Effects of water and nitrogen supply on growth, water-use efficiency and mucilage yield of isabgol (*Plantago ovata* Forsk)

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Abstract

A two-way factorial experiment based on a randomized complete design (RCD) with four replications was used to compare four levels of N supply including control (N0), 60(N60), 120(N120) and 180(N180) mg N per Kg soil and four different water supply including 100(I100), 80(I80), 60(I60) and 40(I40) % FC on growth, water-use efficiency and mucilage yield of isabgol. Under I40, higher N addition led to a significant decrease in root and shoot DM. N addition increased root and shoot biomass especially in I80 treatment followed by I60. The highest (1.71 g plant−1) and the lowest (0.42 g plant−1) seed yield of isabgol observed in I100 at N120 and I40 at N120, respectively. The concentration of soluble sugar increased with N supply at I100 and I40. Water stress led to an increase in proline levels, particularly in the N supply treatments. The addition of N tended to reduce the positive effect of water stress on proline content. Seed water use efficiency (WUEg) of isabgol decreased with the increase in drought stress. N supply significantly increased WUEg from N0 to N120 with no significant differences between N60 and N120 and then significantly decreased by supplying N180. Mucilage water use efficiency (WUEm) is also increased linear in all watering regime at all N supply.

**Keywords:** Nitrogen addition, water stress, mucilage, proline, chlorophyll fluorescence

1. Introduction

Arid regions of the world are generally noted for their low primary productivity which is due to a combination of low, unpredictable water supply and low soil N concentrations (Porter and Nagel, 1999; Banayan et al., 2008). Many studies have found that fertilization often resulted in less biomass to root, increased leaf sensitivity to stress (Palatova, 2002; Liu and Stutzel, 2004), depressed plant growth (Liu and Stutzel, 2004), and caused high seedling mortality (Puri and Swamy, 2001; Song et al., 2010) under drought conditions. Therefore, many believed that nutrient stress may enhance the tolerance of plants to drought and possibly some other stresses as well (Palatova, 2002; Arora et al., 2001). In contrast, many other studies have noticed that increased N application could improve water-use efficiency, alleviate drought stress effects on plant growth in arid systems by preventing cell membrane damage and enhancing osmoregulation (Andrews et al., 1999; Saneoka et al., 2004). In addition, no significant interactions between N supply and drought stress for root dry mass, root/shoot ratio and WUE have been also found (Song et al., 2010; Rahimi et al., 2011). It was clear that additional amounts of N did not always play a positive
role in alleviating the adverse effects of drought on plant growth (Ashraf 
\textit{et al.}, 2001), but the role of N addition on physiological responses, particularly in relation to WUE and drought tolerance, remained unclear (Saneoka \textit{et al.}, 2004). Water and N are essential requirements for plant growth and survival. The photosynthetic rate, chlorophyll content, and concentrations of proline and soluble sugar vary in plants subject to different moisture conditions and nutritional status. Drought stress can increase organic compounds required for osmotic adjustment, such as soluble sugars and proline (Andrews \textit{et al.}, 1999; Garg \textit{et al.}, 2001; Molinari \textit{et al.}, 2007).

Isabgol (\textit{P. ovata}) is native to the Mediterranean region, and is found in the surrounding areas of India, Pakistan and Iran (Sharma, 2004). Isabgol seed husk has the property of absorbing and retaining water which accounts for its utility in stopping diarrhea. It is a diuretic, alleviates kidney and bladder complaints, gonorrhea, arthritis and hemorrhoids (Zargari, 1990). Some studies have shown that black cumin (Mozzafari \textit{et al.}, 2000) and isabgol (Rahimi \textit{et al.}, 2011) are able to tolerate moderate levels of water stress. Agricultural production in Iran decreased by 9.1% in 2006, and a further decrease in 2007 (FAO, 2008) due to drought. Iran is climatically regarded as an arid and semi-arid region in the world, where the lack of precipitation and its inappropriate distribution, high temperature and extensive evaporation makes the irrigation the main way for meeting plants water demand. Few researches have been carried out on responses of isabgol (Nadjafi, 2001; Banayan \textit{et al.}, 2008; Rahimi \textit{et al.}, 2011) to different irrigation intervals but the combination effect of drought stress and N addition has not been studied specially in our region. This study was aimed to (1) evaluate the effects of N addition and water stress on isabgol growth, WUE and physiological responses; (2) determine whether increased N alleviates the effects of water stress.

\section{Materials and methods}

\subsection{Plant material and growth conditions}

A pot experiment was conducted on February 20, 2010 in a temperature-controlled greenhouse located at the Agriculture College, Vali-e-Asr University of Rafsanajn, Iran (30\textdegree24' N, 55\textdegree60' E). Experiment was carried out with seeds of isabgol (\textit{Plantago ovata} Forsk) supplied by medicinal plant research, Jahad-e-Daneshgahi, Iran which cultivated in Iran as rainfed and irrigated system. The experiment consisted of a completely randomized factorial combination of four levels of N supply including control, 60, 120 and 180 mg N per Kg soil indicated as N\textsubscript{0}, N\textsubscript{60}, N\textsubscript{120} and N\textsubscript{180}, respectively and four soil water supply including 100, 80, 60 and 40\% field water capacity (FC) indicated as I\textsubscript{100}, I\textsubscript{80}, I\textsubscript{60} and I\textsubscript{40}, respectively. Accordingly, the average soil volumetric water content was kept 19.4\pm0.3\%, 14.8\pm0.7\%, and 7.5\pm1.1\% under 80, 60 and 40\% of FC water supply regimes during the experimental period, respectively. Each treatment combination was replicated four times. Each treatment pot was paired with a pot without a seedling that served as a control to correct soil evaporation when determining WUE. The watering and N treatments were initiated on March 10, 2010 after the seedlings were established. To avoid N rapid loss, the nitrogen solution was applied in half at 3-4 leaf stage and flowering stage in the form of NH\textsubscript{4}NO\textsubscript{3} solutions. Evaporation from soil surface was minimized by covering the pots with a 2-cm layer of quartz gravel. Transpiration water loss was measured gravimetrically by weighing all pots and re-watering with distilled water every other day at 18:00 h. The watering amount for each pot was determined according to the difference between the weight of a re-watered pot and the weight of the pot 48 h later. The experimental layout was surrounded with a single row of border plants to protect the experimental seedlings from external influences. All pots were rotated weekly to provide for random distribution in a greenhouse. Weight of the pot plus seedling at field capacity was adjusted accordingly. The experiment was terminated on
July 6, 2010. Ten seeds were sown in each pot (30 cm high and 25 cm in diameter) containing 7 kg of a loamy sandy soil (Table 1). One week after sowing, thinning was carried out to four plants per pot. Temperature was 30°C/20°C day/night. Nutrients were applied with irrigation prior to the drying cycle at a rate corresponding to P (Super phosphate) and K (Potassium sulfate) of 80 and 70 kg ha\(^{-1}\), respectively.

2.2. Growth parameters

In July 6, 2010, plants were harvested to determine final root and shoot dry weight and grain yield. Roots were separated from shoots by severing the seedling at the root collar, and were then carefully washed clean of growth media. Shoot and root were oven-dried separately for at least 72 h at 80°C and the dry mass of each fraction was determined.

2.3. Determination of leaf N content

Total nitrogen content was determined by a modified Kjeldahl method using concentrated sulphuric and salicylic acid and Na\(_2\)SO\(_4\), K2SO\(_4\) and Se in a ratio of 62:1:1 (w/w/w) as a catalyze (Sadasivam and Manickam, 1992).

2.4. Chlorophyll content and chlorophyll fluorescence

Chlorophyll was extracted from samples taken from the center of fresh leaves, using 95% (v/v) ethanol. Absorption of the filtrated extract was measured at 665 nm, 649 nm and 470 nm, and chlorophyll content calculated according to the Lichtenthaler formula (Lichtenthaler and Wellburn, 1983). Chlorophyll fluorescence was measured with a portable chlorophyll fluorimeter (Hansatec pocket PEA chlorophyll fluorimeter, England) at midday on the same leaves used for gas exchange measurements. The maximal PS II photochemical efficiency (Fv/Fm) was determined in leaves pre-adapted to dark for 15 min according to (Krause and Weis, 1991).

2.5. Determination of soluble sugar and proline content

The concentration of soluble sugar was determined in extracts obtained from fresh leaves. Soluble sugars was estimated by the anthrone method with glucose as the standard (Yemm and Willis, 1954). Free proline was extracted in aqueous sulphosalicylic acid and measured using ninhydrin according to Bates et al. (1973). The concentrations of soluble sugars, and proline were calculated on a dry weight basis (mg g\(^{-1}\)).

2.6. Determination of WUEg and WUEm

WUEg (Water use efficiency for seed yield) and WUEm (Water use efficiency for mucilage yield) for each treatment was determined by the ratio of seed and mucilage yield to water transpired during the experiment, respectively. While calculating the amount of water transpired during the experiment, evaporative loss from the pots was taken into account by subtracting the average amount of water loss from the control pots without plants from each watering treatment. Mucilage percentage of isabgol and French psyllium measured according to Sharma and Koul (1986).

2.7. Statistical analysis

All the variables from measurements were analyzed using General Linear Model (GLM) with water and N supply regimes and their interaction. When significant differences were noted, LSD multiple range test was used to determine where differences existed (Puri and Swamy, 2001). Relationships among variables were determined using the Pearson’s correlations coefficient test at 0.05 levels. All data were presented as mean ± SD. The regression model that best fitted the data, evaluated by an F-test, was chosen. All of the statistical analyses were performed using SAS 8.1 software (Version 8.0, SAS Institute Inc., and Cary, NC, USA.)
Table 1. Soil characteristics

<table>
<thead>
<tr>
<th></th>
<th>Bulk density (g cm⁻³)</th>
<th>Field capacity (%)</th>
<th>Gravel content (%)</th>
<th>Soil organic matter (g kg⁻¹)</th>
<th>Electronic conductivity (µS cm⁻¹)</th>
<th>Available N (mg kg⁻¹)</th>
<th>Available P (mg kg⁻¹)</th>
<th>CaCO₃ (g kg⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>8.69</td>
<td>1.31</td>
<td>18.24</td>
<td>22.51</td>
<td>9.76</td>
<td>81.23</td>
<td>10.53</td>
<td>8.07</td>
</tr>
</tbody>
</table>

Table 2. The effects of water regime, N supply and the interaction between water regime and N supply on growth characteristics using factorial analysis of variance.

<table>
<thead>
<tr>
<th></th>
<th>Root dry matter</th>
<th>Shoot dry matter</th>
<th>Root/Shoot</th>
<th>Leaf area</th>
<th>Grain yield</th>
<th>Miclilage percentage</th>
<th>Miclilage yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrogen (N)</td>
<td>1.4 **</td>
<td>33.3 **</td>
<td>0.006**</td>
<td>88725**</td>
<td>2.1 **</td>
<td>2.61 ns</td>
<td>91.7**</td>
</tr>
<tr>
<td>Drought stress (D)</td>
<td>0.22 **</td>
<td>6.2 **</td>
<td>0.007**</td>
<td>71467**</td>
<td>0.36 **</td>
<td>13.2**</td>
<td>54.1**</td>
</tr>
<tr>
<td>N*D</td>
<td>0.1 **</td>
<td>2.8 **</td>
<td>0.009**</td>
<td>6358*</td>
<td>0.19 **</td>
<td>4.27**</td>
<td>17.7**</td>
</tr>
<tr>
<td>Error</td>
<td>0.01</td>
<td>0.16</td>
<td>0.001</td>
<td>2089</td>
<td>0.09</td>
<td>3.4</td>
<td>5.5</td>
</tr>
</tbody>
</table>

* p < 0.05. ** p < 0.01.

3. Results and Discussion

3.1. Growth of the plant

A significant interactive effect of N addition and water stress treatment on root and shoot dry weight and R/S ratio of isabgol were found as described in Table 2. N addition stimulated a significant increase root and shoot DM differently related to different water stress (p<0.01). These two parameters markedly decreased under low water condition compared with that of the plants in Iₘ₀ and I₁₀₀, whereas N addition ameliorated the reduction in Iₘ₀ (Low drought stress) and I₆₀ (Medium drought stress) while it had negative effect on root and shoot DM in severe drought stress (I₄₀) (Table 3). The significant changes were detected in shoot (Total above-ground biomass) by N addition under unstressed, low and medium water stress (Iₘ₀ and I₆₀) conditions (p<0.01). Under severe drought stress treatment (I₄₀), higher N addition (N₁₂₀ and N₁₈₀) also led to a significant decrease in root and shoot DM (p<0.01). N addition increased root and shoot biomass especially in Iₘ₀ treatment followed by I₆₀. Soil water and N addition had advantaged effects on R/S ratio. The ratio of the root to shoot biomass (R/S) showed a significant decrease in severe drought stress (I₄₀) treatment compared with in the others (Table 3). But no obvious changes were detected in R/S ratio by N addition in severe drought stress (I₄₀), suggesting that N addition might not affect biomass allocation of isabgol under I₄₀. N addition stimulated the growth of the isabgol under different soil water conditions, as reflecting by a significant increase in plant growth parameters such as leaf area, root and shoot growth, R/S ratio (Table 3). Similar reports of N addition on plant growth have been reported for isabgol (Karimzadeh and Omidbaigi, 2004; Arun et al., 2012) and other crops (Andrews et al., 1999; Ashraf et al., 2001; Puri and Swamy, 2001; Song et
The growth response of the plants to N addition and soil water suggested that N supply could amplify the positive effects of elevated soil moisture on plant growth (Puri and Swamy, 2001; Song et al., 2010). These results indicated that N addition might alleviate the negative effects of drought stress manipulation on whole-plant growth of the plant. It is demonstrated that N addition might play a key role in maintaining plant productivity under different soil water conditions in the arid land. The ratio of root and shoot DM (R/S) was an indicator that represented demand-supply balance for environmental stresses (Andrews, 1999; Palatova, 2002). Nutrient limitation and drought stress were found to increase carbon translocation from the leaves to the roots, thereby increased the R/S ratio (Andrews et al., 1999; Poorter and Nagel, 1999). Similar result was presented in our study, as the R/S ratio increased with decreasing soil water content (Table 3), which supported the assumption that reduced soil water content could lead to carbohydrate accumulation in the roots of plants (Andrews et al., 1999). Our results provided the evidence that N addition drive an alternation in the ratio of the shoot, root DM and biomass allocation for isabgol in water limited condition.

**Table 3.** Mean comparison of root Dm, shoot Dm, R/S, Leaf area and grain yield of isabgol under different water and N supply.

<table>
<thead>
<tr>
<th>Water and N Supply</th>
<th>Root dry matter (g plant⁻¹)</th>
<th>Shoot dry matter (g plant⁻¹)</th>
<th>Root/Shoot</th>
<th>Leaf area (cm² plant⁻¹)</th>
<th>Seed yield (g plant⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N₀</td>
<td>0.76</td>
<td>3.3</td>
<td>0.17</td>
<td>259.2</td>
<td>0.97</td>
</tr>
<tr>
<td>N₆₀</td>
<td>1.29</td>
<td>5.1</td>
<td>0.16</td>
<td>609.3</td>
<td>1.58</td>
</tr>
<tr>
<td>N₁₂₀</td>
<td>1.28</td>
<td>5.6</td>
<td>0.15</td>
<td>585.5</td>
<td>1.72</td>
</tr>
<tr>
<td>N₁₈₀</td>
<td>1.45</td>
<td>6.9</td>
<td>0.14</td>
<td>565.6</td>
<td>1.62</td>
</tr>
<tr>
<td>LSD</td>
<td>0.41</td>
<td>1.55</td>
<td>0.02</td>
<td>25</td>
<td>0.95</td>
</tr>
<tr>
<td>N₀</td>
<td>1.07</td>
<td>2.6</td>
<td>0.31</td>
<td>294.9</td>
<td>0.87</td>
</tr>
<tr>
<td>N₆₀</td>
<td>1.17</td>
<td>4.7</td>
<td>0.33</td>
<td>421.7</td>
<td>1.51</td>
</tr>
<tr>
<td>N₁₂₀</td>
<td>1.39</td>
<td>5.7</td>
<td>0.26</td>
<td>592.9</td>
<td>1.60</td>
</tr>
<tr>
<td>N₁₈₀</td>
<td>1.55</td>
<td>6.3</td>
<td>0.22</td>
<td>413.8</td>
<td>0.81</td>
</tr>
<tr>
<td>LSD</td>
<td>0.13</td>
<td>2.2</td>
<td>0.018</td>
<td>36</td>
<td>0.89</td>
</tr>
<tr>
<td>N₀</td>
<td>0.65</td>
<td>2.4</td>
<td>0.45</td>
<td>199.3</td>
<td>0.78</td>
</tr>
<tr>
<td>N₆₀</td>
<td>0.92</td>
<td>3.8</td>
<td>0.55</td>
<td>359.8</td>
<td>1.09</td>
</tr>
<tr>
<td>N₁₂₀</td>
<td>0.86</td>
<td>2.8</td>
<td>0.33</td>
<td>261.6</td>
<td>0.81</td>
</tr>
<tr>
<td>N₁₈₀</td>
<td>0.66</td>
<td>2.5</td>
<td>0.26</td>
<td>276.4</td>
<td>0.78</td>
</tr>
<tr>
<td>LSD</td>
<td>0.32</td>
<td>1.65</td>
<td>0.052</td>
<td>21</td>
<td>0.75</td>
</tr>
<tr>
<td>N₀</td>
<td>0.43</td>
<td>1.5</td>
<td>0.33</td>
<td>169</td>
<td>0.55</td>
</tr>
<tr>
<td>N₆₀</td>
<td>0.66</td>
<td>1.8</td>
<td>0.37</td>
<td>121.7</td>
<td>0.47</td>
</tr>
<tr>
<td>N₁₂₀</td>
<td>0.33</td>
<td>1.9</td>
<td>0.36</td>
<td>176.4</td>
<td>0.42</td>
</tr>
<tr>
<td>N₁₈₀</td>
<td>0.22</td>
<td>1.2</td>
<td>0.21</td>
<td>179.1</td>
<td>0.56</td>
</tr>
<tr>
<td>LSD</td>
<td>0.15</td>
<td>0.4</td>
<td>0.042</td>
<td>18</td>
<td>0.36</td>
</tr>
</tbody>
</table>
3.2. Seed yield and Mucilage content

Seed yield of isabgol was significantly affected ($p<0.01$) by drought stress, N supply and their interaction (Table 2). The highest (1.71 g.plant$^{-1}$) and the lowest (0.42 g.plant$^{-1}$) seed yield of isabgol observed in I$_{100}$ at N$_{120}$ and I$_{40}$ at N$_{120}$, respectively. Generally seed yield reduced, as irrigation was limited especially in I$_{40}$. Although no significant variances were observed in seed yield under I$_{100}$ and I$_{50}$ regardless of N supply (Table 3). Under low drought stress (I$_{50}$), the seed yield obtained the largest/smallest with N$_{60}$ and N$_{180}$ levels, respectively (Table 3). Under severe drought stress (I$_{40}$), the seed yield peaked with N$_{30}$ with no significant differences with other N supply. (Table 3). Rahimi et al., (2011) and Arun et al., (2012) also reported that water stress decreased the seed yield of isabgol but no report observed about water stress and N addition interaction in this case. Drought stress, N supply and their interaction highly significantly ($p<0.01$) influenced Mucilage percentage and mucilage yield at the end of experiment (Table 4). Regardless of N$_{0}$, mucilage percentage and mucilage yield linear increase with increasing N supply and more availability of water (Figures 1, 2); on the contrary, N$_{0}$ showed a deviation from linearity with a second order relationship in mucilage percentage and mucilage yield. In this case, the mucilage percentage and mucilage yield limiting factor was nitrogen availability and irrigation supply which proved to be sufficient to achieve maximum mucilage percentage at N$_{60}$ in all irrigation levels (Figures 1, 2). Similarly, Koocheki et al. (2007) also reported that drought stress increased mucilage percentage and reduced mucilage yield.

3.3. Leaf chlorophyll content

Total leaf chlorophyll content (Chl a+b) is a good indicator of photosynthetic capacity. Low concentrations of chlorophyll limit photosynthetic potential directly and lead to a decrease in biomass production in the plants (Molinari et al., 2007; Van den Berg and Perkins, 2004). In this study, the concentrations of leaf Chl (a+b) was significantly influenced by N supply and water treatments in isabgol ($p<0.01$) (Figure 3). N supply had significant positive effects on Chl (a+b) regardless of soil water contents. Chl (a+b) was significantly different in N addition or water treatment (Figure 3). In N addition, the Chl (a+b) was dramatically increased under drought stress (especially at I$_{60}$ and I$_{40}$) treatments (Figure 3). However, the highest N addition (N$_{180}$) significantly decreased of Chl (a+b) at I$_{40}$ (Figure 3). The effects of N addition on chlorophyll were in agreement with the previous findings in suger beet (Shaw et al., 2002; Van den Berg and Perkins, 2004).

Table 4. The effects of water regime, N supply and the interaction between water regime and N supply on growth characteristics using factorial analysis of variance.

<table>
<thead>
<tr>
<th>Source</th>
<th>Chlorophyll</th>
<th>Fv/Fm</th>
<th>Nitrogen content</th>
<th>Soluble sugar</th>
<th>Proline</th>
<th>WUEg</th>
<th>WUEm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrogen (N)</td>
<td>0.09**</td>
<td>0.05**</td>
<td>3472**</td>
<td>0.05**</td>
<td>0.004**</td>
<td>0.018**</td>
<td>0.021**</td>
</tr>
<tr>
<td>Drought stress (D)</td>
<td>0.14**</td>
<td>0.02*</td>
<td>744**</td>
<td>0.09ns</td>
<td>0.06**</td>
<td>0.017**</td>
<td>0.019*</td>
</tr>
<tr>
<td>N*D</td>
<td>0.11**</td>
<td>0.02*</td>
<td>14.2**</td>
<td>0.05**</td>
<td>0.006**</td>
<td>0.006ns</td>
<td>0.03**</td>
</tr>
<tr>
<td>Error</td>
<td>0.01</td>
<td>0.007</td>
<td>29.1</td>
<td>0.004</td>
<td>0.0005</td>
<td>0.003</td>
<td>0.005</td>
</tr>
</tbody>
</table>

* $p<0.05$. ** $p<0.01$. 

Figure 1. Regression fits between mucilage Yield and water supply. Bars represent means of 4 replications ± standard deviations.

\[ Y \text{ (N0)} = -0.0027x^2 + 0.2007x + 15.835 \]
\[ R^2 = 0.84 \]

\[ Y \text{ (N60)} = 0.208x + 14.54 \]
\[ R^2 = 0.92 \text{ (N60)} \]

\[ Y \text{ (N120)} = 0.174x + 12.52 \]
\[ R^2 = 0.93 \text{ (N120)} \]

\[ Y \text{ (N180)} = 0.1405x + 10.49 \]
\[ R^2 = 0.761 \]

Figure 2. Regression fits between mucilage Yield and water supply. Bars represent means of 4 replications ± standard deviations.

\[ Y \text{ (N0)} = -0.0027x^2 + 0.2007x + 15.835 \]
\[ R^2 = 0.84 \]

\[ Y \text{ (N60)} = 0.208x + 14.54 \]
\[ R^2 = 0.92 \]

\[ Y \text{ (N120)} = 0.174x + 12.52 \]
\[ R^2 = 0.93 \]

\[ Y \text{ (N180)} = 0.1405x + 10.49 \]
\[ R^2 = 0.7612 \]
3.4. Chlorophyll fluorescence parameters

Water and N addition and their interaction had significant effect \((p<0.01)\) on chlorophyll florescence of isabgol (Table 4). N addition particularly in \(I_{60}\) and \(I_{40}\) stimulated a significant decrease of \(Fv/Fm\) ratio related to \(N_0\) \((p<0.01)\) (Figure 4a). Quantum yield, as indicated by \(Fv/Fm\), was in the range of 0.47 to 0.65 for \(N_{180}\) and \(N_{0}\) at \(I_{40}\), respectively (Figure 4a). A significant, but not dramatic decline of about 12\% occurred for plants irrigated with at \(I_{60}\) in \(N_{60}\) and \(N_{120}\) compared to \(N_0\) and \(N_{180}\). Concomitantly with this decrease the rate of electron transport dropped by about 41 and 44\% for \(I_{60}\) and \(I_{40}\) at \(N_{180}\) compared to \(N_0\). Quantum yield at \(I_{40}\) was significantly decreased by N addition with no drastically differences between N supplies which revealed the occurrence of a dynamic photo inhibition, which seemed to be effective in protecting the photosynthetic apparatus from the high risk of photo damage occasioned by the superimposed stresses to which the plants were subjected under drought stress conditions (Ranjbarfordoei et al., 2006). In the present research, we observed that \(Fv/Fm\) showed significant differences between the water treatments at given nitrogen supply (Table 4). This is in agreement with most of the data reported in the literature regarding water deficit (Ranjbarfordoei et al., 2006). They found that variable and maximal Chlorophyll fluorescence and fluorescence quenching were not affected by drought stress, and that other fluorescence parameters showed little difference.

3.5. Nitrogen content

As described in Table 4, drought stress, N supply and their interaction significantly \((p<0.01)\) influenced Nitrogen content of isabgol plants at the end of experiment. Drought stress obviously diminished Nitrogen content, but increased by N supply in all watering regimes (Figure 4b). The highest Nitrogen content in \(I_{60}\) (3.2 \%) and \(I_{80}\) (3.7\%) observed in \(N_{180}\). Under severe drought stress \((I_{40}\), the leaf N concentration obtained the largest/smallest with \(N_{120}\) and \(N_{180}\) levels, respectively (Figure 4b). Under low water stress \((I_{80}\), the leaf N concentration peaked with \(N_{180}\) with no significant differences with other N supply. (Figure 4b).
3.6. The concentration of soluble sugars and proline

Water stress, N supply and their interaction significantly affected leaf soluble sugar and proline content of isabgol (Table 4). The concentration of soluble sugar increased with N supply at I100 and I40. In contrast, the concentration of soluble sugars decreased with N addition in I80 with no significant difference between N120 and N180 (Figure 4c). The content of soluble sugars at N120 is significantly higher than other N supply in all irrigation levels except at I80 (Figure 4c). Conversely, water stress led to an increase in proline levels, particularly in the N supply treatments. The addition of N tended to reduce the positive effect of water stress on proline content (Figure 4d). In the present study, N supply significantly increased the concentration of soluble sugar and proline. Soluble sugars and proline play an important role in osmotic adjustment and may protect plants against oxidative stress (Foyer and Noctor, 2005; Molinari et al., 2007). In the present study, the concentration of soluble sugars and proline...
increased with N addition, suggesting that N input could not altered organic carbon allocation. Water stress can increase the soluble sugar and proline contents. For example, water stress increased total soluble sugar and free proline contents in Moth Bean and Mulberry plants (Garg et al., 2001; Molinari et al., 2007). In our study, water-stressed plants showed higher proline content than well-watered plants, particularly under low N and moderately increased N treatments. In contrast, there were no noticeable differences between I80 and I60 watering regimes in the concentration of soluble sugars.

3.7. Water-use efficiency

WUE, the functional indicator strongly related to plant growth and health under water deficit condition, is dependent on the amount of water used for growth and biomass production (Liu and Stutzel, 2004; Monclus et al., 2006). Many previous studies have observed that WUE was improved under water limitation (Liu et al., 2005; Binghua et al., 2012), but some others have found the inverse case and thought that the species employed a prodigal water-use strategy (Clavel et al., 2005). In this study, a significant effect of N addition and water treatment on WUEg and their interaction on WUEm of isabgol were found as described in Table 4. WUEg of isabgol was decreased with the increase in drought stress as described in Figure 5a. This might be attributed to low biomass production under severe drought condition. N supply significantly increased \((p < 0.01)\) WUEg from N0 to N120 with no significant differences \((p > 0.05)\) between N60 and N120 and then significantly decreased by supplying N180 (Figure 5a).

WUEm increased with the increase of N supply under all watering regimes. Compared with those under well-watered condition \(I_{80}\) and medium drought stress \(I_{60}\), plants under severe drought stress \(I_{40}\) exhibited the lowest WUEm under \(N_{180}\) followed by \(N_{120}\) and the highest WUEm in all watering regimes observed in \(N_{60}\) (Figure 6). WUEm increased linear in all watering regime at all N supplying compared with \(N_0\). The relationships between watering regimes and WUEm are reported in Figure 6. The results of the statistical regression analysis showed a close relationship between watering regimes and WUEm. With decreasing water stress, the slope of the regression line decreased, as well as R2 value (Figure 6). The slopes of the regression lines, which present the increment of WUEm for unit increment of water availability, were similar over different N supply, while the intercepts varied widely. From the equations reported in Figure 6, the basal N supply to increase WUEm can be derived at \(N_{60}\) in all watering regimes.

The graph of isabgol WUEm versus watering regimes (Figure 6) shows the linearity of the regression for the \(N_{60}\), \(N_{120}\), and \(N_{180}\) treatments, with the slightly higher slope in \(N_{60}\) rate. On the contrary, \(N_0\) treatment showed a deviation from linearity with a second order relationship. In this case, the mucilage yield limiting factor was nitrogen availability and irrigation supply which proved to be sufficient to achieve maximum WUEm value at \(N_{60}\) in all irrigation levels which is highlighting the fact that after a water use threshold, the soil nitrogen content became the grain yield and consequently WUEm limiting factor (Figure 6). Tolk and Howell (2003) reported that the slope of the relationships and cross-over point are affected by climate, soil properties and irrigation practices. Higher values were obtained at the \(N_{60}\) rate, but at \(I_{140}\) and \(I_{180}\) there were no differences between the irrigation levels.
Figure 5. WUEg of *P. ovata* under drought stress conditions (a) and different N addition (b). Bars represent means of 4 replications ± standard deviations.

Figure 6. Regression fits between WUEm and water supply. Bars represent means of 4 replications ± standard deviations.
4. Conclusion

Increasing availability of soil N may have led to the increase of leaf area and then biomass production, which could contribute to a higher WUE; but it is also increased the allocation to above-ground components relative to root structures, which finally resulted in a lower R/S ratio. As yet, high R/S ratios are advantageous to dry-adapted plants as drought stress occurring, because greater water absorptive component (root) can sustain more water supplies for transpiration (Binghua et al., 2012). There might be a balance between N supply and WUE or drought tolerance as suggested by Binghua et al. (2012). Even so, our results suggested that appreciable N supply might enhance the adaptability of isabgol to dry condition by getting better growth characters, increasing biomass production and WUE.

Our hypothesis that supra N supply could improve adaptability of isabgol under dry condition was only partly evidenced as discussed above in this study. Drought stress dramatically decreased the growth and biomass production of isabgol, although N supply altered biomass allocation to belowground, increased leaf area, WUEg and WUEm. It seems that Nsup could enhance the ability of isabgol adapted to drought condition by stimulating plant growth, gaining more biomass, increasing grain yield, HI and WUE, although Nlow showed the inverse performance. Thus, appropriate or low N supply was recommended for the planting of isabgol, but excess N supply should be avoided. Additionally, it seems that drought showed to be a stronger stress factor than single N-depositions in production of isabgol.

References


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