

Maize Response to Nitrogen and Irrigation Regimes

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Abstract: Drought is the most important limiting factor of the agricultural plants production in Iran and the world. Nitrogen application management under water limited condition needs to be refined for low yielding environments. Therefore, to see how restricted irrigation and different nitrogen fertilizer affect yield and chlorophyll content of corn, an experiment was conducted in a semi-arid area in Fars, Iran in 2011. Split-plot experimental design was used, based on a complete randomized block design with four replications. The main plots consisted of four moisture depletion: MD1 (20% FC), MD2 (40% FC), MD3(60% FC) and MD4 (80%FC). Each subplot received four rates of nitrogen fertilizer application: 150, 200, 250 and 300 kg ha⁻¹. The results showed that the MD1 treatment resulted in significantly higher grain yields, 1000-kernel weight, grain numbers per ear, harvest index, biological yield, chlorophyll index, chlorophyll a, b than the other treatments. However, the highest contribution of stem and leaf dry matter remobilization in grain yield were obtained in MD4 and 300 kg N ha⁻¹ and the lowest one was found in the MD1 treatment and 150 kg N ha⁻¹. Nitrogen application increased all traits; however there were no significant difference between 250 and 300 kg N ha⁻¹.

Key words: Corn • Irrigation regimes • Nitrogen fertilizer • Chlorophyll content

INTRODUCTION

Drought stress is one of the most important environmental stresses affecting agricultural productivity worldwide and can result in considerable yield reductions [1]. The physiological mechanisms involved in cellular and whole plant responses to water stress, therefore, generate considerable interest and are frequently reviewed [2]. Numerous physiological and biochemical changes occur in response to drought stress in various plant species [3]. Drought occurs when moisture around the roots is so reduced that a plant is not able to absorb enough water, or in other words with transpiration of water absorption [4]. Drought limits plant growth and productivity more than any other environmental factor in the arid and semi-arid area. Water deficit reduces the economic yield of crop to different degree during its growth stage [5-7]. Water stress tolerance of crop is largely dependent on the crop genotype [8]. Maize (*Zea mays* L.) is the third most important cereal crop

in the world after wheat and rice which is frequently subject to environmental stress, particularly periods of long-term water shortage [9]. Approximately 8 to 10% of the corn crop is used as food for human consumption. It is not only a source of food, fodder and feed but also many by products like glucose, starch and corn oil, etc are prepared from it. Maize is extensively grown in temperate, subtropical and tropical regions of the world. The productivity of maize not only on the accumulation of dry matter, but also on its effective contribution to plant parts of economic importance and this is a key to yield stability. Nitrogen is a nutritive element required by plants in large amounts while its use efficiency is always low in dry land regions, which has become one of the most nutritional-limiting factors for increasing crop yield and improving crop quality in Asia [8]. Nitrogen is the most important element for plant growth and development. Nitrogen is an integral component of many compounds essential for corn growth processes including chlorophyll and enzymes. Nitrogen also mediates the utilization of

potassium, phosphorous and other element in plants [10]. The optimal amount of these elements in the soils cannot be utilized efficiently if nitrogen is deficient in plants or excess can result in reduced plants yields. El-Bana and Gomaa, [11] found a significant increase in grain yield as a results of increasing nitrogen levels from 100 to 125kg N. In addition to supplying a nutrient for plant growth, N application could improve drought tolerance of plants to enhance yields [12-14]. Edmeads *et al.* [15] asserted that corn yield in tropical regions decreased on average by about 17% as a result of drought but depending on the severity and time of occurrence of the drought, yield reduction could reach 80%. According to [16] maize is more resilient to water shortage during the vegetative stage rather than during the reproductive phase. Water shortage not only affects leaves and stems at the vegetative stage but also affects other important developmental events such as the emergence of four male flowers, silk ear in the fifth week and the start and end of linear growth in the grain filling stage [2]. Nesmith and Rithcie, [17] reported delay in the emergence of male flowers, silk and cob development (linear growth) and pollen in corn plants due to a week of water deficiency. Extreme water stress at different stages of crop development has been reported to reduce the yield significantly [18]. Water stress has been found to reduce leaf area; photosynthesis, leaf chlorophyll contents and consequently grain yield [19]. Biomass yield is also affected by drought conditions and is an indicator of reduced yield; under low moisture regimes biomass yield was reported within the range of 75 to 61% of that obtained under favorable irrigation treatments while pre and post-anthesis moisture deficit significantly reduced grain yield by 49 and 66%. Leta *et al.* [20] and Karimian *et al.* [21] reported that drought stress at the vegetative growth stage had a minimal effect and that drought stress caused a greater decrease in grain yield at the grain filling stage. Fatemi *et al.* [22] and Khalili *et al.* [23] reported that the yield decrease under drought stress at the reproductive stage was greater than that at the vegetative and grain filling stages.

The purposes of the present study were to clarify the responses of a maize genotype under water deficit and effects of different nitrogen rates in water-stressed plants. The interaction effects of genotype, water and nitrogen in relation to chlorophyll a, b, carotenoids content and remobilization of dry matter from stem and leaf to the grain are also examined.

Table 1: Metrological statistics in the experiment location in month of experiment

Months of Experiment	Average maximum temperature°C	Average minimum temperature°C	Rainfall (mm)
July	30.33	15.3	0
August	35.5	14.59	0
September	30.88	9.38	0
October	26.31	3.99	0
November	16.92	1.9	13.7

MATERIALS AND METHOD

Plant Material, Growth Conditions

Nitrogen and Water Stress Treatments: A filed experiment with a hybrid corn variety SC-260 was conducted in research field of the Islamic Azad University of Shiraz, Iran, in 2011. The climate is semi-arid with 1350 meters altitude from sea level (Table1). The investigation was arranged as split-plot experiment based on the randomized complete block design with four replications. Main-plots were assigned to four irrigation regimes (20%, 40%, 60% and 80% moisture depletion from field capacity which was 26%) and sub-plot were also four nitrogen levels; 150, 200, 250 and 300 kg N ha⁻¹ in forms of urea (46% N). Plots were sown on 7 July 2011 with a four rows planting machine and were 6 m long and 4.5 m wide, with 6 rows 0.75 m apart. The recommended cultural practices, except methods of irrigation and nitrogen levels, were adopted to raise the crop during growth season. Irrigation treatments were applied 30 days after planting. To determine the soil characteristics 5 samples from 30 cm depth were collected and analyzed for physical and chemical properties (Table 2). One third of nitrogen was given at the time of land preparation and remaining was applied as topdressing at ZGS-23 and ZGS-60. During the growth period, all plots were weeded manually. No serious incidence of insect or disease was observed and no pesticide or fungicide was applied. Drip irrigation system was used in the study. The soil water content measurements were done one day before irrigation until harvest in four replications for all treatments by gravimetric sampling in 0–0.30 m as follows:

$$V = \frac{(FC - Om) \times Pb \times Droot \times A}{Ei}$$

where;

- V = Volume of irrigation water per cubic meter
- FC = Percent moisture content at field capacity
- Om = Percent moisture content before irrigation

Table 2: Soil physical and chemical characteristics of the experimental site

Variable										
	pH	EC dS m ⁻¹	OM%	N%	P mgkg ⁻¹	K mgkg ⁻¹	Fe	Zn	Mn	Cu
Silty-Clay	7.64	0.57	1	0.02	8.5	250	3.1	0/78	6.5	0./98

pb = Soil bulk density (gcm⁻³)

A = Irrigated area per square meter

D_{root} = Depth of root development according to meters

$E I$ = Irrigation efficiency

At silking and grain physiological maturity leaf and stem were harvested for contribution of dry matter remobilization. All plant parts were oven dried at 65°C to constant weight for dry weight determination.

Contribution of DM Remobilization to Grain (%) = (DM remobilization / grain yield) × 100.

Chlorophyll Index: Chlorophyll content of five ear leaves in each plot was measured at anthesis stage by the chlorophyll content meter device (Hansatech Instruments - model-CI-01, Tokyo, Japan).

Chlorophyll A, B and Carotenoids Content: In this experiment, chlorophyll a, b and carotenoids of the plant was measured at the fully expanded ear leaves at tasseling stage. Three leaves collected per plant from five plants (therefore, 15 leaves) per treatment. Leaves were frozen in liquid N₂, stored at -80°C for no longer than 10 d, then freeze dried. Freeze-dried tissue (1 g) punched fresh leaf sample was grinded along with 40 mL acetone 80% (v/v) until it was well smoothed. The resulted green liquid was transferred through Whatman paper No. 2. Eventually, the final liquid volume using acetone 80% reached to 100 ml. Light densities of Chlorophyll extract were read using spectrophotometer at 645, 663 and 452 nm wavelengths. Chlorophyll a, b as mg g⁻¹ leaf fresh weight was calculated according to Dhopte and Manuel, [24]:

Mg chlorophyll a = [12.7(D663) - 2.69 (D645)] × V/1000 w

Mg chlorophyll b = [22.9(D645) - 4.68(D663)] × V/1000 w

where, D, Light densities of Chlorophyll extract, V, final volume of chlorophyll extract in acetone 80% and w is leaf fresh weight as gram.

Grain yield values were adjusted to 15.5% moisture content.

In addition, 1000-kernel weight, grain number per cob and harvest index values were also evaluated. Harvest index (HI) is calculated as the ratio of the grain yield (GY) to above-ground dry matter yield (DM) at harvest stage.

Statistical Analysis: Data were analyzed by analyses of variance using the general linear model (GLM) procedure provided by SAS [25]. When significant differences were found ($p = 0.05$), the Duncan's multiple range test (DMRT) was carried out.

RESULTS AND DISCUSSION

Yield and Yield Components: Corn grain yield, above ground dry-matter yield and yield components data are summarized in Table 3. Variance analysis of the grain yield data indicated that irrigation treatments significantly affected the grain yield ($P < 0.05$). Duncan classification showed that the plots receiving MD1 (20% FC) resulted in significantly higher grain yields than the other treatments (Table 3). Grain yield varied from 6533 to 8877.5 kg ha⁻¹ among the treatments. The highest average grain yield was observed in MD1 treatment as 8877.5 kg ha⁻¹ and the lowest yield was found in MD4 (80% FC) treatment as 6533 kg ha⁻¹. The highest and lowest grains yield in consumption of 250 kg N ha⁻¹ was 8877.5 kg ha⁻¹ and in 150 Kg N ha⁻¹ was 6533 kg ha⁻¹. Nitrogen fertilizer application under water deficit treatments increased grain yield. Water deficit treatments at the highest nitrogen fertilizer treatment increased grain yield to 17% (Table 3). There were no significant difference between MD3 and MD4. Corn grain yields were reported to vary from 1.05 t ha⁻¹ for non-irrigated to 10.02 t ha⁻¹ for full irrigated treatment [26] form 3.26 t ha⁻¹ in dry-treatment to 8.51 t ha⁻¹ for the full irrigation for sprinkler irrigated corn [27] varying between 9.19–10.79 t ha⁻¹ in full irrigation treatment for surface irrigated corn in the first and second year [28] and from 6.18 t ha⁻¹ for water deficit to 9.79 t ha⁻¹ for full irrigation for drip-irrigated corn in the same experimental site [29].

In the study, there was significant difference in 1000-kernel weight among different irrigation treatments. 1000-kernel weight varied from 202 to 261 g. The highest 1000-kernel weight was observed in MD1 (20% FC) treatment as 261.9 g and the lowest one was found in MD4 (80% FC) treatment as 202 g. Water deficit reduced 1000-kernel weight; however, there were not significant differences between MD3 (60% FC) and MD4 (80% FC) treatments. Water shortage led to smaller kernels compared to those gained from the full irrigation cases. Generally, the 1000-kernel weight production with full

Table 3: Mean values of the traits as affected by moisture depletion and nitrogen fertilizer

Treatments		No. of rows per ear	No. of grains per ear	1000-kernel weight (g)	Grain yield Kg ha ⁻¹	Biological yield Kg ha ⁻¹	Harvest index (%)
Moisture depletion	Nitrogen fertilizer						
	150	14.77 ab	501.9 ab	221.3 ab	7556.1 b	13801 c	54 ab
	200	14.99 ab	514 a	239.0 ab	8171.3 ab	14110 b	57 a
	250	15.44 ab	541.3 a	260.9 a	8700.8 a	16732 a	51 b
MD1	300	15.77 ab	549.3 a	261.9 a	8877.5 a	15152 ab	58 a
	150	16.44 a	498.6 ab	228.8 a	7229.2 b	13441 c	53 ab
	200	14.89 ab	501.7 ab	235.6 ab	8055.6 ab	14332 b	56 a
	250	15.79 ab	514.9 a	240.7 ab	8410.2 a	15952 ab	52 b
MD2	300	15.32 ab	522.1 a	243.1 ab	8432.2 a	16200 ab	52 b
	150	15.77 ab	483.5 b	212.0 b	6914.1 c	14332 b	48 c
	200	15.33 ab	460.9 b	225.6 ab	7521.3 b	13831 c	54 ab
	250	15.10 ab	485.7 b	231.9 ab	8330.2 a	14460 b	57 a
MD3	300	16.44 a	491 ab	214.8 b	7521.3 b	13832 c	54 ab
	150	15.44 ab	403.3 b	202.0 c	6533.2 c	13171 c	49 c
	200	15.10 ab	481.2 b	219.1 ab	7033.0 b	13841 c	50 ab
	250	14.55 b	460.4 b	223.0 ab	7395.6 b	14072 ab	52 ab
MD4	300	14.22 b	464.4 b	203.1 c	7428.7 b	14101 ab	52 ab
Sources of variation	Moisture depletion (MD)		ns	*	*	*	* ns
	Nitrogen fertilizer (N)	*	*	*	*	*	**
	MD × N	*	*	*	*	*	*
	CV (%)	5.18	8.02	9.37	7.09	7.66	8.91

*, ** Significant at 0.05 and 0.01 probability levels, respectively.

Same letters in columns are not significantly different at $P \leq 0.05$.

irrigation is higher than the deficit irrigation treatments. Results showed that significant difference exists among different nitrogen fertilizer levels in 1000-kernel weight. The highest 1000-kernel weight was obtained at 300 kg N ha⁻¹ (261.9 g) and the lowest was in 150 kg N ha⁻¹ (202 g). This indicated that 1000-kernel weight was increased by 24% in 300 kg ha⁻¹ than 150 kg ha⁻¹ (Table 3). However, there were no significant difference between 250 and 300 kg N ha⁻¹.

Irrigation treatments had a significant effect on grain number per ear ($P < 0.05$). The highest grain number per ear was observed in MD1 (20% FC) treatment as 549.3, while the lowest was found in MD4 treatment as 202. As the applied irrigation amount increased, the grain number per ear was also increased (Table 3). Mean comparison showed that application of nitrogen at the rate of 300 kg ha⁻¹ produced the highest grains number per ear as 549.3 but there was no significant difference between 250 and 300 Kg N ha⁻¹. Therefore, applying nitrogen as 250 kg ha⁻¹ was better than 300 kg N ha⁻¹ (Table 3). Nitrogen affected cell metabolism and enzyme activity and regulates cell osmosis and photosynthesis. These results confirm the findings of other studies reported by Jun-chen and Dai-Junying [19], El-Sheikh [30] and Vicente *et al.* [31].

Biological Yield: The highest biologic yield was observed in MD1 (FC 20%) treatment as 16732 Kg ha⁻¹ and the lowest was found in MD4 treatment as 13171 kg ha⁻¹ (Table 3). Generally, the dry matter production under the 20% FC was significantly higher ($P < 0.01$) than those under the other irrigation treatments. The reason for higher biologic yield in the MD1 treatment can be attributed to favorable soil water conditions created in MD1 plots, which enhanced the vegetative development. The highest and lowest biomasses obtained, respectively at 300 kg N ha⁻¹ (16732 kg ha⁻¹) and 150 kg N ha⁻¹ (13171 kg ha⁻¹). However, there was no significant difference between 250 and 300 kg N ha⁻¹. Wiebold and Scharf, [32] indicated that nitrogen increased yield of biologic yield in corn.

Harvest Index: Harvest index (HI), known as the proportion of grain yield to the biological yield for the different treatments, is presented in Table 3. The highest harvest index was observed in MD1 treatment as 58% and the lowest one was found in MD2 treatment as 48%. Harvest index was affected by soil water deficit that developed during the grain filling period following anthesis. It appears that irrigation treatments had significant effect on harvest index ($P < 0.05$). HI values were reported to vary from 0.20 to 0.43 [26] from 0.31 to

Table 4: Mean values of the traits as affected by moisture depletion and nitrogen fertilizer

Treatments		LDMR†	SDMR‡	Chlorophyll A (mg g ⁻¹ Fw ⁻¹)	Chlorophyll B (mg g ⁻¹ Fw ⁻¹)	Chlorophyll Index (%)	Carotenoids (mg g ⁻¹ Fw ⁻¹) ¹
Moisture depletion	Nitrogen fertilizer						
	150	0.66 a	3.67 c	1.630 c	0.985 c	14.18 c	1.252 b
	200	0.90 a	3.60 c	2.483 b	1.550 ab	20.15 b	1.288 b
	250	2.93 ab	4.47 ab	3.480 ab	1.642 ab	25.70 a	1.391 b
MD1	300	3.09 ab	5.56 b	3.768 a	2.127 a	26.41 a	2.100 a
	150	1.24 b	3.79 c	1.438 c	0.840 c	13.53 c	1.117 b
	200	1.81 b	4.00 bc	2.457 b	1.480 b	19.64 b	1.205 b
	250	1.84 b	4.01 bc	3.480 ab	1.620 ab	23.49 ab	1.370 b
MD2	300	3.97 a	4.04 bc	3.706 a	1.911 a	25.25 a	1.664 ab
	150	1.91 b	4.14 bc	1.347 c	0.266 c	13.27 c	1.105 b
	200	1.92 b	4.59 b	2.425 b	1.410 b	18.09 bc	1.204 b
	250	2.90 ab	4.76 b	3.444 ab	1.443 b	21.73 b	1.33 b
MD3	300	3.96 a	5.30 ab	3.605 a	1.702 ab	24.94 a	1.511 ab
	150	2.13 b	4.16 bc	1.341 c	0.108 c	12.26 c	0.902 c
	200	2.07 b	4.75 b	2.405 b	1.250 b	16.65 bc	1.071 c
	250	2.72 ab	4.76 b	3.30 ab	1.487 b	18.25 bc	1.220 b
MD3	300	4.02 a	6.54 a	3.44 ab	1.487 b	20.08 b	1.377 b
Sources of variation	Moisture depletion (MD)	*	*	*	*	*	Ns
	Nitrogen fertilizer (N)	*	*	*	*	*	Ns
	MD × N	*	*	*	*	*	**
	CV (%)	9.02	8.87	15.87	10.24	11.58	13.68

†: Leaf Dry Matter Remobilization, ‡: Stem Dry Matter Remobilization.

Same letters in columns are not significantly different at $P \leq 0.05$.

0.55 [27] from 0.33 to 0.42 [29] in the same experiment station. Effect of N application as well as water stress on harvest index was also significant. The optimum dose of N for maximum harvest index was N 250 (Table 3), but there was no significant difference between 250 and 300 kg N ha⁻¹.

Contribution of Stem and Leaf Dry Matter Remobilization in Grain Yield: Results showed that contribution of leaf dry matter remobilization in grain yield was significantly affected by nitrogen levels and moisture depletion (Table 4). Increasing in nitrogen fertilizer levels and moisture depletion increased contribution of dry matter remobilization from stem to the grain (Table 4). Interaction effect of moisture depletion and nitrogen levels showed that the highest and lowest contribution was observed in MD4 × 300 kg N ha⁻¹ and MD1 × 150 kg N ha⁻¹ with average of 6.54% and 3.67%, respectively (Table 4). Increasing drought stress and nitrogen application increased contribution of dry matter with average of 14.79 and 22.81% in normal condition and severe drought, respectively [33-35].

Chlorophyll a and B Content: The effects of different irrigation levels on chlorophyll a and b values were significant (Table 4). Drought stress had significant difference between chlorophyll a and b values. Table 4

shows that increasing in severity of drought decreased chlorophyll synthesis. So that, the highest values of chlorophyll a and b obtained at MD1 treatment, with an average of 3.768 and 2.127 mg g⁻¹ fresh weight of leaves and the lowest values with an average of 1.341 and 0.108 mg g⁻¹ in MD4 treatment (Table 4). Similar Results were obtained by Sakinejad, [36]. The effects of different amounts of nitrogen on chlorophyll a and b values were significant (Table 4). Increasing in nitrogen application increased chlorophyll a and b values. The highest values of chlorophyll a and b were related to 300 Kg N ha⁻¹ treatment, while the lowest values belonged to the application of 150 Kg N ha⁻¹ (Table 4). Considering the fact that the nitrogen in the chlorophyll molecule is involved in building, it seems that under nitrogen deficiency conditions, due to the loss of leaf nitrogen, chlorophyll production decreased. The interaction between irrigation and nitrogen on chlorophyll a and b content was significant (Table 4). The values of chlorophyll a and b increased under drought stress and nitrogen application, (Table 4). The highest chlorophyll a and b with the averages 3.75 and 2.12 belonged to the MD1 (20% FC) and 300 Kg N ha⁻¹ treatment and the lowest values with averages of 1.34 and 1.08 were related to MD4 (80% FC) and 150 Kg N ha⁻¹ treatment. Sakinejad, [36] reported that, severe reduction in soil moisture limits nitrogen uptake and this would be resulted

to decrease in chlorophyll a and b values. Other studies, Rashidi [37] and Mujtaba and Alam Nia [38] reported that nitrogen limitation reduce the positive effects of increased nitrogen fertilizer when soil moisture is lost.

Chlorophyll Index (%): Chlorophyll content was influenced by N fertilization, moisture depletion (MD) and interaction $N \times MD$. Increasing in severity of drought decreased chlorophyll synthesis. Increasing nitrogen level increased chlorophyll index from 16.11 to 23.71, but there was no significant difference between 250 and 300 Kg N ha⁻¹ (Table 4). The highest values of chlorophyll index obtained at MD1 (20% FC) and 300 kg N ha⁻¹ and the lowest values at MD4 (80% FC) and 150 kg N ha⁻¹ treatment with an average of 26.41 and 12.26, respectively (Table 4.) This is in agreement with those reported by Boutraa *et al.* [13]. So, it seems that drought stress caused inhibition of chlorophyll synthesis and increasing its breakdown. Zhao *et al.* [39] stated that severe water shortages caused the stoppage of chlorophyll making. In water deficit conditions, all enzymes activities, such as nitrate reducing enzyme, were often reduced.

Carotenoids Content: Results also showed that the effects of different irrigation levels and moisture depletion were not significant on carotenoids content (Table 4). But, increasing nitrogen levels increased average of carotenoids content (Table 4). The interaction between moisture depletion and nitrogen was significant difference ($P < 0.01$). The highest and lowest values of carotenoids obtained at MD1×N4 and MD4×N1 treatment, respectively, with an average of 2.100 and .902 mg g⁻¹ fresh weight of leaves (Table 4).

CONCLUSION

Nitrogen fertilizer and irrigation regimes are the important factors to yield and yield contributing characters of maize. In the experiment, moisture depletion had effect on grain yield, 1000-kernel weight, grain number per cob, harvest index, biological yield, chlorophyll index, chlorophyll a, b. Nitrogen application increased all traits, however there was not significantly difference between 250 and 300 kg N ha⁻¹. Moisture depletion and nitrogen level separately were not affected on carotenoids but interaction between them had effect on it. The highest contribution of stem and leaf dry matter remobilization in grain yield were obtained in MD4 and 300 kg N ha⁻¹ and the lowest one was found in the MD1 treatment and 150 kg N ha⁻¹. Results are in agreement with other

researchers [20, 21] who reported that drought stress at the vegetative growth stage had a minimal effect, but caused a greater decrease in grain yield at the grain filling stage. Fatemi *et al.* [22] and Khalili *et al.* [23] reported that decreasing in grain yield under drought stress at the reproductive stage was greater than the vegetative and grain filling stages. Shoaie Hosseini *et al.* [40] reported that in a drought stress condition grain number per row and ear length, yield and plant height were decreased.

ACKNOWLEDGMENT

The authors wish to express their appreciation to the Islamic Azad University of shiraz, Iran for providing field experiment and laboratory of the research.

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