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Effects of Nitrogen Application on Growth of Irrigated Chickpea (*Cicer arietinum* L.) under Drought Stress in Hydroponics Condition

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Abstract: To study the effects of N application on growth and biomass of a local variety (cv. Kabouli) of chickpea under water deficit, a study was carried out hydroponically in growth chamber using three concentrations of N (0.25, 0.5 and 1 Mm) and four levels of drought stress (0, -0.3, -0.6 and -0.9 MPa) in three replications in the form of a completely random block design in 2007, Ardebil, under the Iran conditions. Water deficit stress were evaluated for leaf water content, leaf water potential, membrane stability index, chlorophyll content, leaf area, root area, root/shoot ratio, nodule water content, nodule number and biomass. According to observed data, N application was increased the leaf water content, membrane stability, chlorophyll, leaf water potential, leaf area, nodule water content, nodule number and biomass. The experiment showed that N fertilizer application (with a concentration of 1 Mm) can increase leaf and nodule Relative Water Content (RWC), leaf water potential, membrane stability index, leaf chlorophyll content, leaf area and biomass under water deficit condition. Therefore, it seems that mineral nitrogen application can mitigate the adverse effects of water deficit stress and improve growth and biomass in chickpea. Consequently, nitrogen application after moisture stress decrease negative effects drought.

Key words: Drought stress, irrigate chickpea, RWC, chlorophyll, leaf area and biomass

INTRODUCTION

Chickpea (*Cicer arietinum* L.) is one of the most important legumes in the world which is cultured in both irrigated and dry farming systems. Drought stress is the most important and common environmental issue which limits agricultural production and decreases the efficiency of dry lands (Soltani *et al*., 2001). Water deficit affects the metabolism, physiology and morphology of crops. Leaves are the most effective weapons in the crop economy and eventually their yield under the drought stress. The water deficit stress conditions decrease yield of the chickpea and shortens the vegetative period and reduces the produced biomass. Early stress affects the biomass and yield more strongly than late stress (Leport *et al*., 2006). This, in turn, causes the conversion of oxygen by excess electron produced through photosynthetic reactions in chloroplast to toxic forms like hydrogen super oxide radicals. It has been found that these oxygen derivatives injure the cells and disrupt the growth under stress (Cakmak, 2005). Leaf chlorophyll content is an important factor in determining the photosynthesis rate and Dry Matter (DM) production (Ghosh *et al*., 2004). Water deficit stress significantly decreased the amount of chlorophyll a. They suggested that the decrease in chlorophyll concentration under stress was due to the effects of chlorophylls, peroxides and phenol compounds

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and thus the decomposition of chlorophyll. Overall, drought stress decreases crop chlorophyll content (Chandrasekar *et al*., 2000). Ghosh *et al*. (2004) reported an increase in chlorophyll content under stress due to the application of fertilizer. Drought stress decreases the RWC, too (Chandrasekar *et al*., 2000). Costa-Franca *et al*. (2000) indicated that the RWC of beans (*Vicia faba* L.) leaf decreased due to drought. Drought stress has a significant effect on the decline of leaf RWC and membrane rigidity (Sairam and Srivastava, 2001). In an experiment on chickpea and beans, it was revealed that water deficit stunted the growth of both crops, which in turn affected their yields and decreased leaf and root water potential (Grzesiak *et al*., 1997). In another experiment, Antolin *et al*. (1995) observed that the water potential of crops fed with nitrate was significantly different from those relied on nitrogen fixation and it seemed that nitrate application increased the concentration of metabolites which are effective in maintaining leaf water potential. Drought stress in chickpea leads to the increase in the length of roots which penetrate into the soil. Chickpea has the ability of matching its root distribution with weather condition and can occupy larger surface area per root weight (Benjamin and Nielsen, 2006). Under the water deficit the ratio of root area to its weight increases and hence, makes the chickpea to have thinner root system compared to stress-free condition. Under water deficit, roots occupy larger surface area per root weight (Benjamin and Nielsen, 2006). Nitrogen is an important element needed by crops, since it is one of the constituents of nucleic and amino acids, proteins, peptides, chlorophyll and alkaloids (Mengel, 1992) and nitrogen application increases shoot growth and decreases of R/S ratio (Shangguan *et al*., 2004). Two important sources of nitrogen for legumes are fixed nitrogen and mineral one. Nitrogen is fixed by root nodules which supplies an important part of required nitrogen. When crops commence the growth without accessing mineral nitrogen, inoculation by rhizomes and establishment of nitrogen fixing systems decelerates the growth (Silsbury *et al*., 1986). Many researchers have shown that legumes which use nitrate or ammonium have better performance in leaf area and number, shoot and root nitrogen content and nodule number and growth than crops which depend on fixed N_2 (Sprent and Thomas, 1984; Silsbury *et al*., 1986). Athar and Johnson (1996) showed that the decrease in soil water potential from -0.3 to 1 MPa led to the decrease in plant total dry weight by 65% and their highest alfalfa (*Medicago sativa* L.) yield compared to control treatment (no-fertilizer) in all drought stress levels was obtained in 15 kg N ha^{-1} treatment. In another experiment, it was shown that plants fed by nitrate had higher RWC than N-fixing plants at the same water potential (Antolin *et al*., 1995).

The aim this study is the evaluation of effects nitrogen application on better growth of chickpea (cv. Kabouli) and improvement of its resistance in water deficit in hydroponics condition.

MATERIALS AND METHODS

The study was carried out on a local variety of chickpea (*Cicer arietinum* L.) (cv. Kabouli) under hydroponics condition in growth chamber with day/night temperature of 25/18°C, lightness/darkness duration of 16.8 h and a humidity of 40%. This 3×4 factorial design (a factorial experiment (using RCB design)) was a design with three replications in which humidity stress was in four levels including 0, -0.3, -0.6 and -0.9 and nitrate was in three levels including 0.25, 0.5 and 0.75 Mm, Ardebil, Iran with longitude of 48° 15' and latitude of 38° 15' in 2007. Ardabil region has very cold winters, rainy spring, dry and warm summers and with mean precipitation of 400 mm yearly. The soil bed was sterilized and composed of prelates and washed sand. Before planting, seeds were sterilized by alcohol, washed five to six times and then smeared with inoculums *Rhizochickpea* which included *Mesorhizobium ciceri* (Karimi, 2001). Nitrogen-free, modified Hoagland solution (pH = 6.5) was used for feeding the crops. After crop establishment, polyethylenglycohol (PEG) 6000 was used for exerting water stress on them. To determine RWC, the following formula was used (Rosales-Sernaa *et al*., 2004; Clavel *et al*., 2006):

$$RWC = \left[\frac{(E_W - D_W)}{(T_W - D_W)}\right] \times 100$$

The RWC was measured before noon about one week before harvest. Besides, some of the full-developed leaves were cut from the end of stem (third leaf from the end) and weighed with 0.001-precision scale to obtain leaf fresh weight (F_W). Then, they were floated in twice distilled water under dark condition in laboratory temperature (26±1.5°C) for 4 h. Afterwards, their turgidity weight (T_W) was measured. To measure their DW, they were put in electrical drier at a temperature of 85°C for 24 h. PEG 6000 was used for determining the membrane stability (Saneoka *et al*., 2004). In this method, some young and full-developed leaves are sampled. Samples were taken from each plot as control and the same numbers of leaves were taken as treatment. Then, they were washed in twice distilled water three times to wash their surface electrolytes. In the case of control, 20 mg of distilled water and in the case of treatment, 20 mg of PEG 6000 was into each test tube and the samples were floated in them at a temperature of 4°C for 24 h. After reaching the temperature of samples to the surrounding temperature, their electrical conductance was measure by EC-meter (in terms Mm cm^{-1}) (Saneoka *et al*., 2004). Then, they were autoclaved under the temperature of 115°C for 25 min and after cooling, their electrical conductance was measured again according to Saneoka *et al*. (2004). Afterwards, the injuries to membrane and its stability percentage were assessed by the following formula:

$$\text{Membrane injury (\%)} = \frac{1-\frac{T_1}{T_2}}{1-\frac{C_1}{C_2}} \times 100$$

$$MSI = 1 - MIP$$

Leaf water potential was determined by model pressure chamber (ELE, England). In this measurement, full-developed leaves from the end of stem were used. Leaf chlorophyll difference was the difference between leaf chlorophyll before water stress and that after stress. Leaf chlorophyll was measured by model source chlorophyll meter (Minolta-SPAD). Leaf area and root area were measured after harvest by (SCANMAN) model C1202 made in the US. To measure RWC of nodules, immediately after cutting the roots and washing them by distilled water, 10 nodules were cut from each plant, quickly dried by absorbent paper and weighed by 0.001-precision scale (to gain fresh weight) (Rosales-Sernaa *et al*., 2004). Then they were put in distilled water for 4 h in darkness and temperature of 5°C and afterwards they were weighed again (to gain turgidity weight). To measure the DW of nodules, they were put in oven with a temperature of 75°C. RWC of leaves was measured by the following formula in terms of percent (Rosales-Sernaa *et al*., 2004):

$$RWC = \left[\frac{(F_W - D_W)}{(T_W - D_W)}\right] \times 100$$

To measure plant DW, all organs of plants were separated after full maturity and were weighed after putting in oven with a temperature of 80°C (Rosales-Sernaa *et al*., 2004). Data was subjected to a standard analysis of variance using SAS statistical software. Least significant differences (Duncan) tests were done at the ($p<0.05$) confidence level using Microsoft® Excel software.

RESULTS AND DISCUSSION

Nitrogen fertilizer application had a significant effect ($p>0.01$) on leaf water potential and it was found that there was a significant difference among different levels of N applications (Table 2).

Table 1: Effect of nitrogen application on leaf water potential, relative water content leaf and nodule, leaf area, root area, nodule number, of chickpea affected from water stress

Factor		Ψ_W (MPa)	LA (cm^2 plant^{-1})	RWC (%) (leaf)	Nodule No.	RA (cm^2 plant^{-1})	RWC (%) (nodule)
Water stress ratio	-0.90	-1.61a	99.83d	73.82d	11.22d	206.74a	83.32d
	-0.60	-1.46b	112.65c	78.07c	15.11c	130.08b	84.56c
	-0.30	-1.21c	133.60b	85.06b	21.11b	107.60c	89.45b
	0.00	-0.96d	163.41a	94.85a	34.11a	97.37d	97.11a
Nitrogen (Mm)	0.25	-1.36a	121.08c	80.93c	17.66c	141.62a	87.58c
	0.50	-1.31b	127.37b	82.46b	20.16b	135.85b	88.66b
	1.00	-1.25c	133.67a	85.46a	23.33a	128.87c	89.59a

Means with the same letter in a column (in each factor) are not statistically different at p = 0.05

Table 2: Interaction effects of plant density and nitrogen level on measured traits

Interactions effects	Ψ_W (MPa)	LA (cm^2 plant^{-1})	RWC (%) (leaf)	Nodule No.	RA (cm^2 plant^{-1})	RWC (%) (nodule)
0.9 MPa×0.25 Mmol N	-1.99a	95.58k	70.06h	9.66h	213.00a	82.71j
0.9 MPa×0.5 Mmol N	-1.60ab	100.53j	72.40g	11.00h	207.80b	83.34ij
0.9 MPa×1.00 Mmol N	-1.56b	103.39j	79.00e	13.00g	199.43c	83.89hi
0.6 MPa×0.25 Mmol N	-1.52c	106.76i	76.00f	14.00g	139.23d	82.84h
0.6 MPa×0.5 Mmol N	-1.47d	112.43h	78.13e	14.66g	132.12e	84.52gh
0.6 MPa×1.00 Mmol N	-1.39e	118.77g	80.10e	16.66f	118.91f	85.05g
0.3 MPa×0.25 Mmol N	-1.29f	124.41f	83.66d	18.00f	113.86g	87.17f
0.3 MPa×0.5 Mmol N	-1.22g	133.29e	85.30cd	21.00e	106.70h	89.72e
0.3 MPa×1.00 Mmol N	-1.13h	143.11d	86.23c	24.33d	102.23i	91.48d
Control×0.25 Mmol N	-0.98i	157.57c	94.00b	29.00c	100.40i	96.34c
Control×0.5 Mmol N	-0.96j	163.24b	94.03b	34.00b	96.80j	97.04b
Control×1.00 Mmol N	-0.94k	169.42a	96.53a	39.33a	94.93j	97.96a

Numbers with the same letters in each column, have no significant differences to each other

The highest leaf water potential (-0.946 MPa) was obtained under no-stress and 1 Mm N application. By applying 0.25 Mm N fertilizer and exerting stress, the lowest leaf water potential was obtained (-1.66 MPa). It was observed that with the rise of drought stress from -0.3 to -0.6 and -0.9 MPa, leaf water potential was considerably decreased (Table 1). In addition, Goicoechea ***et al***. (1997) showed that N application under water deficit increased adaptation metabolites and leaf water potential.

Leaf area was affected by water deficit stress, too (Table 1). Severe water deficit stress led to the decrease in leaf growth, area and number. N application mitigated the adverse effects of drought stress on plants. There was a significant interaction ($p>0.01$) between water deficit and mineral nitrogen, so that the highest leaf area (169.42 cm^2 plant^{-1}) was obtained under no-stress condition and 1 Mm N application. The lowest leaf area (94.58 cm^2 plant^{-1}) was obtained under -0.9 MPa water stress and 0.25 Mm N application (Table 2). The study indicated that leaf area expansion is highly sensitive to moisture deficit. In a study on pea and broad bean, it was found that leaf area of both crops (cultivars of field bean and field pea) considerably decreased under moisture stress (Grzesiak ***et al***., 1997). In addition, Pagter ***et al***. (2005) have reported that the development of water deficit stress had remarkably decreased leaf area. Present results showed that N application under drought condition increased leaf area which matches with the results of other experiments (Lindmann and Glovir, 2003).

Leaf water content was significantly decreased by the increase in water deficit. 1 Mm N application increased leaf area by 4.53% compared to applying 0.25 Mm N (Table 1). There was a significant interaction ($p>0.05$) between water deficit and N application levels. The highest leaf water content (96.53%) was obtained under control (no-stress) treatment and 1 Mm N application (Table 2). In contrast, the lowest leaf water content (70.06%) was obtained under -0.9 MPa stress and 0.25 Mm N application. Sairam and Srivasta (2001) reported that water deficit stress significantly decreased leaf water content which has been confirmed by other studies (Chandrasekar ***et al***., 2000; Rosales-Sernaa ***et al***., 2004). N application under drought stress exhibited the highest leaf water content in crops depended on N fixation (Antolin ***et al***., 1995).

Nodule water content was affected by water deficit, too (Table 1). The decrease in nodule water content under water deficit stress was not as much as that in leaf water content. There was a significant interaction ($p>0.01$) between water deficit and mineral nitrogen. Like the variations of leaf water content, the highest nodule water content (97.96) was obtained under optimum moisture condition and application of 1 Mm N (Table 2). Also there was a significant interaction ($p>0.01$) between water deficit and mineral nitrogen. The highest nodule number (39.33) was obtained in no-stress treatment and application of 1 Mm N. The lowest relative nodule water content and nodule number (82.71 and 9.66, respectively) were obtained under -0.9 MPa stress and application of 0.25 Mm N (Table 2). Drought significantly decreases nodule number and relative NWC (Guerin *et al*., 1990; Athar and Johnson, 1996). Water deficit affects N application which in turn affects nodule activities (Sprent and Thomas, 1984). The nodulating and nodular activity is in the highest level when seeds procure their required N either from N fertilizer supplies of soil or from application of N fertilizer in the first weeks of establishment (Marschner, 1995).

The results showed that drought stress affected root area so that the increase in soil moisture from -0.9 MPa to optimum level led to the decrease in root area (Table 1). The increase in N application from 0.25-1 Mm led to the decrease in root area, too (Table 2). There was a significant difference ($p>0.01$) among root area, different levels of N application and water deficit. The greatest root area (213 cm^2 $plant^{-1}$) was obtained under water stress of -0.9 MPa and application of 0.25 Mm N and the smallest one (94.93 cm^2 $plant^{-1}$) was obtained under optimum irrigation and application of 0.25 Mm N. Benjamin and Nielsen (2006) in experiments on chickpea showed that water deficit led to the increase in root area and penetration of a greater area of roots to the depths of soil. The result that root growth was decreased by application of N matches with the results of Shangguan *et al*. (2004).

According to these results and measurement of root and shoot DW under N deficit and availability of N in different levels, a significant difference ($p>0.01$) was observed in R/S ratio (Table 3). Shoot growth decreases under drought, but N application has a positive effect on increasing its growth. The highest R/S ratio (1.11) was obtained under severe stress (-0.9 MPa) and application of 0.25 Mm N. The lowest one (0.66) was obtained under optimum irrigation and application of 1 Mm N (Table 4). Shangguan *et al*. (2004) showed that root growth had a negative correlation with N application, N application increased shoot DW and water deficit decreased shoot growth. N has a significant effect on root and shoots growth as well (Silsbury *et al*., 1986).

Water deficit is one of the limiting factors of crop production (Sadras, 2002). Water deficit stress significantly decreases membrane stability (Table 3). Short periods of drought stress reduce carbon exchange rate and plant growth which reduces turgidity pressure (Brevedan and Egli, 2003). The results showed that there was a significant difference ($p>0.01$) between stress levels and N levels in terms of membrane stability. The highest membrane stability (97.4) was associated with application of 1 Mm N under optimum irrigation and the lowest one (71.56) was associated with application of 0.25 Mm N under over -0.9 MPa stress (Table 4). Water deficit stress decreases membrane stability (Chandrasekar *et al*., 2000; Sairam and Srivastava, 2001). In water stress conditions, nitrogen application increased growth and membrane stability (Antolin *et al*., 1995).

The difference between chlorophyll amount before and after stress was affected by water deficit stress. N application under drought condition had a significant effect ($p>0.01$) on leaf chlorophyll (Table 3) so that there were significant differences among different levels of N (Table 4). No significant difference was observed between 0.5 and 1 Mm N. Overall, water stress decreases plant chlorophyll content (Chanrasekar *et al*., 2004). Also, Ghosh *et al*. (2004) reported that chemical fertilizer application increased plant chlorophyll content.

Chickpea growth is deeply affected by water deficit stress, so that stress severity decreases biomass (Table 3). The results showed a significant difference ($p>0.01$) between biomass under different levels of N application and water stress. The highest biomass (1.113 g $plant^{-1}$) was obtained

Table 3: Effect of nitrogen application on root/shoot, membrane stability, chlorophyll and biomass of chickpea affected from water stress

Factor		R/S	MSI (%)	Chlorophyll*	Biomass (g plant^{-1})
Water stress ratio	-0.90	1.04a	73.15d	19.90a	0.78d
	-0.60	0.91b	76.34c	11.45b	0.89c
	-0.30	0.80c	83.92b	1.40c	0.95b
	0.00	0.68d	95.94a	-0.84d	1.08a
Nitrogen (Mm)	0.25	0.90a	80.63c	6.52b	0.90c
	0.50	0.85b	82.27b	8.95a	0.93b
	1.00	0.83c	84.11a	8.45a	0.95a

Means with the same letter in a column (in each factor) are not statistically different at p = 0.05. *The difference between chlorophyll amount before and after stress

Table 4: Interaction effects of plant density and nitrogen level on measured traits

Interactions effects	R/S	MSI (%)	Chlorophyll*	Biomass (g plant^{-1})
0.9 MPa×0.25 Mmol N	1.11a	71.56k	16.53b	0.76l
0.9 MPa×0.5 Mmol N	1.02b	73.16j	21.23a	0.78k
0.9 MPa×1.00 Mmol N	0.98c	74.73i	21.93a	0.81j
0.6 MPa×0.25 Mmol N	0.95d	75.30i	8.76d	0.88i
0.6 MPa×0.5 Mmol N	0.90e	76.20h	13.83c	0.89h
0.6 MPa×1.00 Mmol N	0.88f	77.53g	11.76c	0.91g
0.3 MPa×0.25 Mmol N	0.83g	81.23f	1.9e	0.92f
0.3 MPa×0.5 Mmol N	0.80h	83.73e	1.53ef	0.95e
0.3 MPa×1.00 Mmol N	0.78h	86.80d	0.76efg	0.98d
Control×0.25 Mmol N	0.70i	94.43c	-1.10g	1.05c
Control×0.5 Mmol N	0.68j	96.00b	-0.80fg	1.09b
Control×1.00 Mmol N	0.66k	97.40a	-0.63fg	1.11a

Numbers with the same letters in each column, have no significant differences to each other. *The difference between chlorophyll amount before and after stress

under application of 1 Mm N without exerting stress and the lowest one (0.761 g plant^{-1}) was obtained under severe stress (-0.9 MPa) and application of 0.25 Mm N (Table 4). The adverse effect of water deficit stress on biomass and yield is stronger when the stress occurs at the final stages of growth than when it occurs at the first stages (Leport *et al*., 2006). Athar and Johanson (1996), also reported that the decrease in water potential from -0.3 to -1 MPa led to a decrease in biomass by 65%. In a greenhouse experiment on chickpea, it was shown that water deficit reduces grain growth and number (Behboudian *et al*., 2001). Water stress reduces N_2 fixation and stunts the growth. Mineral nitrogen application improves growth and increases leaf area, leaf number and shoot and root N content (Silsbury *et al*., 1986). Plants fed by nitrogen have greater transpiration efficiency and thus have optimum growth under water stress (Hubick, 1990).

CONCLUSION

Water deficit stress affects plant growth and physiological activities. The decrease in N fixation under stress decreases growth and yield and disrupts key processes of plant. Legumes themselves fix nitrogen, but severe water deficit adversely affects N fixation. Under this condition, mineral nitrogen application can mitigate adverse effects of stress on plants. In addition, nitrogen increases the adaptability of metabolites like proline, soluble sugar, amino acids, nucleic acids, etc. The increase in metabolite concentrations, in turn, improves the plant resistance to drought. The results showed that the increase in application of mineral nitrogen from 0.25 Mm (as starter) to 0.5 and 1 Mm increased RWC by 4.53%, leaf area by 12.56%, plant water potential by 0.11%, membrane stability by 3.48%, nodule RWC by 2.01%, chlorophyll by 1.96% and biomass by 0.05%. The increase in nitrogen availability decreased the R/S ratio because nitrogen availability increases shoot growth. Obtained results also suggested that application of mineral nitrogen under water deficit increases plant resistance to stress.

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