Maize (*Zea mays* L.) Response to Nitrogen Fertilizer under Drought Stress at Vegetative and Reproductive Stages

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ABSTRACT

In the sub-tropics, water and nitrogen are the most important factors limiting the grain yield of maize. The effect of nitrogen (N) rates and drought stress at different growth stages of maize were investigated. Nitrogen treatments consisted of 100, 150, and 200 kg N ha-1 from the urea source while water stress treatments were composed of irrigation-off since the early 10-leaf stage to emergence of 50% tassel (vegetative), irrigation-off since the emergence of 50% tassel to the end of pollination (reproductive), and well watered plots. Drought condition at both vegetative and reproductive growth stages reduced grain yield 35% and 45%, respectively. Number of kernel per ear and kernel weight also reduced significantly with drought stress especially in reproductive stage. The highest response of maize yield to N fertilizer was observed in treatment with optimal irrigation; however, the increase of N particularly in vegetative stress somewhat led to decrease of drought stress effects on grain yield. Drought stress at vegetative and reproductive stages increased resource limitation with the rate of 67.1% and 77.25%, respectively. Drought stress at both stages caused significant decrease in chlorophyll content and leaf relative water content. In general, the highest negative effect of drought stress on grain yield and physiological traits occurred at reproductive stage and increasing the amount of nitrogen cannot compensate these reductions.

Keywords: *Chlorophyll, Corn, Water deficit, Yield.*

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INTRODUCTION

Maize is one of the valuable and important grains in temperate and subtropical areas in the world (Siadat *et al*., 2013; Modhej *et al*., 2014). In the subtropics, water and nitrogen are the most important factors that limit the grain yield of maize. Maize produces higher yields under sufficient water and soil fertility; however, this crop has the least tolerance to unfavorable conditions (Muchow, 1989). According to CIM-MYT (International Maize and Wheat Improvement Centre), estimations the major causes of losses are low soil fertility (predominantly N deficiency) and drought (Edmeades and Deutsch, 1994). Nitrogen plays a key role in plant morphology, which directly influence the total grain yield (Nagi, 2008; Modhej *et al.*, 2014). Optimal use of N increases leaf area index, plant green area duration, and biomass resulting in the increase of received resources such as water, light, carbon dioxide, and other nutrients that finally results in increase of grain yield (Boote, 1996; Abbas *et al*., 2003; Akmal *et al*., 2010). Previous studies have reported that water deficit limits the growth of plant (Hung *et al*., 2005), by reducing the leaf area, height, dry weight, stomatal closure, photosynthesis and chlorophyll contents, enzymes degradation, and accumulation of amino acids (Hassani and Omide Beigi, 2001). Drought has more severe effects on plant at the beginning of vegetative growth stage (Ahmed Amal *et al*., 2005), and Post-flowering drought stress causes accelerate of leaf senescence and decrease the current photosynthetic activities (Efeoğlu *et al*., 2009). After pollination, the most significant sinks are grains (Bonnett and Incoll, 1992), therefore, the rate of sink demand is the most important component in determining mobilization rate of stem reserved assimilates. If transport of assimilates to grains decreases because of drought stress and poor soil fertility, the source limitation will increase. Studies previously have suggested that the deleterious effects of drought could be mediated by application of nutrients, which may enhance plant ability to tolerate the drought stress (Aslam *et al*., 2013). However, it is still unclear whether increase in N application and uptake under drought conditions at different stages of plant life cycle has compensatory effects on final productivity or not. Thus, the present study aims to investigate the effect of different N rates on grain yield and physiological parameters of maize under drought condition at both vegetative and reproductive stages.

MATERIALS AND METHODS
Field and Treatments Information

The experiment were carried out in South-west of Iran at 31°3' N latitude, 47°52' E longitude, and 67 m above the sea level. The region had temperate winters with hot and dry summers. The soil texture was clay loam with 0.5% N and approximately 1% organic matter. The experiment was arranged as factorial on the basis of randomized complete block design (RCBD) with three replicates. Different rates of N fertilizer including 100, 150, 200 N kg.ha^{-1} from urea (46% N) source and water stress treatments including irrigation-off since the early 10-leaf stage to emergence of 50% tassel (vegetative), irrigation-off since the emergence of 50% tassel to the end of pollination (reproductive), and well watered plots were examined. Each plot contained 7 rows (10 m long) and 75 cm distance between the rows. Prior to planting, the experimental field was fertilized with 150 kg P_2O_5 ha^{-1} and 120 K_2O ha^{-1} in the form of single super phosphate and potassium sulfate, respectively. Half of N fertilizer was ap-

plied before sowing and the remaining N was added as a top dressing at the as banding at 6-8-leaf stage and irrigation began immediately.

Relative water content (RWC) and Chlorophyll (Chl)

Leaf relative water content was calculated according to Catsky (1960) from the samples immediately excised from the leaves harvested between 12 to 2 pm. The fresh samples were weighted and placed in distilled water for 5 hours and then the weight of saturated samples was measured. The samples placed in the oven for 48 hours at 72°C and then the dry weight of samples was determined. The rate of RWC was calculated using the following equation:

Equ 1. $RWC (\%) = [FW-DW)/(TW-DW] \times 100$

RWC: Relative water content, FW is fresh leaf weight, DW is leaf dry weigh, and TW is total saturated leaf weight.

To evaluate the rate of *Chl a* and *b* and carotenoids, 0.5 g of fresh leaf was ground in 4.5 cc acetone (80%) using porcelain mortar. The mixture was brought to the volume of 20 ml by adding distilled water. The final solution was exposed to the wavelengths of 645 and 663 nm to calculate the concentration of *Chl a* and *b* respectively and 470 nm for carotenoids using spectrophotometer. Chlorophyll concentration per mg of fresh weight was determined based on Arnon (1975) method via the following formula:

Equ 2. Chlorophyll a (mg.g^{-1}) $= 12.7 (663$ nm D) $- 2.69$ (at 645 nm D) $\times \dfrac{V}{1000 \times W}$

Equ 3. Chlorophyll b (mg.g^{-1}) $= 22.9 (645$ nm D) $- 4.68$ (at 663 nm D) $\times \dfrac{V}{1000 \times W}$

Equ 4. Total chlorophyll (mg.g^{-1}) = chlorophyll a + chlorophyll b

Equ 5. Carotenoids $= [(1000 \times 470nm\ D) - 1.82\ Chl\ a - 85.25\ Chl\ b]/198$

Where, D is optical density of carotenoids and chlorophyll extract in a certain wavelength, V is final volume of extract at acetone 80%, W is sample leaf fresh weight (g). Since the amount of *Chl*, carotenoids and leaf relative water content at vegetative and reproductive stages were separately measured in relation to control treatment in the same stage, the reduction percentage of each trait relative to control treatment was measured to have a similar trend.

Source limitation and yield components

To measure the rate of source limitation, half of the ear omitted one week after pollination. The ear was cut in the third line of planting lines where the control treatment was not manipulated. After calculating the grain weight in the control treatments and cut-off Ears, the rate of source limitation was calculated through the following equation (Ma *et al.*, 1996):

Equ 6. $S.L (\%) = (a/b - 1) \times 100$.

Where, S.L is source limitation, *a* is kernel weight in cut-off ears, and *b* is kernel weight in control ears.

The final harvest was applied in two rows of each plot. In the harvest process, 25 plants from each plot were randomly chosen and the yield components including the number of rows in the maize, the number of grains in the maize and the weight of kernels were calculated.

Statistical analysis

Statistical analysis (ANOVA) was applied with using the SAS software (Ver.8). The differences between traits means were assessed by Duncan's Multiple Range Test at 5% probability level. Pearson correlation analysis also was conducted among different variables.

RESULTS AND DISCUSSION

The results exhibited that the effect of drought stress and interactive effect of N and drought stress on grain yield were significant ($p<0.01$), while the effect of N on grain yield was not significant (Table 1). Although the effect of N on grain yield was not significant, the increase in N fertilizer from 100 to 200 kg.ha^{-1} increased the grain yield by 18.9 % (Table 1). The highest and the lowest grain yield were obtained from well-watered treatment and drought stress at reproductive stage respectively (Table 1). The difference among all three treatments of drought stress was significant in terms of the grain yield. Decrease of grain yield in stress treatments at vegetative stage was due to decrease of number of kernels and kernel weight. Drought stress occurrence at reproductive stage caused reduction of fertile florets and number of kernels resulted in a decline in the grain yield. The interactive effect of N and water stress indicated that increase of N at stress treatments slightly led to increase of the grain yield (Table 2).

Table 1. Mean comparison of seed yield and its components

Treatments	Seed yield (g.m^{-2})	Kernels per row	Kernel rows per ear	1000 kernel weight (g)	S.L (%)
Nitrogen (kg.ha^{-1})					
100	326[‡]a	32a	14a	179b	35a
150	351a	33a	14a	231a	26b
200	402a	34a	14a	234a	19ab
MS	13547ns	16.04ns	0.703ns	8695**	741.6**
Irrigation					
Optimal	495a	43a	14a	222a	9c
Vegetative	317b	35b	14a	202b	29b
Reproductive	268c	22c	14a	219a	42a
MS	128016**	1020**	0.701ns	1081**	301.4**

[‡]In each column, the means with similar letters are not significantly different at 5% probability level.

Ms: Mean square. ns, * and **: non-Significant, significant at the 0.05 and 0.01 probability level, respectively.

The increase was observed mostly in treatments subjected to the water stress at vegetative stage which probably can be related to stimulating role of N in development of root system and vegetative organs. Dinh *et al.* (2013) showed that the peanut (*Arachis hypogaea* L.) genotypes which have higher levels of drought tolerance were able to take up more nutrients than those with lower levels and the absorbed nutritional elements contributed to the yield production. There was a positive correlation between grain yield and number of kernel per row (Table 3), which was consistent with the results of some researches (Ashofteh, 2011). Regression trends of grain yield in treatments of different N levels and irrigation showed that water stress at different growth stages decreased the grain yield in comparison to the control treatment (Fig. 1). The effect of increase in nitrogen on the grain yield under optimal irrigation conditions was more than those treatments with stress conditions. The higher yield at optimal water condition likely are because of improvement in N uptake, extensive translocation of N from vegetative parts to grains, and larger leaf area index which were found positively correlated with grain yield under non-water stress conditions (Eghball and Maranville, 1991).
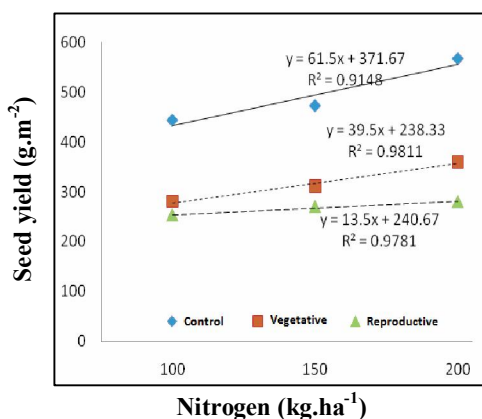
Fig. 1. Changes trend of seed yield in treatments of irrigation and different levels of Nitrogen fertilizer

1000 kernel weight (TKW) in drought stress treatment at vegetative stage compared with other treatment decreased more than that of control treatment and irrigation after drought stress (Table 1). It seems that plants failed to have complete compensation after stress conditions to improve leaf area index which negatively affects net photosynthetic rate and consequently reduces the assimilates in sources. Average of TKW reductions because of drought at vegetative and reproductive stages were estimated about 9.3% and 1.3%, respectively. The TKW of drought-stressed plants under vegetative phase was significantly lower than that of well-watered plants. TKW reduction at vegetative stress probably is because of fewer reserved carbohydrates in vegetative organs before pollination caused by decrease in leaf area duration resulting in shortening the grain-filling period. At both stress treatments, TKW was improved by increasing in rate of N application in both water stress treatments (Table 2). Previous report of a significant reduction of grains weight due to water deficit stress by Osborne *et al.* (2002) confirmed our findings. The effects of the treatments on the number of

rows per ear were not significant (Table 1). Since environmental change and assimilate distribution pattern in plants do not influences the number of the ear at the time of determining the number of rows per ear (Vitale *et al.*, 2007), this yield component was not significantly altered by the stress treatments. The kernel number was significantly affected by the interaction of water stress and N rate treatments (Table 2). Drought stress at vegetative and reproductive stages decreased the number of kernels per row by 18.6% and 48.8% respectively compared to the control treatment. Previous reports also stated that the kernel number was the most affected yield components under water deficit (Lorens *et al.*, 1987). Drought condition at growth stage of 50% tassel to pollination, lack of adequate water causes a decrease in the number of fertile pollen grains and also dry up most stigmas, which consequent in the reduction of floret inoculations led to decrease in the number of kernel per row (Ghooshchi *et al.*, 2008; Mansouri-Far *et al.*, 2010; Asmatullah *et al.*, 2007; Shakarami and Rafiee, 1990). Higher nitrogen rates under drought conditions, however, insignificantly increased the number of kernel per row (Table 2), indicating the positive effect of N consumption on the number of grain per ear (Rahmati, 2012; Osborne *et al.*, 2002). Positive effect of increase of N consumption on number of grains per Ear at optimal irrigation conditions was probably due to N uptake at optimal water regime of soil. The results of this part of research show that application of N fertilizer had a positive effect on number of filled kernels, too although the increase was not significant (Table 2). Regression trends of the number of grains per row in different N levels and irrigation-off treatments exhibited the highest reaction to N increase at the

control treatment with optimal irrigation followed by drought at vegetative and productive stages respectively (Fig. 1). The higher grain yield at control treatment can be justified with provided the necessary conditions for N uptakes in the soil. Since grains are filling at vegetative stage, when N increases this trend is accelerating and as N was used before the stress, maize hybrid did not have the chance to make use of it to increase number of grains per row. At the time of pollination, inoculation and grain filling would occur. However, in vegetative stage, plant had the chance to use and absorb N that resulted in increase of number of grains per row.

Source Limitation

The effects of drought stress and nitrogen rates on source limitation were significant (Table 2). In average, the highest and the lowest rates of source limitations were belonged to treatments with 100 and 200 nitrogen kg.ha^{-1} respectively. Reduction of nitrogen rate from 200 to 100 kg.ha^{-1} increased source limitation by 43.57% (Table 1). Voltas *et al*. (1997) concluded that endosperm cells were significantly affected by the rate of assimilates at early growth stages of grain, and deficit in nitrogen before pollination stage may cause a decrease in endosperm cell reaction to increase the source and source limitation through reduction of assimilate storage after pollination. However, it seems like that in the present experiment the studied conditions have been quite be different. Water stress at the vegetative growth stage increased the source limitation to 67.1% and at the reproductive growth stage to 77.25% (Table 1).

Table 2. Mean comparison interaction effect of treatments of seed yield and its components

Treatments		Seed yield (g.m^{-2})	Kernels per row	Kernel rows per ear	1000 kernel weight (g)	S. L (%)
Nitrogen (kg.ha^{-1})	Irrigation					
100	Vegetative	281[‡]de	20c	14a	129c	36bc
	Reproductive	253e	34b	14a	204b	51a
	Optimal	444b	42ab	14a	203b	18e
150	Vegetative	311cd	24c	14a	226ab	29d
	Reproductive	270de	35ab	14a	238ab	42b
	Optimal	473b	42ab	14a	228ab	7f
200	Vegetative	360c	24c	14a	251a	22e
	Reproductive	280de	36ab	14a	217ab	34cd
	Optimal	567a	44a	14a	236ab	4f
MS		2069**	18.31*	0.7ns	2749**	1004**

[‡] In each column, the means with similar letters are not significantly different at 5% probability. Ms: Mean square.
ns, * and **: non-Significant, significant at the 0.05 and 0.01 probability level, respectively.

Increase in source limitation could be attributed to growth acceleration, decrease in the grain growth duration, reduction of leaf area, and early senescence of the leaves (Wardlaw, 1980). Scott *et al*. (1990) stated that stress via decreasing source activity caused decrease of grain performance. In source limitation conditions, sink strength (sum of reservoir activity and size) is higher; thus, because of physiological relationship between the source and the sink, the source provides required materials to the sink via increasing of photosynthetic activities. Drought stress at reproductive stage also increased the source limitation because of earlier leaves senescence at the end of growth

season causing photosynthetic sources limitation and consequently decline in the transformation of nutrition to the grains. The interactive effect of stress and N showed that increase of N consumption relatively decreased source limitation (Table 2) because of better development of leaves and root systems and increase in plant chlorophyll through the increase of N. Source limitation had a negative significant correlation with total kernel weight (Table 3).

Concentration of Chlorophyll (*Chl*) and Carotenoids

It was consider that the rate of chlorophyll is different between maize growth stages. Thus, chlorophyll reduction percentage in each growth stage

was assessed and compared to the well-watered treatment at the same stage. The results exhibited that the chlorophyll content was altered by different treatments (Table 2). The highest reduction of *Chl a* was belonged to the treatment with 100 kg N ha^{-1} and irrigation-off treatment at vegetative stage while the lowest reduction was observed at treatment with 200 N kg.ha^{-1}. Efeoglu *et al.* (2009) reported that the *Chl a*, *Chl b*, total *Chl* (*a+b*) and carotenoid contents of maize hybrids were significantly reduced under drought which confirms our findings. Drought stress at the different growth stages causes stomatal closure and led to decrease the photosynthetic activities and rubisco enzyme reconstruction.

Table 3. Correlation coefficients between seed yield, its components and source limitation

Traits	Seed yield	Biological yield	Harvest index	Kernel rows per ear	Kernels per row	1000 kernel weight
Kernel rows per ear	0.114^{ns}	-0.39^{ns}	0.103^{ns}	-	-	-
kernels per row	0.555*	0.215^{ns}	0.586*	0.337^{ns}	-	-
1000 kernel weight	0.307^{ns}	0.261^{ns}	0.264^{ns}	0.21^{ns}	0.199^{ns}	-
Source limitation	0.248^{ns}	0.158^{ns}	0.265^{ns}	0.166^{ns}	0.212^{ns}	-0.580*

$^{ns, *, **}$: respectively indicate non-significant difference, significant difference at 5% and 1% probability levels.

Lack of sufficient chloroplast activities because of insufficient water supply causes the leaf senescence that decrease the leaf area (Modhej and Fathi, 2008). Consequently, it limits the photosynthetic capacity of the plant, reduces the dry matter accumulation. Producing more assimilates increase the cell division and cell size and ultimately leaf area index increases. Previous results also demonstrated that reduction of *Chl a* concentration in drought stress treatments (Mujeeb Ur Rahman *et al.*, 2004; Mansouri-Far *et al.* 2010) that supports our findings. As the rate of N increased, reduction of *Chl* significantly decreased at all irrigation treatments (Table 5).

The highest amount of *Chl* belonged to the well-watered treatment and 200 N kg.ha^{-1}. The lowest reduction of *Chl b* belonged to treatment of 200 N kg.ha^{-1} and reproductive stress (Table 4). Disorder in synthesis of chlorophyll a and b due to drought stress and sensitivity of photosynthetic process to water restriction via destruction of various components such as *Chl a* and *b*, enzymes, and proteins have been previously reported (Zeidi *et al.*, 2008; Mansouri-Far *et al.*, 2010). Thus, it can be concluded that the decrease of moisture at different growth stages caused destruction and decrease of *Chl b* although the reduction in reproductive stress was more at

vegetative stage due to the lack of proper re-growth after re-watering and loss of plant photosynthetic potential. The highest percentage of *Chl a+b* reduction was observed in treatment with the lowest level of N (Table 4). The lowest percentage of carotenoids reduction belonged to treatment with 200 N kg.ha^{-1} and reproductive drought (Table 5). *Chl b* and carotenoids act as auxiliary pigments protecting *Chl a* and have an effective role in absorbing and transferring received light energy to *Chl a*. Moreover, carotenoids absorb light wavelengths, which cause photo-oxidation of *Chl* and protect it in this way (Telesinski *et al*., 2008). In average, lower rates of N had higher percentage of RWC reduction (Table 4).

RWC reduction was higher when maize plants imposed to drought stress in reproductive phase. Plants receiving higher N rate maintain high RWC during the drought stress by better osmotic adaptation both in vegetative and reproductive drought stresses (Table 5). It has suggested that N affects osmotic regulation, cell wall elasticity, carbohydrates metabolism, and synthesis of drought-induced signal substances in roots (Morgan, 1986). These results were not in agreement with Bennett *et al*. (1986). They reported that, maize was more sensitive to water stress under higher rates of N fertilizer. The basic contradiction may relate to drought intensity or duration of stress occurrence.

Table 4. Mean comparison of Chlorophyll, Cartenoids, RWC (relative water content) reduction under stress treatments compared to well-watered maize

Treatments	Carotenoids	Relative water content	Chl (a+b) %	Chl b	Chl a
Nitrogen (kg.ha^{-1})					
100	37.0[†][a]	19.6[a]	39.9[a]	38.7[a]	49.2[a]
150	22.0[b]	12.8[a]	19.2[b]	11.3[b]	28.6[ab]
200	1.7[c]	5.3[b]	11.6[b]	4.9[b]	17.0[b]
Irrigation					
Vegetative	39.1[a]	9.2[b]	33.8[a]	25.3[a]	49.2[a]
Reproductive	1.4[b]	16.0[a]	13.3[b]	10.8[b]	16.1[b]

[†]In each column, the means with similar letters are not significantly different at 5% probability level.

CONCLUSION

Drought stress at vegetative and reproductive growth stages significantly decreased grain yield of maize in comparison to well-watered plants. Significant decrease in grain yield under drought stress at vegetative stage was due to reduction of number of kernels and kernel weight. Drought stress at reproductive stage led to decrease in grain yield by reducing fertile florets and number of kernels. Drought stress at both stages led to decrease of leaf *Chl* content. Percentage of *Chl* reduction in vegetative drought stress was more than that of reproductive stage. Shortage of N exacerbated *Chl* degradation in drought stress conditions. Nevertheless, as N increased, the amount of *Chl* increased significantly. Irrigation-off at vegetative and reproductive stages increased source limitation by 67.1 and 77.25% respectively. Intensification of source limitation under stress conditions was due to acceleration of growth, decrease of leaf green area duration and decrease of leaf *Chl* and RWC. Nitrogen rates influenced physiological responses of maize to water stress.

Table 5. Mean comparison interaction effect of treatments on RWC, Carotenoids and Chlorophyll

Treatments		RWC	Carotenoids	Chl (a+b) %	Chl b	Chl a
Nitrogen (kg.ha⁻¹)	Irrigation					
100	Vegetative	18.44[‡a]	71.89[a]	49.59[a]	48.80[a]	53.07[a]
	Reproductive	20.85[a]	2.13[c]	30.19[b]	27.34[b]	32.87[b]
150	Vegetative	6.96[bc]	43.01[b]	31.46[b]	18.69[bc]	44.84[ab]
	Reproductive	18.80[a]	1.20[c]	7.03[c]	3.96[d]	12.44[c]
200	Vegetative	4.20[c]	2.50[c]	20.50[b]	8.59[cd]	31.03[b]
	Reproductive	8.55[b]	1.3[c]	2.69[c]	1.33[d]	3.13[c]

[‡]In each column, the means with similar letters are not significantly different at 5% probability level.

Increase of N led to increase of plant ability for holding relative water content in drought stress conditions. Even though the maize yield showed the highest reaction to N fertilizer in optimal irrigation treatment, increase of early application of N particularly in drought stress at vegetative stage somewhat decreased negative effects of drought stress on grain yield.

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