The Impact of Drought Stress on Some Effective Physiological Traits of Corn under Different Managements of Nitrogen Consumption

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ABSTRACT

In order to study the effects of drought stress, different levels and split application of nitrogen on some effective physiological traits of corn (hybrid SC.704), this study was conducted based on three split-plot field experiments, using Randomized Complete Block Design (RCBD). Each of irrigation treatments implemented separately in each experiment were as follows; Optimum irrigation, moderate drought stress and severe drought stress (irrigation after depletion of 30%, 40% and 50% of field capacity respectively). In each experiment, the three nitrogen levels consisted of 140, 180 and 220 kg N ha⁻¹ and were applied in the main plots and subplots which consisted of split application of nitrogen (50% at planting + 50% at V6 stage, 25% at planting stage and + 75% at V6 stage, and 25% at planting + 50% at V6 stage + 25% at V12 stage). The results indicated that drought stress significantly increased leaf rolling rate and decreased chlorophyll a and b concentration, nitrogen percentage of ear leaf, relative water content and grain effective filling period. The increase of nitrogen application especially at optimum irrigation and moderate drought stress increased these traits. Results showed that under severe drought stress, the increase of nitrogen consumption in order to reduce the effect of severe drought stress is not recommended but s₃ treatment (50% at V6 stage + 25% at V12 stage) for improvement of physiological traits is recommended for all the three irrigation conditions.

Key words: Corn, Drought stress, Nitrogen, Split application, Physiological traits

Introduction

Plant response to drought stress can be evaluated by changes in physiological traits. The reduction of chlorophyll synthesis is the most important effect of drought stress on plants [1]. Chlorophyll content of leaf is an indicator of photosynthetic capability of plant tissues [2,3]. Mensah et al., [4] reported that soybean plants grown at the greenhouse and subjected to drought stress from the early seed filling stage to the maturity stage, rapidly lost their leaf chlorophyll content than the control plants.

Schlemmer et al., [1] stated that drought stress had no significant effect on leaf chlorophyll content of maize and decreased turgor pressure, and resulted in changes in the amount of far red radiation which passed through the leaf. In other words, light reflection from leaf was increased with increasing drought stress. Barry et al., [5] reported chlorophyll destruction in barley was affected by water deficit. Xian-He et al., [6] found the same results in wheat. Also, Fotovat et al., [7] found that by exerting severe drought stress on wheat, the chlorophyll content of leaf significantly decreased.

Mujtaba and Alam Nia [8] reported that the chlorophyll content of soybean plants was reduced under drought stress conditions. Pazooki [9] and Rahman [10] stated that with increasing intensity of drought stress, chlorophyll pigments in corn was destroyed more quickly. Relative water content of leaves is a suitable criterion for evaluating plant water status [11]. Schonfeld et al., [12] showed that wheat cultivars with having high relative water content are more resistant against drought stress. Siddique et al., [13] reported that the increase of drought severity, relative water content of wheat flag leaves were significantly reduced. Cosculleula and Fact [14] also claimed that with increasing water deficit, leaf water potential in maize and grain yield decreased. Nitrogen is an essential element for growth and changes its values accessible to the crop yield [15].

Norwood [15] reported that inadequate management of irrigation and nitrogen are considered as the major factors in lowering the growth and yield of corn. Leaf analysis is one of the
methods of determining critical nutrient concentrations in plants. The Overall interpretation of results is based on the assumption that if the element concentration in plant tissues is lower than the critical level, its uptake is not sufficient from the soil. In critical concentrations, method, time and location of sampling for advice on the proper interpretation of nitrogen fertilizers are very important [16,17,18]. According to Vetsch and Randall [19] to determine the ear leaf chlorophyll content at the silking stage, forming one of the ways to detect nitrogen uptake by corn is enough. Jonse et al., [20] reported optimum percentage of ear leaf nitrogen in silking stage, respectively 2.6-4 and 2.7-3 percent.

Luis et al., [21] reported that with increasing nitrogen fertilizer, the percentage of ear leaf nitrogen linearly increased. Isfan et al., [22] and Jokela [23] reported nitrogen uptake by corn in different amounts of nitrogen is positively correlated with leaf nitrogen concentration at the silking stage. Subedi and Ma [24] stated that, supplied corn nitrogen requirement of the eight leaf stage onwards, and due to higher N uptake by plants, the chlorophyll content was increased. Moursi and Saleh [25] mentioned that the reduction in leaf nitrogen under water stress conditions, indicating nitrogen limitation in this situation. Grain weight is one important component of yield, and the main factor affecting grain weight are grain filling rate and grain filling period.

Banziger et al., [26] reported that the late application of nitrogen fertilizer due to positive effects on leaf area duration increased the corn grain filling period. Khalifa [27] expressed that the split application of nitrogen increased the leaf longevity, current photosynthetic and the grain filling period. Due to high sensitivity to changes in physiological traits, environmental conditions and crop management are also closely associated with the traits grain yield. This study investigated the changes in the physiological traits affecting plant growth under different irrigations and nitrogen managements.

Materials and Methods

This study was conducted in two cropping seasons (2005 and 2006) in the research field of Khuzestan Natural Resources and Agricultural Sciences University (20 m above sea level, 32° N, 40° E). The experimental field had a Clay loam soil. This research consisted of three separated experiments, each one performed in the form of split plot in a Randomized Complete Block Design with three replications. In each experiment, one level of irrigation treatment (I) including I1 (optimum irrigation), I2 (moderate drought stress) and I3 (severe drought stress) was exerted. 1, 2, and 3 were evacuation of 30, 40 and 50 percent of the field capacity respectively. Until the four to five leaf stage (the stage of established plantlet) Irrigations was performed according to 30 percent of the field capacity in all treatments and after this stage, the irrigation treatments were performed precisely. In each experiment, nitrogen was the main treatment including 3 levels, N1, N2 and N3, consisting of 140, 180, 220 kgNHa⁻¹. The secondary treatment had three distribution levels including S1:50% at the time of planting +50 percent at the time of six leaf stage, S2:25% at the time of planting +65% at the time of six leaf stage and S3:25% at the time of planting +50% at the time of six leaf stage +25% at the time of planting +50% at the time of six leaf stage +25% at the time of 12 leaf stage that were accomplished in random in the main and the subplots.

The growth stage of plant at the time of topdressing fertilization according to the suggested crown system of Ritchie and Hanvey [28] was specified, when more than 50 percent of the field was in this growth stage. Each subplot, had seven planting rows, each of row had seven meters length with a distance of 75 cm from each other. In the middle of November, the harvest was performed manually.

The irrigation time was when weight humidity in treatments I1, I2, I3, in 0-30 cm deep reached to 17.1, 14.6, and 12.2 percent and in 30-60 cm deep reached to 17.4, 14.9 & 12.4, respectively.

After reaching the soil weight humidity content to the determined point, the total irrigation required was calculated through the relationship suggested by Alizadeh [11]. The related parameters are:

\[
V = \frac{(F_C - \theta_m) \times pb \times D_{root} \times A}{E_i}
\]

\[V = \text{Volume of irrigation water per cubic meter}\]
\[F_C = \text{Percent moisture content at field capacity}\]
\[\theta_m = \text{Percent moisture content before irrigation}\]
\[pb = \text{Soil bulk density grams per cubic centimeter}\]
\[A = \text{Irrigated area per square meter}\]
\[D_{root} = \text{Depth of root development according to meters}\]
\[E_i = \text{Irrigation efficiency}\]

Thus, the required volume of water per irrigation time was calculated for each treatment and water distribution based on 90- percent efficiency using a pump. In order to calculate relative water content, leaf fresh weight samples were weighed; then, they submerged in distilled water and finally dried at 70 °C for 48 hours and they were finally
weighed again. RWC was calculated according to Dhopte and Manuel [29]:

\[
RWC = \frac{FW - DW}{TW - DW} \times 100
\]

Where, FW is fresh weight, DW is dry weight and TW is turgor weight of leaf samples. In order to calculate the leaf chlorophyll content, 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 652 nm wavelengths. Chlorophyll a, b and total as mg g⁻¹ leaf fresh weight were calculated according to Dhopte and Manuel [29]:

\[
\text{Mg chlorophyll a} = [12.7(D663) - 2.69(D645)] \times \frac{V}{1000w}
\]
\[
\text{Mg chlorophyll b} = [22.9(D645) - 4.68(D663)] \times \frac{V}{1000w}
\]

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

To determine the percentage of ear leaf nitrogen, the leaves after being dried at 65 degrees of temperature for about 48 hours, the percentage of leaf nitrogen was determined using the instrument Kjeldahl.

Statistical analysis was made by the SAS statistical program. Mixed ANOVA was used and differences between trait means were measured using Duncan's Multiple Range Test for each individual environment. Stepwise was made between grain yield and yield components. Pearson correlation analysis was also conducted among the different variables.

Results and Discussion

Relative Water Content and Leaf Percentage Rolling:

The results showed that the effects of irrigation treatments on relative water content and leaf percentage rolling were significant. With increasing severity of drought stress, relative water content was significantly reduced; the highest mean value was 91 percent at the optimum irrigation treatment and the lowest was in the severe drought stress treatment, with an average of 78 percent (Table 2). Rafiee [30] and Sakinejad [31] reported similar results for maize under drought stress. Mensah et al., [4] reported that with decreasing irrigation, relative water content of leaf in Sesame decreased from 79.8 to 66.5%. Vildabadi et al., [32] reported that the reduced relative water content of leaves under drought stress conditions showed that the inflammation in plant cells was low. The results indicated that drought stress significantly increased the leaf rolling percentage. The maximum reduction of the leaf rolling percentage under the severe drought stress treatment was estimated by 29% and its minimum value associated with optimum irrigation was 7% (Table 2). The correlation between leaf rolling percentage and relative water content of leaf was negative (Table 4). Other effects of treatments on leaf relative water content and leaf rolling percentage were not significant (Tables 1 and 2).

Nitrogen percentage of ear leaf:

The effect of irrigation treatments on the ear leaf nitrogen percentage was significant (Table 1). Considering the negative correlation between the severity of stress and ear leaf nitrogen (Table 4), with the rise in drought stress, the percentage of ear leaf nitrogen decreased. Thus, the highest and the lowest percentages of ear leaf nitrogen were 2.65% and 2.02% under optimum irrigation and severe drought stress respectively (Table 2). The reduction in leaf nitrogen under drought stress may be due to the reduction of nutrients uptake by the plant roots under drought stress conditions. Fapohunda and Hussein [33] expressed that the availability and uptake of nitrogen are influenced by the availability of water.

The effects of different nitrogen rates on the ear leaf nitrogen percentage were significant (Table 1). The positive correlation between nitrogen treatments and ear leaf nitrogen (Table 4) showed that with increasing nitrogen application, the percentage of leaf nitrogen increased so that the highest and lowest percentages of leaf nitrogen were obtained by using 220 and 140 kg N ha⁻¹ respectively (Table 2).

Luis et al., [21] reported that with the increase in nitrogen fertilizer, the percentage of ear leaf nitrogen increased. On the other hand, Fox et al., [34], Isfan et al., [22] and Jokela and Randall [23] reported that there was a significant positive correlation between nitrogen fertilizer application and nitrogen uptakes by corn. The interaction between irrigation and nitrogen on ear leaf nitrogen was significant (Table 1). This situation indicated that nitrogen application under different irrigation treatments have different effects on leaf nitrogen content so that under severe drought stress, nitrogen intake had no positive effects on ear leaf nitrogen (Table 3).

Burman et al., [35] and Martin et al., [36] reported that nitrogen is extremely influenced by the availability of water. Thus, in case of severe soil moisture, nitrogen uptake by plants is impaired and the required nitrogen for sensitive growth stage will not be supplied. The effect of split application of nitrogen on ear leaf nitrogen percentage was significant (Table 1). The application of more nitrogen percentage of the total nitrogen used in the linear growth stage plants, the percentage of leaf nitrogen significantly increased. Although the effects
of $S_1$ and $S_2$ treatments on ear leaf nitrogen percentage were not significant, but the $S_3$ treatment was more effective than the $S_2$ treatment (Table 2).

Spellman et al., [37] expressed that in corn due to more rapid absorption of nitrogen from the six leaf stage onwards, the possibility of loss through leaching was lower, and resulting in nitrogen, more time was given to corn roots. Bair et al., [38] reported the consumption of nitrogen during rapid growth stage of corn crop increased nitrogen uptake by plant roots. Muthukumar et al., [39] reported with increasing nitrogen application from 12 leaves to the next stage, ear leaf nitrogen percentage increased. The effect of other treatments on the percentage of ear leaf nitrogen was not significant.

*Chlorophyll a and b Content:*

The effects of different irrigation levels on chlorophyll a and b values were significant (Table 1). Drought stress had significant negative correlation with a and b chlorophyll values (Table 4) showed that, with the increase in severity of drought, chlorophyll synthesis decreased. So that the highest values of chlorophyll a and b at the optimum irrigation treatment, respectively, with an average of 3.89 and 1.96 mg/g fresh weight of leaves and the lowest values of chlorophyll a and b, respectively, with an average of 1.11 and 0.58 mg/g in treatment of severe drought stress were obtained (Table 2). The results of Sakinejad [31] were consistent with the results of this study. The effects of different amounts of nitrogen on chlorophyll a and b values were significant (Table 1). By increasing the nitrogen application, the chlorophyll a and b values increased. The highest values of chlorophyll a and b were related to the treatment of 220 kg, while the lowest chlorophyll a and b values belonged to the application of 140kgNha$^{-1}$ (Table 2). The correlation between the amount of ear leaf nitrogen with chlorophyll a and b values increased. The highest values of chlorophyll a and b were related to the treatment of 220 kg, while the lowest chlorophyll a and b values belonged to the application of 140kgNha$^{-1}$ (Table 2). The correlation between the amount of ear leaf nitrogen with chlorophyll a and b values were positive (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 values were significant (Table 1).

Under optimum irrigation and moderate drought stress, with increasing nitrogen application, the values of chlorophyll a and b increased (Table 3). The highest chlorophyll a and b, respectively, with averages 3.5 and 1.8 belonged to the optimum irrigation treatment and nitrogen consumption of 220 kg and the lowest values of chlorophyll a and b, respectively, with averages of 1.13 and 0.59 were related to the treatment of severe drought stress and consumption of 140kgNha$^{-1}$. Sakinejad [31] reported that due to severe reduction in soil moisture, nitrogen uptake is limited. Thus, the effects of nitrogen on chlorophyll a and b values would decrease. Rashidi [40] and Bock [41] and Mujtaba and Alam Nia [8] reported that nitrogen limitation reduce the positive effects of increased nitrogen fertilizer when soil moisture is lost. The split application of nitrogen on chlorophyll a and b values were significant (Table 1).

Considering the correlation values of chlorophyll a and b with the ear leaf nitrogen, chlorophyll a and b values increased in the $S_3$ treatment implying more nitrogen uptake in this treatment.

Subedi and Ma [25] indicated that nitrogen supply is required at the eight-leaf stage of corn to the next because of more nitrogen uptake by plants, chlorophyll increased.

To determine the chlorophyll content at the silking stage, one of the ways to detect sufficient nitrogen uptake is sufficient by corn in response to side dressing [19].

*Grain filling rate:*

The grain filling rate was not affected by the different treatments (Tables 1 and 2). It seems that higher grain filling rate is controlled by plant genetic [42]. On the other hand, non-sensible changes in grain filling rate of corn, during drought stress conditions, were thus interpreted: although canopy photosynthesis of corn under drought stress conditions was reduced, due to the reduction of the number of grains per ear under these conditions, the relative contribution of each grain of current photosynthesis and parallel to increase the contribution of material from other sources was caused, grain filling rate under drought stress conditions than optimum soil moisture conditions did not change much.

*Grain filling period:*

The Effect of irrigation on the effective grain filling period was significant (Table 1). According to the negative correlation between the severity of drought stress with the effective grain filling period (Table 4), with increasing drought intensity, duration of grain filling was reduced so that the highest and lowest effective grain filling periods with a mean of 36.6 and 30.6 days respectively belonged to the optimum irrigation and severe drought stress (Table 2) Jee [43] reported that the shorten in of the grain filling period of maize in the water deficiency stress conditions was due to the effect of drought stress on reducing the current photosynthesis and accelerated leaf senescence. The effective duration of grain filling was affected by nitrogen application (Table 1).

The positive correlation between nitrogen intake and the grain effective filling period (Table 4), showed that with increasing nitrogen application,
grain filling period increased, so the most effective grain filling periods were in 180 and 220 kg N ha\(^{-1}\) treatments respectively (Table 2). By increasing nitrogen consumption, the amount of dry matter production and leaf area duration and thereby current photosynthesis during grain filling period increased. The results showed that the interaction effect of irrigation and nitrogen on grain filling period was significant (Table 1). With increasing nitrogen application at optimum irrigation and moderate drought stress, the grain effective filling period was significantly increased. Nitrogen effects in severe drought stress conditions on grain filling period were not significant (Table 3). Severe drought stress conditions, due to nitrogen limitation and the reduction of positive effect of nitrogen on LAI and parallel to the prevention of delayed leaf senescence and thus the reduction of leaf area duration reduced the effective grain filling period was expected. The effect of split application of nitrogen treatment on the effective grain filling period was significant (Table 2). The positive effect of split application of nitrogen in the S3 treatment on leaf area index, leaf longevity the and durability of current photosynthesis significantly increased the grain filling period compared to the S1 treatment. The results of Banziger et al., [26] were consistent with this result.

Khalifa [27] pointed that the method of split application of nitrogen increased the leaf longevity, current photosynthetic, and the grain filling period.

**Conclusions:**

Results showed that the growth physiological traits were affected by drought stress and all traits decreased with increasing the severity of drought stress significantly.

The increase of nitrogen application especially in optimum irrigation and moderate drought stress caused an increased in these traits. These traits in the S3 treatment were more than the S1. Results showed that under drought severity stress, the increase of nitrogen consumption in order to reduce effect of severity drought is not recommended but the S3 treatment for improvement of physiological traits is recommended for all the three irrigation conditions.

| Table 1: Summary of combined analysis of variance for some physiological traits |
|---------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|----------|
|                          | S.O.V           | df              | RWC             | Nitrogen of ear leaf (%) | Chlorophyll a content (mg/g FW) | Chlorophyll b content (mg/g FW) | Rate of grain filling (mg/day) | Duration of grain filling (day) |
|                          |                 |                 |                 |                             |                                        |                                        |                    |                     |
| Year                     | n               | ns              | ns              | ns                          | ns                                      | ns                                      | ns                  | ns                  |
| Irrigation               | 2               | **              | **              | ns                          | **                                        | **                                      | ns                  | **                  |
| Y*I                     | 2               | ns              | ns              | ns                          | ns                                      | ns                                      | ns                  | ns                  |
| R*I                     | 12              | 59.62           | 40              | 3.49                        | 6                                        | 2                                        | 16                  | 7.69                |
| Nitrogen                 | 2               | ns              | ns              | ns                          | ns                                      | ns                                      | ns                  | ns                  |
| Year* Nitrogen           | 2               | ns              | ns              | ns                          | ns                                      | ns                                      | ns                  | ns                  |
| N* I                     | 4               | ns              | *               | ns                          | **                                      | **                                      | ns                  | **                  |
| N*N* Y                   | 4               | ns              | ns              | ns                          | ns                                      | ns                                      | ns                  | ns                  |
| Ea                       | 24              | 18              | 24              | 1.55                        | 5                                        | 2                                        | 18                  | 2.31                |
| Split of nitrogen        | 2               | ns              | **              | ns                          | **                                      | ns                                      | ns                  | ns                  |
| Y* S                     | 2               | ns              | ns              | ns                          | ns                                      | ns                                      | ns                  | ns                  |
| I *S                     | 4               | ns              | ns              | ns                          | ns                                      | ns                                      | ns                  | ns                  |
| Y * I *S                 | 4               | ns              | ns              | ns                          | ns                                      | ns                                      | ns                  | ns                  |
| N* S                     | 4               | ns              | ns              | ns                          | ns                                      | ns                                      | ns                  | ns                  |
| Y * N * S                | 4               | ns              | ns              | ns                          | ns                                      | ns                                      | ns                  | ns                  |
| I * N * S                | 8               | ns              | ns              | ns                          | ns                                      | ns                                      | ns                  | ns                  |
| Y * I * N * S            | 8               | ns              | ns              | ns                          | ns                                      | ns                                      | ns                  | ns                  |
| Eb                       | 72              | 8.08            | 11              | 0.56                        | 3                                        | 1                                        | 10                  | 2.19                |

ns, *and,**: Not significant, significant at probability levels 5% and 1% respectively
Mean of Chlorophyll a and Chlorophyll b Content has been multiplied by 100 and mean nitrogen of ear leaf has been multiplied by 100

| Table 2: Mean comparison for measured morphological traits |
|---------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|                          | RWC             | Nitrogen of ear leaf (%) | Leaf rolling rate (%) | Chlorophyll a content (mg/g FW) | Chlorophyll b content (mg/g FW) | Rate of grain filling (mg/day) | Duration of grain filling (day) |
| Irrigation               |                 |                             |                       |                                        |                                        |                    |                     |

Means with same letter in each column are not significantly different at probability level of 5% using DMRT

Table 3: Comparison of average interaction N×I on Nitrogen of ear leaf, Chlorophyll a Content, Chlorophyll b Content, duration of grain filling

<table>
<thead>
<tr>
<th>Treatment (N×I)</th>
<th>Nitrogen of ear leaf (%)</th>
<th>Chlorophyll a content (mg/g FW)</th>
<th>Chlorophyll b content (mg/g FW)</th>
<th>duration of grain filling (day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Irrigation (I)</td>
<td>Nitrogen (kg N ha⁻¹)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>140</td>
<td>2.53b</td>
<td>3.5b</td>
<td>1.8b</td>
<td>34.5b</td>
</tr>
<tr>
<td>optimum irrigation</td>
<td>2.69a</td>
<td>4.01a</td>
<td>2.03a</td>
<td>37.7a</td>
</tr>
<tr>
<td>220</td>
<td>2.75a</td>
<td>4.16a</td>
<td>2.06a</td>
<td>37.8a</td>
</tr>
<tr>
<td>moderate drought stress</td>
<td>2.35c</td>
<td>1.98d</td>
<td>0.95d</td>
<td>32.3c</td>
</tr>
<tr>
<td>180</td>
<td>2.49b</td>
<td>2.3c</td>
<td>1.2c</td>
<td>34.2b</td>
</tr>
<tr>
<td>200</td>
<td>2.54b</td>
<td>2.41c</td>
<td>1.26c</td>
<td>36.6b</td>
</tr>
<tr>
<td>intense drought stress</td>
<td>2.02d</td>
<td>1.10e</td>
<td>0.57e</td>
<td>30.6d</td>
</tr>
<tr>
<td>180</td>
<td>2.04d</td>
<td>1.12e</td>
<td>0.59e</td>
<td>30.9d</td>
</tr>
<tr>
<td>200</td>
<td>2.04d</td>
<td>1.13e</td>
<td>0.59e</td>
<td>30.2d</td>
</tr>
</tbody>
</table>

Means with same letter in each column are not significantly different at probability level of 5% using DMRT

Table 2: Correlation coefficients between some traits related to redistribution and current photosynthesis

<table>
<thead>
<tr>
<th>Drought stress</th>
<th>Nitrogen</th>
<th>RWC</th>
<th>Leaf rolling rate</th>
<th>nitrogen of ear leaf</th>
<th>Chlorophyll a content</th>
<th>Chlorophyll b content</th>
<th>Rate of grain filling</th>
</tr>
</thead>
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<tr>
<td>RWC</td>
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<td>leaf rolling rate</td>
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<td>ns</td>
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<td>nitrogen of ear leaf</td>
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<tr>
<td>Chlorophyll a Content</td>
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<tr>
<td>Chlorophyll b Content</td>
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<td>rate of grain filling</td>
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<td>duration of grain filling</td>
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ns, *and, **: Not significant, significant at probability levels 5% and 1% respectively

Acknowledgments

The author would like to acknowledge the help by Agronomy Department of Islamic Azad University, Ahvaz Branch.

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