The Effects of Drought Stress and Nitrogen Levels on Yield, Stomatal Conductance and Temperature Stability of Rapeseed (Canola) Genotypes

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INTRODUCTION

Rapeseed (canola) is one of the most important oilseed plants which is the greatest source of oil production in the world after soybean and oil palm and is a good alternative for wheat by 40-44 % oil [9]. This plant has a particular stand for production in arid areas due to having cholesterol-free polyunsaturated oil, low physiological zero, high efficiency of water consumption [2] and moderate tolerance to soil salinity [38]. Moreover, rapeseed is now the largest source of biodiesel throughout the world. About one-third of the world land is classified as arid and semiarid areas and the rest are subject to seasonal or regional water fluctuations [7]. Worldwide food shortage in recent years has made agricultural authorities around the world concentrate on making optimal use of arid areas talents [5]. One of the most important characteristics of arid and semiarid areas is the occurrence of drought stress there. Drought is the most common peripheral stress which nearly restricts production of 25% of the land around the world. This stress is the most important factor restricting the production of rapeseed in semiarid areas of the world [34]. Drought stress is one of the hardest stresses for growth and production of plants and is one of the major factors of reduction of crop yield throughout the world which decreases the yield 50% or more [57]. The average annual precipitation in Iran is 240 mm which is about one-third of global annual precipitation (700 mm), and it has arid and semiarid climate [56]. Moreover, rainfall pattern in most areas of Iran is very irregular and unpredictable and plants might be exposed to drought stress even during the common rainfall periods [15]. It has been observed that drought stress can disrupt a number of...
metabolic and physiological processes in plants [31]. For example, nutrient uptake is reduced under drought stress conditions through reduction of transpiration, active transport damage, and membrane permeability disorder due to reduction of root absorption strength [55]. Plants reaction to drought in different drought levels significantly depends on severity and duration of stress and also the plant species and it growth stage [10]. On the other hand, nitrogen is the main component of protein and thus plants need adequate amount of it in order to grow naturally [33]. Plants access to nitrogen and its absorption is affected by available moisture; therefore, adjusting the rate of fertilizer for feeding plants in arid and semiarid areas is based on available moisture regime in the root zone [14]. It has been identified that even when the moisture is limited, lack of nutrients can decrease water consumption efficiency. Since the efficiency of nitrogen absorption from the soil is low, the optimal yield of rapeseed is obtained when remarkable amount of this element is used [17,18]. Since nitrogen is an effective element in the success of rapeseed production [24], it is the first element whose shortage in arid and semiarid areas is mainly discussed [33]. Rapeseed genotypes have different reactions to agronomic factors [27]. Moreover, many researchers have referred to a lot of differences between rapeseed characteristics under stress conditions such as the difference in yield and yield components [41,45,16], number of branches per plant [41], percentage of oil [45,40], percentage of fatty acids [40], sensitivity and tolerance towards drought [1]. Therefore, determining the best drought tolerant genotypes and also managing nitrogen consumption in dealing with stress in arid and semiarid areas are quite necessary.

MATERIALS AND METHODS

This experiment was carried out in 2010-2011 in the research field of Islamic Azad University Shoushtar branch. The experiment site was at latitude of 32°3’ N and 48°47’ E and 383 m above the sea level. Soil texture was silt loam.

The annual precipitation of this region is 322 mm and it has semiarid climate. It was a split split plot experiment in the form of randomized complete block design. The main plot included drought stress in two levels: without drought stress (control) and drought stress. The sub plot included different amounts of nitrogen in four levels: without nitrogen, 35, 70, 105 kg/ha pure nitrogen from urea source, and also sub sub plot, rapeseed genotypes is including Hyola 401 (control), SG1-87182 and SG9-87182 lines. In order to apply drought stress since the beginning of pods growth stage (consistent with 6/1 Sylvester-Bradley Makepeac scale), the irrigation was cut and the rain water was prevented from reaching the desired plots by installing a shelter. Each plot contained 4 rows and each row contained two planting lines as long as 3 m. The seeds were planted at a depth of 1 cm and they were spaced from each other 5 cm and irrigation was done immediately. In order to prepare the land, after smoothing the land it was plowed by moldboard. Then the lumps were crushed by disk and the land surface was leveled by land leveler. Chemical fertilizers were distributed by centrifuge fertilizer supplier machine and the fertilizers were buried under the soil by the disk and finally furrow and ridge were made by furrower. Immediately after planting, irrigation was done by means of hydro flume.

In order to determine plant temperature stability at the beginning of grain formation stage (code 6/1 according to Sylvester-Bradley scale) plant canopy temperature was measured, so that minimum and maximum temperature of plant were measured by an infrared thermometer equipped with laser radiations before the sunrise (minimum temperature) and between 12-2 pm (maximum temperature) and plant stability was calculated via the following equation [43].

$$\text{CTS} = \frac{(T_{a(max)}-T_{a(min)})}{(T_{c(max)}-T_{c(min)})}$$

Here, $T_{a(max)}$, $T_{a(min)}$, $T_{c(min)}$, and $T_{c(max)}$ are respectively minimum air temperature, maximum air temperature, leaf minimum temperature, and leaf maximum temperature. In order to determine the rate of stomatal conductance, prometer, made by Ajkalcamp Company, Netherland was used. Measurements were done between 12 to 2 pm, 25 days after applying drought stress. The data was analyzed by SAS software version 9.2, and the diagrams were drawn by EXCEL software.

**Crop Temperature Stability (CTS):**

In this experiment, it was observed that temperature stability of the plant was affected by the interaction between peripheral and genotypic factors (Table 1).

When nitrogen was not used, temperature stability of plants grown in optimal moisture conditions was higher than drought stress which was possibly due to presence of sufficient water in plant tissues. As the rate of nitrogen increased, the difference between temperature stability of plants decreased. In other words, nitrogen increased temperature stability under drought stress conditions. Nitrogen as one of the main elements needed by plants plays an important role in internal metabolism of plant cells and improves practical and biological situation of the cells. Therefore, by enhancing cellular metabolism and recycling more energy, it increases temperature stability under drought stress conditions. Rapeseed genotypes had different temperature stability (Figure1) and temperature fluctuation in Hyola 401 was less than other two genotypes in this regard.
Table 1: Variance analysis of yield and physiological traits.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>df</th>
<th>Grain yield</th>
<th>CTS</th>
<th>Stomatal conductance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Year(Y)</td>
<td>1</td>
<td>48382</td>
<td>0.05&lt;sup&gt;**&lt;/sup&gt;</td>
<td>1.6&lt;sup&gt;**&lt;/sup&gt;</td>
</tr>
<tr>
<td>Year error</td>
<td>4</td>
<td>1108</td>
<td>0.007</td>
<td>218</td>
</tr>
<tr>
<td>Drought stress(D.S)</td>
<td>1</td>
<td>463882</td>
<td>0.3</td>
<td>19448&lt;sup&gt;**&lt;/sup&gt;</td>
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<tr>
<td>Y*D.S error</td>
<td>4</td>
<td>512</td>
<td>0.02</td>
<td>228</td>
</tr>
<tr>
<td>Nitrogen(N)</td>
<td>3</td>
<td>78334</td>
<td>0.5</td>
<td>6545</td>
</tr>
<tr>
<td>Y* N</td>
<td>3</td>
<td>1152</td>
<td>0.006&lt;sup&gt;**&lt;/sup&gt;</td>
<td>38&lt;sup&gt;**&lt;/sup&gt;</td>
</tr>
<tr>
<td>D.S *N</td>
<td>3</td>
<td>1540</td>
<td>0.007&lt;sup&gt;**&lt;/sup&gt;</td>
<td>7.8&lt;sup&gt;**&lt;/sup&gt;</td>
</tr>
<tr>
<td>Y<em>D.S</em>N error</td>
<td>24</td>
<td>163</td>
<td>0.005</td>
<td>22</td>
</tr>
<tr>
<td>Genotype(G)</td>
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<td>5612</td>
<td>0.01</td>
<td>271</td>
</tr>
<tr>
<td>Y* G</td>
<td>2</td>
<td>450</td>
<td>0.008&lt;sup&gt;**&lt;/sup&gt;</td>
<td>1.7&lt;sup&gt;**&lt;/sup&gt;</td>
</tr>
<tr>
<td>D.S* G</td>
<td>2</td>
<td>496</td>
<td>0.0003&lt;sup&gt;**&lt;/sup&gt;</td>
<td>8.5&lt;sup&gt;**&lt;/sup&gt;</td>
</tr>
<tr>
<td>N* G</td>
<td>6</td>
<td>294</td>
<td>0.005&lt;sup&gt;**&lt;/sup&gt;</td>
<td>5.2&lt;sup&gt;**&lt;/sup&gt;</td>
</tr>
<tr>
<td>Y<em>D.S</em> G</td>
<td>2</td>
<td>32&lt;sup&gt;**&lt;/sup&gt;</td>
<td>0.0006&lt;sup&gt;**&lt;/sup&gt;</td>
<td>1.2&lt;sup&gt;**&lt;/sup&gt;</td>
</tr>
<tr>
<td>Y<em>N</em> G</td>
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<td>92&lt;sup&gt;**&lt;/sup&gt;</td>
<td>0.001&lt;sup&gt;**&lt;/sup&gt;</td>
<td>3.4&lt;sup&gt;**&lt;/sup&gt;</td>
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<tr>
<td>D.S <em>N</em> G</td>
<td>6</td>
<td>146&lt;sup&gt;**&lt;/sup&gt;</td>
<td>0.002&lt;sup&gt;**&lt;/sup&gt;</td>
<td>6.9&lt;sup&gt;**&lt;/sup&gt;</td>
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<tr>
<td>Y*D.S <em>N</em> G</td>
<td>6</td>
<td>1591</td>
<td>0.0002&lt;sup&gt;**&lt;/sup&gt;</td>
<td>11&lt;sup&gt;**&lt;/sup&gt;</td>
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<tr>
<td>Total error</td>
<td>64</td>
<td>75</td>
<td>0.001</td>
<td>9.2</td>
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<tr>
<td>c.v(%)</td>
<td>--</td>
<td>3</td>
<td>3</td>
<td>2.3</td>
</tr>
</tbody>
</table>

Fig. 1: Year and genotype interaction on CTS.

Temperature stability of SG1 and SG9 genotypes was more dependent on environmental and climate conditions. Nitrogen increased temperature stability of different genotypes and SG9 allocated more stable temperature to itself in presence of nitrogen fertilizer (Figure 2). Adjusting internal temperature is an important factor in keeping favorite functional conditions for plant and provides optimal internal space for plant to do necessary and vital reactions and ultimately increases plant potential to deal with stress.

Fig. 2: Stress and nitrogen interaction on CTS.

These features were clearly observed in Hyola 401 genotype. In a similar condition, Nasri [37] showed that higher tolerance of B. napus species to water stress was clear within higher osmotic potential and lower temperature of the leaf which approves of above claim. Internal bed of plant cells is full of enzymes whose reaction rate depends on temperature. Factors which change temperature (like drought stress) inevitably change plant internal metabolism. Plant response to environmental factors can be influenced by the temperature of its tissue. On the other hand, temperature variations will change plant needs, too. Therefore, genotypes might establish various mechanisms in relation to regulating their internal temperature. Temperature stability in plants can provide an indoor exponential of the plant situation.
Stomatal conductance:

Drought stress, nitrogen, and genotype had a significant effect on stomatal conductance of rapeseed leaf (Table 1). Stomatal conductance under drought stress conditions was significantly less (16%) than optimal moisture conditions (Figure 3). Decrease of turgor pressure due to lack of water is the main reason of stomatal closure under drought stress conditions [35]. Decrease of stomatal conductance under drought stress conditions is mainly due to two reasons: first, the decrease of water in stomatal cells and second, plant defensive mechanisms.

![Graph showing effect of stress on stomatal conductance.](image)

**Fig. 3:** Effect of stress on stomatal conductance.

Although carbon enters plant through stoma, plants try to close it in dealing with stress in order to prevent the waste of water. Abscisic acid and potassium ion exit through protective cells are the main reasons of stomatal closure. Setting stomatal conductance in plant is one of its vital processes because it should be set in such a way that it could uptake CO$_2$ and prevent evaporation and transpiration, as well. Mechanisms involved in opening and closing stomata make it influenced by environment and genotype.

As nitrogen consumption increased, stomatal conductance increased as much as 21% (Figure 4).

Through cell expansion and changing the concentration of anions and cations of cytoplasmic environment, nitrogen can change stomatal conductance. On the other hand, this element increases the leaf growth and development and enhances the number of stomata and thus can help stomatal conductance. The increase of stomatal conductance due to presence of nitrogen has been reported in the findings of some researchers [12,49]. Nitrogen can help provide the needed signaling for opening the stomata and make cation and anion balance in cells and ultimately can increase stomatal conductance. Hyola 401 genotype had less stomatal conductance than other genotypes (Figure 5) and this process helps it keep its internal water particularly in dealing with stress. Decrease of plant internal water loss in Hyola 401 genotype can be another reason for its better development in drought stress conditions.

![Graph showing nitrogen effect on stomatal conductance.](image)

**Fig. 4:** Nitrogen effect on stomatal conductance.

Stomatal conductance had the highest correlation with rapeseed yield (r = 0.95). This correlation might have been made in different ways. Its first reason could be attributed to the increase of carbon formation in case the stomata are open. It is obvious that when there is more CO$_2$ in intracellular environment, production and growth will increase. Another reason could be more water absorption by plant when the stomata are open. When the stomata are open the movement slope of water from the ground towards plant tissue increases. In other words, the exit of water from the stomata makes potential of plant water more negative and increases water absorption in plant. More absorption of water improves plant internal metabolism and enhances
physiological processes which in turn increases plan yield and performance. The presence of constant water in plant moderates temperature of its tissues and provides a suitable ground for the reactions and mobilization of assimilates. In this experiment, a positive significant correlation has been observed between stomatal conductance and temperature stability \((r = 0.87)\). Previously, the direct relationship between stomatal conductance and rapeseed yield had been reported, too [28,29,51]. Pasban Islam et al. [43] reported a positive and significant correlation between stomatal conductance and traits such as temperature stability and grain yield in rapeseed.

Fig. 5: Effect of genotypes on stomatal conductance.

Grain Yield:

Rapeseed yield was severely affected by the interaction of crop year, drought stress, nitrogen, and genotype (Table 1). Before this research, the interactive effect of nitrogen fertilizer and irrigation on the yield of rapeseed had been reported [28,13,42]. Rapeseed yield in the second year was significantly (12%) more than that of the first year. The effect of nitrogen in the second year was slightly more than the first year so that the rate of grain yield via nitrogen fertilizer in the second year increased 2% more than the first year (Figure 6). It seems that climate and weather changes have affected nitrogen efficiency in determining the yield. Temperature, accessible water, and soil microorganism life depend on weather conditions and thus plant access to soil nitrogen can change.

Fig. 6: Effect of nitrogen on yield.

In both crop years, the increase of nitrogen caused the increase of rapeseed yield so that the highest rapeseed yield belonged to the treatment with application of 105 kg/ha nitrogen and in irrigation condition (Figure 7). Previously, it had been observed that the yield components of rapeseed such as number of pods, number of grains per pod, and 1000-grain weight had a positive response to the increase of nitrogen and generally, if the yield components improved, ultimate yield would increase too [29,13]. Moreover, changes trend of rapeseed yield towards nitrogen in optimal moisture conditions and drought stress was almost the same and in all levels of nitrogen, irrigation increased the grain yield of rapeseed (Figure 7).

Different interactions between the treatments applied in this experiment show that just nitrogen does not determine the yield of rapeseed, but other factors such as climate changes and crop season and soil moisture conditions are involved in ultimate yield of rapeseed. In fact, the yield of rapeseed in this experiment was the result of several factors.

The results indicate that in order to achieve optimal yield of rapeseed within the conditions of this experiment, 105 kg/ha nitrogen is needed. This is consistent with the findings of Rathke et al. [44] and Jackson [23] that showed optimal grain yield of winter rapeseed would be obtained within the range of 180-240 kg/ha nitrogen fertilizer. The slight differences between the results might be due to weather conditions of cultivation.
environment. In another experiment, it was reported that the increase of nitrogen consumption up to 190 kg/ha increased the grain yield and nitrogen levels over 240 kg/ha did not increase the grain yield significantly [14]. Generally, the increase of rapeseed yield due to nitrogen fertilizer is consistent with the reports of many researchers [8,23,50,20,11,20,19,44,45].

Fig. 7: Stress and nitrogen interaction on yield.

In all nitrogen levels, drought stress decreased the yield of rapeseed, but the reduction was less when nitrogen fertilizer was provided for plant so that the yield reduction due to drought stress in control treatments (without fertilizer) and 35 kg urea was 35% and in treatments with 70 and 105 kg urea was 32% (Figure 7). Yield drop due to drought stress is also influenced by yield components of rapeseed. Drought stress in this experiment occurred during the flowering stage of rapeseed and thus the reproductive parts of rapeseed such as flower and pod were damaged. Mendham and Salisbury [36] stated that adequate water supply at flowering and podding stages would lead to development and growth of pods, increase of number of grains per pod and also the increase of grain yield per area unit. In this experiment, the drop of yield components such as number of pods, number of grains per pod and 1000-grain weight was 39, 27, and 15% respectively which ultimately led to drop of grain yield.

The increasing trend of yield in all genotypes in presence of nitrogen was observed, but Hyola 401 genotype had a better response than other genotypes (Figure 8).

Fig. 8: Genotype and nitrogen interaction on yield.

Application of 105 kg/ha nitrogen increased hyola yield nearly 34% more than lack of application of fertilizer while in such condition their yield had increased 32 and 31% respectively. In both soil moisture conditions, Hyola 401 genotype had a higher yield than SG1 and SG9 (Figure 8). The results show that Hyola 401 genotype is more stable than other two genotypes to soil water changes and is more efficient in nitrogen fertilizer application. Srivastava et al. [52] have stated that a genotype is tolerant to drought which has less drop of yield in comparison to other genotypes and within the same conditions. According to the researchers’ ideas and with regard to the results of present research it can be said that hyola 401 genotype is better than SG1 and SG9 because its yield is respectively 6% and 2% more than that of SG1 and SG9 in optimal conditions and 5% and 3% more than that of SG1 and SG9 under drought stress conditions (Figure 9). Amiri Oughan et al. [3] had formerly studied grain yield in rapeseed genotypes and reported that hyola 401 genotype had yield stability and this point is confirmed by present results.
Among the yield components, ultimate yield of rapeseed had the highest positive and significant correlation with the number of pods ($r = 0.96$) and the number of grains per pod ($r = 0.94$). This shows that the number of pods formed in rapeseed is more important in rapeseed yield. In a similar research, Sedaqut et al. [47] reported that there was a positive and significant correlation between number of pods and rapeseed yield. Since the number of pods in rapeseed is the first component of plant yield any factor which decreases its number or reduces its capacity will inevitably influence other yield components such as the number of grains per pod and 1000-grain weight and will ultimately reduce the yield. High correlation between number of pods and yield confirms this point, as well. It can be concluded that drought stress and shortage of nitrogen not only limit rapeseed resources but also restrict assimilates reservoirs and will be followed by reduction of primary reservoir (number of pods), reduction of secondary reservoirs (number of grains per pod and grain capacity) which will finally lead to decrease of ultimate yield. It seems that in this experiment reservoir restriction has been more than the source restriction because drought stress has been applied in plant since flowering stage and has limited yield components formation; on the other hand, irrigation at critical stages of rapeseed growth (formation of flower and pod) has led to the increase of reservoir capacity in plant and has increased the yield.

**Conclusion:**

Drought stress reduced stomatal conductance and temperature stability in plants. Consequently, stomata got closed, carbon formation process disrupted, and ultimately the yield decreased. Nitrogen consumption moderated the effect of drought stress and increased the grain yield, so that grain yield with consumption of 105 kg pure nitrogen increased 32.5% in comparison to the treatment without consumption of nitrogen. Among the studied genotypes, Hyola 401 had the highest yield by producing 293.88 g/m² grain.

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