ABSTRACT: Drought stress and nutrient deficiency are the major limiting factors in crop production around the world. In order to investigate the effects of nitrogen on physiological characteristic and yield of two winter wheat varieties under drought stress, a pot experiment was carried out in a factorial experiment in randomized complete block design with three replications at the University of Kurdistan during 2008. Treatments included drought stress (irrigation at soil water potential -3 bar as control and irrigation at soil water potential -13 bar as water deficit), nitrogen fertilizer (control treatment as non fertilizer application, 0.35 and 0.70 g nitrogen per pot), applied on Zarrin and Azar2 of wheat cultivars. Results showed that despite of lower values of SPAD in drought stress condition, it was increased in each of nitrogen application. Under drought stress treatment, however we observed a reduced amount of photosynthesis ($P_n$), intercellular CO$_2$ concentration ($C_i$), transpiration ($E$) and mesophyll conductance ($MC$). After exposure to recovery condition, the levels of photosynthesis and mesophyll conductance in Azar2 was significantly higher than Zarrin cultivar. Nitrogen fertilizer application under non stress condition caused in increasing trend of mesophyll conductance and concentration of soluble proteins was also increased.

Keywords: photosynthesis, transpiration, drought stress, wheat

Abbreviations: $C_i$: intercellular CO$_2$ concentration; $E$: transpiration; $MC$: mesophyll conductance; $P_n$: Net Photosynthesis

INTRODUCTION

Insufficient water is the primary limitation to wheat production world-wide (Ashraf and Harris, 2005). Drought stress and nitrogen deficiency are in the most restrictive environmental factors that limited the plant growth and crop type selection in a wide range of agricultural ecosystems (Krček et al., 2008) Water deficiency not only alters plant morphology, but it also affects the metabolism and thus causes a permanent decrease of yield (Nazemosadat and Kazemini, 2008). Stress, called to effects of each environmental factor that potentially affect the survival and function of living organisms. In fact, stress leads to physiological and morphological processes that disrupt equilibrium (Yamaushi et al., 2002). Drought stress inhibits plant growth and development, with impact of various biochemical and physiological processes such as photosynthesis, respiration, nutrient transport, carbohydrates and ion absorption and metabolism of nutrient (Farooq et al., 2009; Rafieet et al., 2003)

Drought stress is described by decrease in leaf relative water content, leaf water potential, turgescence pressure, stomatal closure, and reduce in cell enlargement and reduction (farooq et al., 2009) Severe drought stress reduces photosynthesis and plant metabolism and can lead to death (Abdul Jaleel et al., 2009) When wheat plants exposed to drought stress Occurs a decrease in the rate of photosynthesis (Olszewski et al., 2008) Stomatal conductance and increase intercellular carbon dioxide concentration (Nazemosadat and Kazemini, 2008). Plant
species under water stress show a significant difference in yield. Plant responses to drought, mainly depends on the species, growth stage, severity and duration of stress (Abdul Jaleel et al., 2009). Nitrogen is the most limiting nutrient in wheat yield. Nitrogen, affects the grain yield, grain weight, dry matter remobilization and leaf size, leaf biochemical and biological activities of wheat (Mustafavi-rad et al., 2007).

Plants growth and photosynthesis is affected by various environmental conditions such as drought stress. Plant's ability to survive and continue its growth and photosynthesis in the environmental stresses is dependent on the genetic potential of plant that indicates as the molecular and physiological responses (Mohsenzadeh et al., 2003). Farshadfar et al. (2008), reported that there is a negative correlation between grain yield and proline, soluble sugars and total grain protein under dry conditions. Soluble proteins with high molecular weight (over 100 kDa) are reduced under stress conditions While the proteins with low molecular weight, are increasing. Sio-Se Marde et al. (2006), reported that drought stress reduced photosynthetic capacity of wheat. Thus, the carbon dioxide enters the leaf can not be used properly. Drought stress effects on plant morphological characteristics such as leaf area, plant height, biomass weight, seed weight and finally yield and physiological characteristics of the plant, such as leaf gas exchange and chlorophyll fluorescence, photosynthetic activity, leaf nitrogen concentration, leaf water status, free amino acids and soluble proteins, activity of some enzymes such as nitrate reductase and finally restrict plants growth (Sio-Se Marde et al., 2006; Mohammadkhani and Heidari, 2007; Abdel-hadi, 2007; Zhen Zhu and Zhou, 2006; Mohammadi et al., 2005; Tas and Tas, 2007). In irrigated agriculture, nitrogen management so inevitably depend on irrigation management. Indeed, nitrogen uptake and its efficiency largely depends on plant water available and water stress can have a negative effect on its absorption and efficiency (Zhen-Zhu and Zhen-Wen, 2006).

Mustafavi-rad et al. (2007) reported that different nitrogen fertilizer, have a significant effect on grain yield, grain weight and leaf and stem dry matter remobilization in wheat varieties. Also the effect of combined treatments of varieties and fertilizer on grain yield, flag leaf length and leaf dry matter remobilization was significant. There is a conflict between nitrogen and drought on crop production, therefore, the maximum biomass and grain production, is obtained by increasing the leaf area index and leaf area duration and Stomatal openness that this specificity is obtained by more Nitrogen use. However under drought conditions, less leaf area and lower leaf surface duration and closure the stomata is more favorable to reduce transpiration. Often in most severe drought conditions, aging is accelerated and this matter is severe especially in high nitrogen. Therefore there is a relationship between stress and nitrogen and led to the belief that nitrogen have a negative effects in drought conditions. Nitrogen is one of the important components of many molecules such as proteins, nucleic acids, chlorophyll and some hormones (Emam et al., 2009).

Photosynthesis and nitrogen metabolism are closely related to each other such that the functional activity of photosynthetic systems depends largely on the availability of nitrogen in plant (Brenna, 1992). Because nitrogen involved in the formation of structural and functional proteins in chloroplasts. (Kumar et al., 1995). The results of the Bahavar et al. (2009), study indicated that Nitrogen application increased leaf relative water content, leaf water potential and membrane stability index, leaf chlorophyll content and leaf area index.

Nitrogen is an important part of any other organic compounds, such as pigments and chlorophyll coenzyme forms and with participation in the chlorophyll building, make definitive and directly effect in Chlorophyll production (Latri-Souki et al., 1998).

MATERIALS AND METHODS

This experiment was conducted under semicontrolled conditions in the Faculty of Agriculture, University of Kurdistan (35°16' N, 47°1' E and 1375 alt. and 492 mm mean annual rainfall), Iran, between January 2009 and June 2010. Two wheat cultivars namely Azar2 (rain-fed cultivar and drought resistant) and Zarin (irrigated cultivar and drought susceptible) were grown in plastic pots (35 cm diameter and 28 cm height) containing 13 kg loam soil. The soil test values indicated a pH of 7.4, 0.17% total N, 25 ppm P and 2.1 ppm Zn. A total of 35 seeds were planted per pot in January 2009 in a greenhouse. At three leaf stages, the pots were transferred and remained outdoors in order to vernalize and thinned to 20 seedlings per pot.

Factorial experiment in randomized complete block design with three replications and three factors including cultivar, irrigation and N fertilizer was conducted. The pots were watered uniformly until the flowering stage. Drought treatment was imposed by restricting irrigation and re-irrigated when soil water potential reached -13 bar. Control pots were irrigated at -3 bar soil water potential. Nitrogen fertilizer, as urea, was applied at stem elongation at three rates of 0, 27 and 54 mg N/kg soil. The experiment thus consisted of 12 treatments and two pots allocated to each experimental unit. To determine the pots soil moisture during irrigation treatments, soil moisture levels at
The results showed that the water level has decreased seed and biomass weight and the nitrogen fertilizer applying in irrigated conditions enhanced yield but in stress condition, it wasn’t effective (Figure 2 and 3). Nitrogen fertilizer increased grain yield and this is probably due to better availability of nutrients for plant roots and better conditions for photosynthesis that increased photosynthesis. (Majidian et al., 2008). With regard to nitrogen increases biomass and leaf area duration, it is expected that grain yield will increases with increasing nitrogen application. But often, the plants in encountering with severe water stress will accelerate aging in leaves. And thus the rate of photosynthesis and dry matter production is reduced (Papastilianon, 1995). In relation to this matter, Zadoks et al. (1974) reported that nitrogen use efficiency increased in sufficient water condition.

Drought stress has significant effects on SPAD chlorophyll and it was decreased in comparison to control conditions (Figure 4). Chlorophyll content is an important factor in determining the mesophile conductance, photosynthesis rate and dry matter production (Majidian et al., 2008; Moghadami, 2008). Reduction in Chlorophyll concentration in dry condition is due to the effect of drought stress on chlorophyll degradation and its oxidation. (Bahavar et al., 2009 and Ahmadi; Sio-se Mardeh, 2004). Under stressful conditions, maintaining Chlorophyll concentration, helps to photosynthesis stability in these conditions (Ahmadi and Sio-se Mardeh, 2004). Nitrogen application significantly increased SPAD chlorophyll in compared to control (Figure 5).

It has been reported that nitrogen fertilizer at different growth stages has a significant effect on chlorophyll SPAD. It is also reported that about 70% of leaf nitrogen accumulate in chloroplasts. And the chlorophyll content, has a large correlation with nitrogen (Majidian et al., 2008). The SPAD chlorophyll changes in two cultivars followed the same model during the period. With this difference that the intensity changes, in Zarrin cultivare was higher in compared with Azar2 cultivare (Figure 6). Nitrogen affected the SPAD chlorophyll rate in both cultivars and the second and third levels of Nitrogen were higher than control (Figure 7 and 8). Study of SPAD chlorophyll trend change, shows that from 10 days after flowering, this rate was reduced in both cultivars with drought stress and progression of aging (Figure 6).Ahmadi and Sio-se Mardeh (2004), reported that the aging process has overshadowed the effect of drought on chlorophyll. Application of Nitrogen in the Zarrin cultivare (Figure 7), increased the leaf chlorophyll SPAD in the early stages with the aging advances, this rate was reduced again, but the application of Nitrogen at Azar2 cultivar was better and SPAD chlorophyll was higher still (Figure 8). This could be due to a larger sink in Zarrin cultivars, and more demand to reserve of materials, such as leaf nitrogen in the grain filling period in compared with the Azar2 cultivar and more remobilization of leaf nitrogen, to the seeds. Because the main reason for the decrease in chlorophyll grains in grain filling period, is nitrogen remobilization of chlorophyll to supply the grain protein (Yang et al., 2000, 2001).

Stomatatal closure is one of the first responses of plants to drought stress, and it seems that the main reason for the reduction of photosynthesis due to drought, because with the stomatal closure, mesophill cells access are limited to the carbon dioxide (Siddique et al., 1999; Siosemardeh et al., 2003). Changes in photosynthesis rate of two varieties in during the growth period almost followed a similar trend with this difference that there was greater stability in Azar2 variety in comparison with Zarrin variety. With aging progression in leaves after the drought resolving, the return of photosynthesis rate in Zarrin variety was less than Azar2 variety and stress damage the photosynthetic system more in Zarrin variety. (Figure 9).

Stress accelerated leaf senescence and remobilization of nitrogen and carbohydrates from the leaves to the grain and this issue reduced photosynthetic capacity of leaves (Majidian et al., 2008; Mohammadi et al., 2005; Mohsenzadeh et al., 2003). In this research, observed that the rate of photosynthesis in the third week after flowering is less than the rate of photosynthesis in second week (Figure 9). Loboda (2000), Reported that during a
seven-day period, the rate of photosynthesis in wheat and barley under stress, were lower than control condition and on the last day of stress (maximum stress), the rate of net photosynthesis in drought conditions reached to the half level in control condition.

Nitrogen fertilizer application in Zarrin cultivar, only in the early stages was affected and improved $P_n$, when the leaves were not much affected by drought and water were available for the photosystems activity. But with drought beginning, $P_n$ more strongly reduced and return of $P_n$ were lower than the control treatment (Figure 10) and nitrogen fertilizer had no effect on the $P_n$. These results can be seen in the twelfth and thirteenth days after flowering and before the irrigation.

Azar2 variety showed a better response versus nitrogen fertilizer and throughout the period, the $P_n$ in the second and third levels of nitrogen treatments, was more than the control. But at the end, the $P_n$ of the three treatments was roughly the same level (Figure 11) and this may be due to remobilization of nitrogen in leaves. It means that stress can accelerate the aging in these leaves and the chlorophyll structure, damage more in compared to the control treatment. It also can be say that in drought conditions, Azar2 variety photosynthesis reaction was better in terms of nitrogen fertilizer

Intercellular CO$_2$ concentration ($C_i$), in drought stress condition was significantly reduced (Figure 12). This may be due to the closure of stomata in drought condition, and the consumption CO$_2$ is not being replaced because in the first plant response to drought stress, leaves stomata are closed and entrance is restricted to carbon dioxide (Siddique et al. 1999). If the stress continues, and lack of processing CO$_2$, $C_i$ may be increase. $C_i$ in Azar2 variety was lower than Zarrin variety (Figure 13). With regard that Zarrin variety is susceptible cultivar to drought, it is possible that with the occurrence of drought, stomata are close early. Also, the rate of transpiration in Azar2 was more than Zarrin cultivar that confirms closure of stomata in drought conditions (Figure 15). On the other hand, according to higher photosynthetic rates in Zarrin cultivare, CO2 Consumptione in this cultivare was more and Sub-stomatal CO$_2$ concentration will be lower.

Figure 14 shows the changes in transpiration rate ($E$) of two wheat cultivars to soil water potential, with respect to that, with decreasing soil water potential, $E$ decreases. It is observed that decrease in soil water potential to -5 bar, causing a sharp reduction of $E$ but more severe stress and further reduce soil water potential has a fewer effect on $E$ reduction. The Mean rate of $E$ in Azar2 variety was higher than Zarrin variety (Figure 15). Given that $C_i$ levels in the Zarrin variety was less than Azar2 variety, indicated that stomatal conductance is less in the Zarrin variety, it can be concluded that in drought stress condition, sensitive varieties plants reacts in order to keep the water and in the first step closes the stomata and then reduced $E$. However, resistant varieties, despite more $E$, have the ability to grow under stress.

Closure of Stomata will keep the water in the leaves but on the other hand CO$_2$ entering into the leaves are limited and leaded to decrease in photosynthesis for plant. In this research observed that the rate of transpiration reversible after re-watering and remove the stress in Azar2 variety is higher than Zarrin variety and Effect of nitrogen application on $E$ trend of change, does not follow the specific pattern in the days after anthesis (Figure 16 and 17). But the remarkable point is that in both studied varieties, in the late of growing period, with decreasing soil water potential and advancement senescence in leaves, $E$ has increased in nitrogen fertilizer treatment in comparison to control treatments and this may be due to a reduced ability of leaves, at stomata closure. Because after the drought, stomata may lose their ability to function normally (Shiferaw and Baker, 1996)

There was a significant and direct relationship between $E$ and $P_n$, and by increasing in $E$, the $P_n$ increased. Higher $E$ is done, when the stomata are open and the openness of stomata, means that CO$_2$ can also easily be entered to the leaf (Figure 18).

In relation to non-stomatal limitation, mesophyll conductance (MC) has been proposed and reported that the main limiting factor for $P_n$ under drought stress, is decrease in MC (Rohi and Siosemardeh, 2008; Fischer et al., 1998). In this experiment the MC reduced under drought stress conditions (Figure 19). This may be due to damage of mesophilic mechanisms that affecting $P_n$, such as the production of enzymes, chlorophyll loss and damage cell membranes of thylacoids and the effects of under drought stress. With decrease in soil water potential, MC decreases (Figure 20). The MC trend of change in two studied varieties shows that this parameter, was relatively higher in the Zarrin varieties that it was more related with $P_n$ in this variety, and it’s related to the role of stomatal in increase of $P_n$ in sensitive varieties in favorable irrigation conditions (Figure 21). Nitrogen fertilizer application, increased the MC in both varieties and application of nitrogen fertilizer increased the spad of chlorophyll. So it can be said that the application of nitrogen fertilizer increased CO$_2$ production (Figure 7 and 8). In the early days after flowering, Nitrogen application increased MC in Azar2 variety more than Zarrin variety but its reversible after the stress resolve in Zarrin was higher (Figure 22 and 23). It is reported that after removing the stress, the chlorophyll content of soybean significantly restored (Karimpour et al., 2010).

314
In Sio-Se Marde et al. (2006) experiment, there was a significant positive correlation between mesophil and chlorophyll content in wheat and it was concluded that probably the reduced of leaf chlorophyll concentration, is an important factor in reducing mesophil conductivity.

Since the nitrogen is a fundamental constituent of chlorophyll and chlorophyll content is a major factor in producing CO\textsubscript{2}. Thus improving in MC with the nitrogen consumption is predictable (Deborah and Bruce, 1998). According to Figure 24, it is clear that between the MC and \( P_n \), there is a significant direct relationship (\( R^2=0.996 \)) and higher \( P_n \) rates occurs at high MC and this means that with the increase in MC, fixing of CO\textsubscript{2} entering the leaf will be more and plant will not limits in CO\textsubscript{2} fixing.

Kulshrestha et al. (1987), have expressed that the protein content can be used as an index to assess drought tolerance and in this research, drought stress reduced leaf protein concentration (Figure 25). reason of decrease in leaf protein concentration in drought stress conditions is reduce in protein synthesis and protein breakdown due to increase in activity of protease enzymes (Fisher, 1981; kumar et al., 1995; Zhen-Zhu and Zhen-Wen, 2006; Moghadami, 2008).

Nitrogen is a constituent element of amino acids, nucleic acids, proteins, nucleotides, and a large number of secondary compounds in plants (Brenna, 1981; Brenna, 1992; Robinson and Hodges, 1981) and appears with the increasing consumption of nitrogen, concentrations of these compounds in the plant will increase. In this experiment, with the nitrogen application, concentration of soluble proteins was also increased, and the third level of nitrogen, had the highest protein content in leaves (Figure 26).

![Figure 1](image1)

Figure 1. The relationship between soil water content and soil water potential

![Figure 2](image2)

Figure 2. The effect of drought stress and nitrogen application on biomass weight of wheat. S1: irrigation condition, S2: drought stress, N1: non nitrogen application, N2: 27 mg kg\(^{-1}\) N, N3: 54 mg kg\(^{-1}\) N. Different letters indicate statistically significant difference at P=0.05.
Figure 3. The effect of drought stress and nitrogen application on grain weight of wheat. S1: irrigation condition, S2: drought stress, N1: non nitrogen application, N2: 27 mg kg⁻¹ N, N3: 54 mg kg⁻¹ N. Different letters indicate statistically significant difference at P=0.05

Figure 4. The effect of drought stress on leaf SPAD number in wheat. S1: irrigation condition, S2: drought stress. Different letters indicate statistically significant difference at P=0.05

Figure 5. The effect of nitrogen application on leaf SPAD number in wheat N1: non nitrogen application, N2: 27 mg kg⁻¹ N, N3: 54 mg kg⁻¹ N. Different letters indicate statistically significant difference at P=0.05
Figure 6. The difference of leaf SPAD number in two wheat cultivars, after anthesis. DAA: days after anthesis, W.P.: water potential.

Figure 7. The effect of nitrogen application on leaf SPAD number in Zarrin cultivar, after anthesis N1: non nitrogen application, N2: 27 mg kg$^{-1}$ N, N3: 54 mg kg$^{-1}$ N. DAA: days after anthesis, W.P.: water potential.

Figure 8. The effect of nitrogen application on leaf SPAD number in Azar2 cultivar, after anthesis N1: non nitrogen application, N2: 27 mg kg$^{-1}$ N, N3: 54 mg kg$^{-1}$ N. DAA: days after anthesis, W.P.: water potential.
Figure 9. The difference of Photosynthesis rate in flag leaf of two wheat cultivars after anthesis, DAA: days after anthesis, W.P.: water potential, I: irrigation

Figure 10. Photosynthesis rate in flag leaf of Zarrin cultivare after anthesis in three levels of N application. N1: non nitrogen application, N2: 27 mg kg⁻¹ N, N3: 54 mg kg⁻¹ N, DAA: days after anthesis, W.P.: water potential, I: irrigation

Figure 11. Photosynthesis rate in flag leaf of Azar2 cultivare after anthesis in three levels of N application. N1: non nitrogen application, N2: 27 mg kg⁻¹ N, N3: 54 mg kg⁻¹ N, DAA: days after anthesis, W.P.: water potential, I: irrigation
Figure 12. The effect of drought stress on intercellular CO2 concentration in wheat. S1: irrigation condition, S2: drought stress. Different letters indicate statistically significant difference at P=0.05.

Figure 13. The difference of intercellular CO2 concentration in two wheat cultivars. S1: irrigation condition, S2: drought stress. Different letters indicate statistically significant difference at P=0.05.

Figure 14. The relationship between transpiration and soil water potential, from anthesis to maturity, in two wheat cultivars.

\[ y = -0.32\ln(x) + 1.883 \]
\[ R^2 = 0.314 \]
Figure 15. The difference of transpiration rate in flag leaf of two wheat cultivars after anthesis, DAA: days after anthesis, W.P.: water potential, I: irrigation

Figure 16. Transpiration rate in flag leaf of Zarrin cultivar after anthesis in three levels of N application. N1: non nitrogen application, N2: 27 mg kg⁻¹ N, N3: 54 mg kg⁻¹ N, DAA: days after anthesis, W.P.: water potential, I: irrigation

Figure 17. Transpiration rate in flag leaf of Azar2 cultivar after anthesis in three levels of N application. N1: non nitrogen application, N2: 27 mg kg⁻¹ N, N3: 54 mg kg⁻¹ N, DAA: days after anthesis, W.P.: water potential, I: irrigation
Figure 18. The relationship between photosynthesis and transpiration, from anthesis to maturity, in two wheat cultivars.

Figure 19. The effect of drought stress on mesophyl conductance of two wheat cultivars S1: irrigation condition, S2: drought stress. Different letters indicate statistically significant difference at P=0.05.

Figure 20. The relationship between mesophyl conductance and soil water potential, from anthesis to maturity, in two wheat cultivars.
Figure 21. The difference of mesophyl conductance rate in flag leaf of two wheat cultivars after anthesis, DAA: days after anthesis, W.P.: water potential, I: irrigation

Figure 22. Mesophyl conductance rate in flag leaf of Zarrin cultivar after anthesis in three levels of N application. N1: non nitrogen application, N2: 27 mg kg^{-1} N, N3: 54 mg kg^{-1} N, DAA: days after anthesis, W.P.: water potential, I: irrigation

Figure 23. Mesophyl conductance rate in flag leaf of Azar2 cultivar after anthesis in three levels of N application. N1: non nitrogen application, N2: 27 mg kg^{-1} N, N3: 54 mg kg^{-1} N, DAA: days after anthesis, W.P.: water potential, I: irrigation
Figure 24. The relationship between mesophyl conductance and photosynthesis, from anthesis to maturity in two wheat cultivars

Figure 25. The effect of drought stress on leaf protein concentration of wheat, S1: irrigation condition, S2: drought stress

Figure 26. The effect of drought stress and nitrogen application on biomass weight of wheat, N1: non nitrogen application, N2: 27 mg kg⁻¹ N, N3: 54 mg kg⁻¹ N. Different letters indicate statistically significant difference at P=0.05

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