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

A. Rahimi^{*1}, F. Sayadi¹, H. Dashti¹, A. Tajabadi pour²

¹Department of Agronomy and Plant Breeding, Agriculture College, Vali-e-Asr University of Rafsanjan, Rafsanjan, Iran. ²Department of Soil Science, Agriculture College, Vali-e-Asr University of Rafsanjan, Rafsanjan, Iran. * Corresponding author: Rahimia@vru.ac.ir

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 (N_0), 60(N_{60}), 120(N_{120}) and 180(N_{180}) mg N per Kg soil and four different water supply including 100(I_{100}), 80(I_{80}), 60(I_{60}) and 40(I_{40}) % FC on growth, water-use efficiency and mucilage yield of isabgol. Under I_{40} , higher N addition led to a significant decrease in root and shoot DM. N addition increased root and shoot biomass especially in I_{80} treatment followed by I_{60} . 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. The concentration of soluble sugar increased with N supply at I_{100} and I_{40} . 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 N_0 to N_{120} with no significant differences between N_{60} and N_{120} and then significantly decreased by supplying N_{180} . 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 *et al.*, 2001), but the role of N addition on physiological responses, particularly in relation to WUE and drought tolerance, remained unclear (Saneoka *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 *et al.*, 1999; Garg *et al.*, 2001; Molinari *et al.*, 2007).

Isabgol (*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 *et al.*, 2000) and isabgol (Rahimi *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 *et al.*, 2008; Rahimi *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.

2. Materials and methods

2.1. 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°24' N, 55°60' E). Experiment was carried out with seeds of isabgol (*Plantago ovate* 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_0 , N_{60} , N_{120} and N_{180} , respectively and four soil water supply including 100, 80, 60 and 40% field water capacity (FC) indicated as 1100, 180, 160 and 140, respectively. Accordingly, the average soil volumetric water content was kept $19.4 \pm 0.3\%$, $14.8 \pm 0.7\%$, and $7.5 \pm 1.1\%$ 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_4NO_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 acid and salicylic acid and Na₂S₂O₄, K₂S₂O₄ and Se in a ratio of 62:1:1 (w/w) as a catalyst (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

	Bulk	Field	Gravel	Soil organic	Electronic	Available	Available	
pH	density	capacity	content	matter	conductivity	N	P	CaCO ₃
	(g cm ⁻³)	(%)	(%)	(g kg ⁻¹)	(μS cm ⁻¹)	(mg kg ⁻¹)	(mg kg ⁻¹)	(g kg ⁻¹)
8.69	1.31	18.24	22.51	9.76	81.23	10.53	8.07	8.80

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.

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

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

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 I_{80} and I_{100} , whereas N addition ameliorated the reduction in I_{80} (Low drought stress) and I_{60} (Medium drought stress) while it had negative effect on root and shoot DM in severe drought stress (I_{40}) (Table 3). The significant changes were detected in shoot (Total above-ground biomass) by N addition under unstressed, low and medium water stress (I_{80} and I_{60}) conditions ($p < 0.01$). Under severe drought stress treatment (I_{40}), higher N

addition (N_{120} and N_{180}) also led to a significant decrease in root and shoot DM ($p < 0.01$). N addition increased root and shoot biomass especially in I_{80} treatment followed by I_{60} . 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 (I_{40}) 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 (I_{40}), suggesting that N addition might not affect biomass allocation of isabgol under I_{40} . 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

al., 2010; Binghua *et al.*, 2012). 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.

		Root dry matter (g plant ⁻¹)	Shoot dry matter (g plant ⁻¹)	Root/Shoot	Leaf area (cm ² plant ⁻¹)	seed yield (g plant ⁻¹)
I ₁₀₀	N ₀	0.76	3.3	0.17	259.2	0.97
	N ₆₀	1.29	5.1	0.16	609.3	1.58
	N ₁₂₀	1.28	5.6	0.15	585.5	1.72
	N ₁₈₀	1.45	6.9	0.14	565.6	1.62
	LSD	0.41	1.55	0.02	25	0.95
I ₈₀	N ₀	1.07	2.6	0.31	294.9	0.87
	N ₆₀	1.17	4.7	0.33	421.7	1.51
	N ₁₂₀	1.39	5.7	0.26	592.9	1.60
	N ₁₈₀	1.55	6.3	0.22	413.8	0.81
	LSD	0.13	2.2	0.018	36	0.89
I ₆₀	N ₀	0.65	2.4	0.45	199.3	0.78
	N ₆₀	0.92	3.8	0.55	359.8	1.09
	N ₁₂₀	0.86	2.8	0.33	261.6	0.81
	N ₁₈₀	0.66	2.5	0.26	276.4	0.78
	LSD	0.32	1.65	0.052	21	0.75
I ₄₀	N ₀	0.43	1.5	0.33	169	0.55
	N ₆₀	0.66	1.8	0.37	121.7	0.47
	N ₁₂₀	0.33	1.9	0.36	176.4	0.42
	N ₁₈₀	0.22	1.2	0.21	179.1	0.56
	LSD	0.15	0.4	0.042	18	0.36

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 ($1.71 \text{ g.plant}^{-1}$) and the lowest ($0.42 \text{ g.plant}^{-1}$) 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_{80} regardless of N supply (Table 3). Under low drought stress (I_{80}), 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_{180} 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 sugar 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.

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

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

Figure1. Regression fits between mucilage Yield and water supply. Bars represent means of 4 replications $\pm$ standard deviations

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

Figure 3. Chlorophyll content of *P. ovata* under drought stress conditions in combination with N addition. Bars represent means of 4 replications $\pm$ standard deviations.

3.4. Chlorophyll fluorescence parameters

Water and N addition and their interaction had significant effect ($p < 0.01$) on chlorophyll fluorescence 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_{60} at I_{40} , respectively (Figure 4a). A significant, but not dramatic decline of about 12% occurred for plants irrigated with I_{60} in N_{60} and N_{120} compared to N_0 and N_{180} were significantly lower/higher than N_0 and N_{180} , respectively. 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).

Figure 4. Chlorophyll fluorescence (a), Nitrogen content (b), soluble sugar (c) and proline (d) of *P. ovata* under drought stress conditions in combination with N addition. Bars represent means of 4 replications $\pm$ standard deviations.

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 I_{100} and I_{40} . In contrast, the concentration of soluble sugars decreased with N addition in I_{80} with no significant difference between N_{120} and N_{180} (Figure 4c). The content of soluble sugars at N_{120} is significantly higher than other N supply in all irrigation levels

except at I_{80} (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 I_{80} and I_{60} 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 N_0 to N_{120} with no significant differences ($p > 0.05$) between N_{60} and N_{120} and then significantly decreased by supplying N_{180} (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 R^2 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_{40} and I_{100} , 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 $\pm$ standard deviations.

Figure 6. Regression fits between WUEm and water supply. Bars represent means of 4 replications $\pm$ 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, WUE_g and WUE_m. It seems that N₆₀ could enhance the ability of isabgol adapted to drought condition by stimulating plant growth, gaining more biomass, increasing grain yield, HI and WUE, although N₁₈₀ 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

- Andrews, M., Sprent, J.I., Raven, J.A., Eady, P.E. 1999. Relationships between shoot to root ratio, growth and leaf soluble protein concentration of *Pisum sativum*, *Phaseolus vulgaris* and *Triticum aestivum* under different nutrient deficiencies. *Plant. Cell. Environ.* 22, 949–958.
- Arora, A., Singh, V.P., Mohan, J. 2001. Effect of nitrogen and water stress on photosynthesis and nitrogen content in wheat. *Biol. Plantarum.* 44, 1, 153–155.
- Arun, T., Upadhyaya, S.D., Upadhyay, A. Preeti Sagar, N. 2012. Responses of moisture stress on growth, yield and quality of isabgol (*Plantago ovata* Forsk). *J. Agric. Technol.* 8, 2, 563-570.
- Ashraf, M., Shabaz, M., Ashraf, M.Y. 2001. Influence of nitrogen supply and water stress on growth and nitrogen, phosphorus, potassium and calcium contents in pearl millet. *Biol. Plant.* 44, 459-462.
- Banayan, M., Nadjafi, F., Azizi, M., Tabrizi, L., Rastgoo, M. 2008. Yield and seed quality of *Plantago ovata* and *Nigella sativa* under different irrigation treatments. *Indust Crops. Prod.* 27, 11-16.
- Bates, L.S., Waldren, R.P., Teare, I.D. 1973. Rapid determination of free proline for water-stress studies. *Plant Soil.* 39, 205–207.
- Binghua, L., Liang, C., Mingjun, L., Dong, L., Yangjun, Z., Fengwang, M. 2012. Interactive effects of water and nitrogen supply on growth, biomass partitioning, and water-use efficiency of young apple trees. *African J. Agric. Res.* 7, 6, 978-985.
- Clavel, D., Drame, N.K., Roy-Macauley, H., Braconnier, S., Laffray, D. 2005. Analysis of early responses to drought associated with field drought adaptation in four Sahelian groundnut (*Arachis hypogaea* L.) cultivars. *Environ. Exp. Bot.* 54,, 219–230.
- FAO. 2008. FAO Global information and early warning system on food and agriculture. Special alert, Near East and South Asia. No. 315.
- Foyer, C.H., Noctor, G. 2005. Redox homeostasis and antioxidant signaling: a metabolic interface between stress perception and physiological responses. *Plant Cell.* 17, 1866–1875.

- Garg, B.K., Kathju, S., Burman, U. 2001. Influence of water stress on water relations, photosynthetic parameters and nitrogen metabolism of moth bean genotypes. *Biol. Plant.* 44, 289–292.
- Karimzadeh, G., Omidbaigi, R. 2004. Growth and Seed Characteristics of isabgol (*Plantago ovata* Forsk) as Influenced by some Environmental Factors. *J. Agric. Sci. Technol.* 6, 103–110.
- Koocheki, A., Tabrizi, L., Nassiri Mahallati, M. 2007. The Effects of Irrigation Intervals and Manure on Quantitative and Qualitative Characteristics of *Plantago ovata* and *Plantago psyllium*. *Asian J. Plant Sci.* 6, 8, 1229–1234.
- Krause, G.H., Weis, E. 1991. Chlorophyll fluorescence and photosynthesis: The basics. *Annu. Rev. Plant Physiol. Plant mol. Biol.* 42, 313–349.
- Lichtenthaler, H.K., Wellburn, A.R. 1983. Determinations of total carotenoids and chlorophylls a and b of leaf extracts in different solvents. *Biochem. Soc. Trans.* 11, 591–592.
- Liu, F.L., Andersen, M.N., Jacobsen, S.E., Jensen, C.R. 2005. Stomatal control and water use efficiency of soybean (*Glycine max* L. Merr.) during progressive soil drying. *Environ. Exp. Bot.* 54, 33–40.
- Liu, F.L., Stützel, H. 2004. Biomass partitioning, specific leaf area, and water use efficiency of vegetable amaranth (*Amaranthus* spp.) in response to water stress. *Sci. Hortic.* 102, 15–27.
- Molinari, H.B.C., Marur, C.J., Daros, E., Campos, M.K.F., Carvalho, J.F.R.P., Filho, J.C.B., Pereira, L.F.P.P., Vieira, L.G.E. 2007. Evaluation of the stress-inducible production of proline in transgenic sugarcane (*Saccharum* spp): osmotic adjustment, chlorophyll fluorescence and oxidative stress. *Physiol. Plant.* 130, 2, 218–229.
- Monclus, R., Dreyer, E., Villar, M., Delmotte, F.M., Delay, D., Petit, J.M., Barbaroux, C., Thiec, D.L., Bréchet, C., Brignolas, F. 2006. Impact of drought on productivity and water use efficiency in 29 genotypes of *Populus deltoides*, *Populus nigra*. *New Phytol.* 169, 765–777.
- Mozzafari, F.S., Ghorbanli, M., Babai, A., Faramarzi, M. 2000. The effect of water stress on the seed oil of *Nigella sativa*. *J. Essential Oil Res.* 12, 36–38.
- Palatova, E., 2002. Effect of increased nitrogen depositions and drought stress on the development of Scots pine (*Pinus sylvestris* L.)– II. Root system response. *J. Forest. Sci.* 48, 6, 237–247
- Poorter, H., Nagel, O. 1999. The role of biomass allocation in the growth response of plants to different levels of light, CO₂, nutrients and water: a quantitative review. *Aust. J. Plant Physiol.* 27, 1191–1191.
- Puri, S., Swamy, S.L. 2001. Growth and biomass production in *Azadirachta indica* seedling in response to nutrients (N and P) and moisture stress. *Agroforest. Syst.* 51, 57–68.
- Rahimi, A., Madah Hoseini, S., Sajjadinia, A.R., Roosta, H.R., Fateh, E. 2011. Water use and water-use efficiency of isabgol (*Plantago ovata*) and French psyllium (*Plantago psyllium*) in different irrigation regimes. *Aust. J. Crop. Sci.* 5, 1, 71–77.
- Ranjbarfordoei, A., Samson, R., Van Damme, P. 2006. Chlorophyll fluorescence performance of sweet almond (*Prunus dulcis* (Miller) D. Webb) in response to salinity stress induced by NaCl. *Photosynthetica.* 44, 4, 513–522.
- SAS Institute 2001. SAS System, 8th ed. SAS Inst., Cary, NC.
- Sadasivam, S., Manickam, A. 1992. Biochemical Methods. New Age International (P) Limited Publisher.

- Saneoka, H., Moghaieb, R.E.A., Premachandra, G.S., Fujita, K. 2004. Nitrogen nutrition and water stress effects on cell membrane stability and leaf water relations in *Agrostis palustris* Huds. Environ. Exp. Bot. 52, 131–138.
- Sharma, R. 2004. Agro-Techniques of medicinal plants. Daya Publishing House.
- Sharma, P.K., Koul, A., 1986. Mucilage in seeds of *Plantago ovata* and its wild allies. J. Ethnopharmacol. 17, 289–295.
- Shaw, B., Thomas, T.H., Cooke, D.T. 2002. Responses of sugar beet (*Beta vulgaris* L.) to drought and nutrient deficiency stress. Plant. Growth. Regul. 37, 77–83.
- Song, C.J., Ma, K.M., Qu, L.Y., Liu, Y., Xu, X.L., Fu, B.J., Zhong, J.F. 2010. Interactive effects of water, nitrogen and phosphorus on the growth, biomass partitioning and water-use efficiency of *Bauhinia faberi* seedlings. J. Arid Environ. 74, 1003–1012.
- Tolk, J.A., Howell, T.A. 2003. Water use efficiencies of grain sorghum in three USA southern Great Plains soils. Agric. Water Manage. 59, 97–111.
- Van den Berg, A.K., Perkins, T.D. 2004. Evaluation of portable chlorophyll meter to estimate chlorophyll and nitrogen contents in sugar maple (*Acer saccharum* Marsh.) leaves. Forest. Ecol. Manag. 200, 113–117.
- Yemm, E.W., Willis, A.J. 1954. The estimation of carbohydrates in plant extracts by anthrone. Biochem. J. 57, 508–514.
- Zargari, A. 1990. Medicinal plants. vol.4, University of Tehran Pub., Tehran, Iran.