (3%), 0.2 cm$^3$ of 20 mM benzidine, and 0.05 cm$^3$ of enzyme extract. The rate of benzidine oxidation was measured at 530 nm.

Superoxide dismutase assay

The total SOD (EC 1.15.1.1) activity was determined by measuring the ability to inhibit the photochemical reduction of nitroblue tetrazolium (NBT) as described by Beauchamp and Fridovich (1971). The reaction mixture consisted of 0.1 cm$^3$ enzyme extract, 50 mM potassium phosphate buffer (pH 7.8), 13 mM methionine, 75 μM NBT, 0.1 mM EDTA, and 2 μM riboflavin. First, the test tubes were shaken and placed 30 cm from three 30 W fluorescent lamps. Then, the reduction in NBT was measured by reading absorbance at 560 nm. In the next procedure, blanks and controls were run similarly without any illumination and enzyme, respectively. One unit of SOD was defined as the amount of enzyme which produced a 50% inhibition of NBT reduction under the assay conditions (Fazeli et al., 2007).

Fatty acid compounds

In order to determine the amount of fatty acids, the oil should be extracted without high temperature in order to avoid possible changes in the fatty acid composition. Consequently, 50 g seed (after grinding) was mixed with hexane (1:4 ratio) and placed on a shaker (160 rpm) for 48 hours (Farhoosh et al., 2009). After separating the solvent, it was added to 7 mL methanol potassium 2 M. Then, each sample was injected into a gas chromatography (GC/MASS; Acme 6000, YOUNG LIN, Korea) with a 100 m-length column, an inner diameter of 0.25 mm, and 0.2 μm thickness. By comparing the peak of samples with standard peak and based on the RRT pick (relative retention time), the type of fatty acids was identified and its values were determined through calculating the surface area under the resulting peak curves.

Statistical analysis

ANOVA was used for analyzing the results of total traits by using the SAS 9.4 procedure PROC GLM. The mean comparison of data was done by Duncan’s multiple range test at 5% probability level. Finally, correlation coefficients were calculated for each of the traits with the SAS 9.4.
Results

Seed yield

As shown in Table 3, the effect of drought, foliar application, cultivar, and interaction effect of drought × foliar application × cultivar on seed yield was significant. The highest seed yield was achieved by using phosphorus nano-chelate and control treatment in Naz cultivar and full irrigation condition (2114.7 and 2120.3 kg.ha⁻¹, respectively). According to the Figure 2, severe drought stress in Naz cultivar had the lowest seed yield (659.4 kg.ha⁻¹). Furthermore, an integrated application of phosphorus nano-chelate and chitosan under severe stress condition caused 4.76 and 10.85% increase in the seed yield of Oltan and Naz cultivars in compared to the mild stress, respectively.

Figure 2. Interaction effect of drought stress × foliar treatments on the seed yield of sesame. (Means in each column followed by at least one similar letter are not significantly different at 5% probability level by Duncan multiple test)

Seed oil

The results of ANOVA indicated that the effect of drought stress, spraying and cultivar was significant on oil percentage and oil yield. In addition, the effect of drought × foliar application and drought × cultivar was significant on yielding seed oil (Table 3). As shown in Table 4, irrigation up to 50% flowering caused the least amount of seed oil. In foliar treatments, the highest oil percentage was observed in using phosphorus nano-chelate as well as the integrated application of phosphorus nano-chelate and chitosan, which increased 12.39 and 14.97%, respectively, compared to the control treatment (Table 5). Further, the oil content in the seed of Naz cultivar was 7.22% higher than that of Oltan cultivar seed (Table 6). Furthermore, as illustrated in Figure 3, based on the mean comparison of interaction effects of drought stress and foliar application, the highest seed oil yield was observed in full irrigation (control) with foliar application of phosphorus nano-chelate (922.3 kg.ha⁻¹) and the lowest mean was achieved in severe drought stress and none-sprayed treatment. Regarding the interaction effect of drought stress in cultivars, the highest oil yield was obtained in Naz cultivar
under full irrigation (924.03 kg.ha\(^{-1}\)). The results indicated that the severe drought stress could reduce oil yield by more than 50%, compared to the control treatment in both cultivars (Fig. 3).

**Figure 3.** Interaction effects of drought stress × foliar treatments and drought stress × cultivars on oil yield of sesame. (Means in each column followed by at least one similar letter are not significantly different at 5% probability level by using Duncan multiple test)

**Seed protein**

As shown in Table 3, drought stress and cultivar could play a role in the percentage and yield of protein. In addition, foliar application, drought × foliar, drought × cultivar, and foliar × cultivar significantly affected protein yield. The severe drought stress level and Naz cultivar had the highest seed protein percentage (23.43 and 22.49%, respectively) (Tables 4 and 6). In addition, full irrigation treatment under the foliar application of phosphorus nano-chelate and chitosan (alone and integrated application) resulted in increasing seed protein yield. On the other hand, severe drought stress and non-sprayed treatment had the lowest seed protein yield, which decreased 57.21%, compared to the control treatment (Fig. 4). Further, based on the mean comparison interaction effect of drought stress in cultivar, the highest mean was observed in full irrigation treatment under the foliar application and drought stress conditions. The results showed that increasing the level of drought stress caused a significant decrease in seed protein yield.

**Figure 4.** Interaction effects of drought stress × foliar treatments and drought stress × cultivars on protein yield of sesame. (Means in each column followed by at least one similar letter are not significantly different at 5% probability level by using Duncan multiple test)
Table 3. Results of ANOVA (mean square) for the effect of drought stress and foliar application of phosphorous nano-chelate and chitosan on quality traits, the activity of antioxidant enzymes, fatty acid contents of two sesame cultivars

<table>
<thead>
<tr>
<th>Sources of variance</th>
<th>df</th>
<th>Seed yield</th>
<th>Oil content</th>
<th>Oil yield</th>
<th>Protein content</th>
<th>Protein yield</th>
<th>Phosphorus content</th>
<th>CAT activity</th>
<th>POX activity</th>
<th>SOD activity</th>
<th>Oleic content</th>
<th>Linoleic content</th>
<th>Palmitic content</th>
<th>Stearic content</th>
</tr>
</thead>
<tbody>
<tr>
<td>Block (B)</td>
<td>2</td>
<td>44149.4 ns</td>
<td>19.3 ns</td>
<td>13893.0 ns</td>
<td>0.57 ns</td>
<td>966.8 ns</td>
<td>0.0016 ns</td>
<td>0.0007 ns</td>
<td>0.0009 ns</td>
<td>0.00002 ns</td>
<td>6.31 ns</td>
<td>9.86 ns</td>
<td>3.98 ns</td>
<td>5.13 ns</td>
</tr>
<tr>
<td>Drought (D)</td>
<td>2</td>
<td>3996516.8**</td>
<td>59.9**</td>
<td>115556.6**</td>
<td>0.0064 *</td>
<td>0.91 **</td>
<td>0.17 *</td>
<td>0.49 **</td>
<td>3.85 ns</td>
<td>4.93 ns</td>
<td>10.48**</td>
<td>10.48**</td>
<td>10.48**</td>
<td>10.48**</td>
</tr>
<tr>
<td>B × D</td>
<td>4</td>
<td>12049.9</td>
<td>3.8</td>
<td>4658.8</td>
<td>0.75</td>
<td>1093.1</td>
<td>0.00005</td>
<td>0.001</td>
<td>0.01</td>
<td>0.003</td>
<td>23.43</td>
<td>16.02</td>
<td>1.06</td>
<td>0.68</td>
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<tr>
<td>Foliar application (F)</td>
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<td>232814.7**</td>
<td>227.2**</td>
<td>114094.2**</td>
<td>15.5 ns</td>
<td>18590.2**</td>
<td>0.20 **</td>
<td>0.05 **</td>
<td>0.11 ns</td>
<td>0.03 ns</td>
<td>10.92 ns</td>
<td>9.49 ns</td>
<td>2.47 ns</td>
<td>7.22 *</td>
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<tr>
<td>Cultivar (C)</td>
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<td>786003.0**</td>
<td>173.0**</td>
<td>324063.0**</td>
<td>54.4 *</td>
<td>90303.6**</td>
<td>0.00004 ns</td>
<td>0.001 ns</td>
<td>0.21 *</td>
<td>0.18 **</td>
<td>2.02 ns</td>
<td>19.17*</td>
<td>6.94 *</td>
<td>16.11 *</td>
</tr>
<tr>
<td>D × F</td>
<td>6</td>
<td>240058.6**</td>
<td>13.5 ns</td>
<td>52273.6**</td>
<td>1.2 ns</td>
<td>16581.4**</td>
<td>0.001 ns</td>
<td>0.01 ns</td>
<td>0.07 ns</td>
<td>0.01 ns</td>
<td>3.28 ns</td>
<td>8.62 ns</td>
<td>3.85 *</td>
<td>5.10 ns</td>
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<tr>
<td>D × C</td>
<td>2</td>
<td>182158.5**</td>
<td>12.9 ns</td>
<td>38123.4*</td>
<td>4.93 ns</td>
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<td>117209.2**</td>
<td>8.1 ns</td>
<td>13825.0ns</td>
<td>3.91 ns</td>
<td>10675.3*</td>
<td>0.001 ns</td>
<td>0.004 ns</td>
<td>0.02 ns</td>
<td>0.036 ns</td>
<td>9.40 ns</td>
<td>22.37**</td>
<td>11.69 **</td>
<td>15.51 **</td>
</tr>
<tr>
<td>D × F × C</td>
<td>6</td>
<td>125747.9**</td>
<td>3.7 ns</td>
<td>20469.2 ns</td>
<td>1.94 ns</td>
<td>7519.0 ns</td>
<td>0.00001 ns</td>
<td>0.003 ns</td>
<td>0.12 ns</td>
<td>0.023 ns</td>
<td>1.79 ns</td>
<td>1.88 ns</td>
<td>1.11 ns</td>
<td>1.67 ns</td>
</tr>
<tr>
<td>Error</td>
<td>42</td>
<td>10.63</td>
<td>10.45</td>
<td>15.77</td>
<td>12.8</td>
<td>18.60</td>
<td>16.53</td>
<td>18.29</td>
<td>21.60</td>
<td>22.43</td>
<td>6.02</td>
<td>5.67</td>
<td>10.42</td>
<td>18.34</td>
</tr>
<tr>
<td>CV (%)</td>
<td>-</td>
<td>10.63</td>
<td>10.45</td>
<td>15.77</td>
<td>12.8</td>
<td>18.60</td>
<td>16.53</td>
<td>18.29</td>
<td>21.60</td>
<td>22.43</td>
<td>6.02</td>
<td>5.67</td>
<td>10.42</td>
<td>18.34</td>
</tr>
</tbody>
</table>

*, ** Significant at P < 0.05 and P < 0.01, respectively

Table 4. The effect of drought stress levels on some of the quality traits and activity of CAT and SOD enzymes

<table>
<thead>
<tr>
<th>Drought stress levels</th>
<th>Oil content (%)</th>
<th>Protein content (%)</th>
<th>Phosphorus content (%)</th>
<th>CAT activity (U.mg protein.min⁻¹)</th>
<th>SOD activity (U.mg protein.min⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Full irrigation (non-stress as control)</td>
<td>44.31 ± 1.05 a</td>
<td>20.51 ± 0.46 b</td>
<td>0.28 ± 0.01 a</td>
<td>0.14 ± 0.01 c</td>
<td>0.46 ± 0.01 c</td>
</tr>
<tr>
<td>Irrigation up to 50% seed ripping (mild stress)</td>
<td>43.11 ± 0.87 a</td>
<td>20.91 ± 0.64 b</td>
<td>0.29 ± 0.02 a</td>
<td>0.21 ± 0.02 b</td>
<td>0.56 ± 0.03 b</td>
</tr>
<tr>
<td>Irrigation up to 50% flowering (severe stress)</td>
<td>36.67 ± 1.21 b</td>
<td>23.43 ± 0.46 a</td>
<td>0.26 ± 0.02 b</td>
<td>0.51 ± 0.01 a</td>
<td>0.75 ± 0.03 a</td>
</tr>
</tbody>
</table>

Means within a column followed by the same letter are not significantly (P < 0.05) different according to Duncan multiple test
Seed phosphorous

As indicated in Table 3, the results of ANOVA indicated that drought stress and foliar application could significantly influence on seed phosphorous content. In addition, drought stress caused a 7.14% decrease in seed phosphorous content, compared to the control treatment (Table 4). Further, the use of phosphorous nano-chelate had the highest seed phosphorous content (0.43%) while control treatment had the lowest mean in this regard (Table 5).

Antioxidants activity

Based on the results in Table 3, drought stress played a significant effect on CAT and SOD activities. The activities of these two antioxidant enzymes are consistent with an increase in drought stress. Thus, severe drought stress had the most activity. Regarding the comparison between the spraying treatments, the most catalase activity was related to chitosan application (Table 5). Furthermore, a significant difference was observed between cultivars in terms of POX and SOD activities. In addition, Oltan cultivar had the highest activities of SOD (0.79 U.mg protein.min\(^{-1}\)) and POX (0.64 U.mg protein.min\(^{-1}\)) enzymes (Table 6).

### Table 5. The effect of foliar application of phosphorous nano-chelate and chitosan on oil content, phosphorous content, and CAT activity of sesame

<table>
<thead>
<tr>
<th>Foliar treatments</th>
<th>Oil content (%)</th>
<th>Phosphorus content (%)</th>
<th>CAT activity (U.mg protein.min(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>38.32 ± 1.43 b</td>
<td>0.19 ± 0.01 d</td>
<td>0.24 ± 0.04 c</td>
</tr>
<tr>
<td>Phosphorus nano-chelate</td>
<td>43.74 ± 1.15 a</td>
<td>0.43 ± 0.01 a</td>
<td>0.26 ± 0.04 c</td>
</tr>
<tr>
<td>Chitosan</td>
<td>38.33 ± 1.35 b</td>
<td>0.25 ± 0.009 b</td>
<td>0.36 ± 0.03 a</td>
</tr>
<tr>
<td>Phosphorus nano-chelate + chitosan</td>
<td>45.07 ± 1.11 a</td>
<td>0.23 ± 0.007 c</td>
<td>0.30 ± 0.04 b</td>
</tr>
</tbody>
</table>

Means within a column followed by the same letter are not significantly (P < 0.05) different according to Duncan multiple test

### Table 6. The effect of cultivars od sesame on oil and protein contents, and POX and SOD activities

<table>
<thead>
<tr>
<th>Cultivars</th>
<th>Oil content (%)</th>
<th>Protein content (%)</th>
<th>POX activity (U.mg protein.min(^{-1}))</th>
<th>SOD activity (U.mg protein.min(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oltan</td>
<td>39.81 ± 1.08 b</td>
<td>20.75 ± 0.51 b</td>
<td>0.79 ± 0.04 a</td>
<td>0.64 ± 0.03 a</td>
</tr>
<tr>
<td>Naz</td>
<td>42.91 ± 0.89 a</td>
<td>22.49 ± 0.39 a</td>
<td>0.68 ± 0.03 b</td>
<td>0.54 ± 0.02 b</td>
</tr>
</tbody>
</table>

Means within a column followed by the same letter are not significantly (P < 0.05) different according to Duncan multiple test

Fatty acid composition

Oleic, linoleic, palmitic, and stearic acids are the most important fatty acids measured in the experiment. The results indicated that the effects of cultivar, as well as the interaction of foliar application in cultivar, were significant on the contents of linoleic, palmitic, and stearic acids (Table 3). As illustrated in Figure 5 and 6, the highest content of saturated fatty acids (palmitic and stearic) was achieved in the non-
foliar application (control treatment) in Oltan cultivar. In addition, the interaction effect of drought stress and the foliar application was significant on palmitic acid percent (Table 3). Further, as shown in Figure 5, the highest palmitic acid percent achieved full irrigation treatment under foliar application in chitosan and compound of phosphorous nano-chelate and chitosan. Regarding the interaction effects of foliar treatment and cultivar, the lowest linoleic percentage was achieved in Oltan cultivar under non-foliar treatment and the highest percentage of the acid was observed in other treatments (Fig. 6).

**Figure 5.** Interaction effects of drought stress × foliar treatments and foliar treatments × cultivars on palmitic acid percentage. (Means in each column followed by at least one similar letter are not significantly different at 5% probability level, using Duncan multiple test)

**Figure 6.** Interaction effects of foliar treatments × cultivars on linoleic and stearic acids percentage. (Means in each column followed by at least one similar letter are not significantly different at 5% probability level, using Duncan multiple test)

**Correlations among traits**

Tables 7 and 8 represent the results of simple correlation in non-drought stress (control) and drought stress conditions, respectively. As shown, qualitative traits such as seed yield, oil content, oil yield, protein content, protein yield, and phosphorous content were positively correlated in both conditions. However, a weak correlation was reported between oil content and protein content although the correlation among grain yield with
oil and protein yield was very high. Regarding drought stress conditions, no significant correlation was observed between fatty acids content and qualitative traits while positive and negative correlations were observed between the traits in the control condition.

**Table 7. Correlation coefficients among physiological and quality attributes of sesame under non drought stress conditions**

<table>
<thead>
<tr>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
<th>13</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quality traits</td>
<td>1.00</td>
<td>0.85 0.75 1.00</td>
<td>0.60 0.32 0.66 1.00</td>
<td>0.94 0.33 0.84 0.88 1.00</td>
<td>0.32</td>
<td>-0.32 -0.56 -0.95 -0.81</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Enzyme activity</td>
<td>0.54 0.45 0.29 0.48 0.31 -0.59</td>
<td>-0.37 -0.29 -0.37 -0.47 0.56</td>
<td>-0.32 1.00 -0.45 1.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fatty acids quality</td>
<td>1.00</td>
<td>-0.89 -0.49 -0.60 -0.52</td>
<td>1.00</td>
<td>0.72 0.51 1.00</td>
<td></td>
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<td></td>
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<td></td>
</tr>
</tbody>
</table>

**Table 8. Correlation coefficients among physiological and quality attributes of sesame under drought stress conditions**

<table>
<thead>
<tr>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
<th>13</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quality traits</td>
<td>1.00</td>
<td>0.52 1.00</td>
<td>0.95 0.75 1.00</td>
<td>0.89 0.48 0.85 0.35 1.00</td>
<td>0.36</td>
<td>0.32 0.32 1.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Enzyme activity</td>
<td>-0.56 -0.49 -0.60 -0.52</td>
<td>-0.62 -0.41</td>
<td>0.72 0.51 1.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fatty acids quality</td>
<td>1.00</td>
<td>-0.29</td>
<td>-0.35 -0.48 1.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

1: Seed yield, 2: Oil content, 3: Oil yield, 4: Protein content, 5: Protein yield, 6: Phosphorous content, 7: CAT activity, 8: POX activity, 9: SOD activity, 10: Oleic content, 11: Linoleic content, 12: Palmitic content, 13: Stearic content
Discussion

Plants are exposed to environmental stresses during their growth. Drought stress is considered as one of the major abiotic stresses which causes a high reduction in plant growth and yield, which can affect the absorption and transfer of nutrients to the plant (Askari et al., 2018). Thus, the response of two sesame genotypes for lasting drought stress was emphasized in the present study. In addition, the originality of the present research resides in the fact that it is used as an integrated approach by combining agromorphological, physiological, biochemical and seed quality traits to identify useful genotypes and interesting traits efficiently for planting in drought-prone environments in the south of Tehran, Iran. Based on the results, the severe drought stress led to a decrease in seed yield, oil percentage, oil yield, and plant phosphorus content. In line with the results of the present study, severe or prolonged drought plays a significant role in the productivity by affecting the number of capsules per plant, grain yield and oil yield and quality despite its relative tolerance to drought stress (Kadkhodaei et al., 2014; Dossa et al., 2017). Drought stress through chlorophyllase and peroxidase enzymes activities in plants resulted in reducing the content of chloroplast destruction and chlorophyll. In addition, these physiological changes lead to a reduction in plant growth and yield (Misra and Sricastatva, 2000; Askari et al., 2018).

Sesame seed quality (oil and protein contents) are adversely affected by environments severe drought (Menshah et al., 2006). The results of this study indicated that opposite responses to the changes in the oil content and protein content were found in response to drought stress as severe drought stress led to an increase in protein content and a decrease in the seed oil content (Table 4). According to Akinoso et al. (2006), the oil percentage varies with genetic and environmental influences. The saturated fatty acid such as palmitic (C16:0) and stearic (C18:0) acids and unsaturated fatty acid such as linoleic acid (C18:2) were insignificantly influenced by cultivar and interaction effect of foliar application in cultivar while the effect of drought stress on fatty acids composition, except palmitic acid content, was insignificant (Table 3). As illustrated in Figures 5 and 6, non-application of nutrient treatment (control) in Oltan cultivar had the highest palmitic and stearic acid contents. In addition, drought stress resulted in decreasing the oil percentage, oleic acid and linoleic acid concentrations of the seeds and increasing the protein percentage, palmitic acid, and stearic acid in safflower seeds (Mohsennia and Jalilian, 2012). Further, genetic, climatic and agronomic factors indicated a significant effect on oil content and composition of sesame. However, oil content and composition are considerably different within different varieties (Yoshida et al., 2007). Fatty acid composition and oil content are exposed to variation by different physiological, ecological and cultural factors. The composition of sesame oil relies on the climatic conditions, soil type, as well as the maturity of plant and variety (El-Khier et al., 2008). Furthermore, the proximate analysis showed a significant variation among the different sesame genotypes which implies that genetic diversity is available among Nigerian sesame (Alege and Mustapha, 2013). Alege et al. (2011) studied three species of sesame from Nigeria by using morphological markers and reported a significant genetic diversity.

The mechanisms of absorption and transfer of nutrients in plants such as mass flow, emission or absorption, and transfer by osmotic phenomena are regarded as all functions of the moisture content of the soil and the expansion of the absorbing root. Regarding the reduced moisture or root expansion, intensity and amount of nutrient uptake undergo some changes (Taiz and Ezeiger, 1998). In the present study, the plant phosphorus
content decreased by increasing drought stress (Table 4). Askari et al. (2018) reported that severe and mild drought stress can reduce phosphorus concentration to 15.96 and 6.96%, respectively, compared to optimal irrigation. The modulation of root development is considered as one of the drought stress effects. On the other hand, root growth is closely related to the absorption of phosphorus and nitrogen from the soil. In such a case, foliar application of phosphorus nano-chelate can compensate for the deficiency of this element and improve the growth, which was consistent with the results of the present study (Askari et al., 2018).

In addition, supplying the plant with its needed nutritional sources could increase its protein and oil percentage. Further, as shown in Table 5, practicing the phosphorus nano-chelate fertilizer increased the oil content in this experiment. Furthermore, phosphorus plays a role in increasing the formation of more capsules, and eventually, the formation of more seeds since it can enhance shooting and flowering (Safari Arabi et al., 2018). As a result, an increase in seeds leads to an increase in the oil content. In addition, it can affect the percentage and the yield of sesame oil since better nutrition leads to better photosynthesis during sesame growth and supplying the needed phosphorus fills the pod and seed.

The production of ROS appears to be involved in stress resistance induction (Gill and Tuteja, 2010; Pongprayoon et al., 2013). In order to prevent or alleviate injuries from ROS, plants have evolved an antioxidant defense system including non-enzymatic compounds like ascorbate, glutathione, tocopherol, carotenoids, flavonoids, and enzymes such as SOD, CAT, POX, ascorbate peroxidase, glutathione reductase, and polyphenol oxidase (Agarwal and Pandey, 2004). Based on the results of this study, the higher activity of SOD and CAT in the severe drought stress level indicated that they have a stronger ability to remove ROS (Table 4). Increased SOD, POX and CAT activities in response to water stress were reported in some studies (Kukreja et al., 2005). Further, Fazeli et al. (2007) and Kadkhodaei et al. (2014) concluded that more increase of antioxidant enzymes in leaves was related to drought resistance of some sesame genotypes. Furthermore, Cho et al. (2008) reported an improvement in the quality of sunflower by increasing the free radical scavenging activity. In general, the higher POX and SOD activities in Oltan leaves than may suggest the higher efficiency of Oltan under drought condition, compared to those of Naz.

Chitosan is a biopolymer with multiple agricultural applications, which has been shown to enhance plant growth and production yield in many species such as rice (Oryza sativa L.), pearl millet (Pennisetum glaucum L.), maize (Zea mays L.) and many orchid species (Pornpienpakde et al., 2010; Kananont et al., 2010). Furthermore, it was proposed as one potential chemical which can induce drought resistance via improving water use efficiency in the plant, as well as inducing drought resistance (Bittelli et al., 2001; Dzung et al., 2011). The same results were observed in the present experiment.

In addition, based on the results of the present experiment, the highest CAT activity was observed in the foliar application of chitosan which an increase of 33.33%, compared to the control treatment (Table 5). The chitosan response in plants is involved in inducing the reactive oxygen species (ROS) scavenging system. The enzyme activity and/or transcript levels of several antioxidant enzymes such as CAT, SOD, and POX were found to be induced by chitosan treatment (Yang et al., 2009; Povero et al., 2011). Further, the application of chitosan prior to drought stress can enhance the antioxidant activities of the SOD and CAT enzymes and decrease electrolyte leakage and production of malondialdehyde (Yang et al., 2009).
Regarding the results of correlation between traits, more negative correlation was observed between antioxidant enzyme activities (CAT and SOD) and qualitative traits (seed yield, seed oil and protein contents, and phosphorous content) in drought stress conditions, compared to non-drought stress conditions (Tables 7 and 8). Dossa et al. (2017) reported a high correlation within the different antioxidative enzyme activities and morphological traits of sesame which is in line with the results of the present study.

Conclusion

In conclusion, the exposure of sesame cultivars to drought resulted in decreasing seed yield, percentage and yield of seed oil, increasing seed protein percentage, CAT and SOD activities, and changing the fatty acids composition. In addition, foliar application of phosphorus nano-chelate and chitosan caused an increase in the seed yield, seed oil yield, and phosphorous content. Further, Naz cultivar was superior to most of the studied traits, compared to Oltan cultivar. Furthermore, the results indicated that sesame planting in warm and dry conditions could produce the appropriate grain yield and oil yield, due to the improvement of the physiological traits including the synthesis of oil and protein, the activity of antioxidant enzymes, and the like. Due to an increase in the content of phosphorus seeds and the improvement of oil and protein content of seeds, phosphorus nano fertilizer increased the quantitative and qualitative attributes of sesame. Finally, planting Naz cultivar under irrigation limited to the foliar application of phosphorus nano-chelate and chitosan is highly recommended to achieve higher performance in warm and dry climates. Study of changes in the quality of fatty acids and their relationship with physiological traits for future research is recommended.

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