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The Effect of Cytokinin and Iron on Biological Nitrogen Fixation in Chickpea Cultivars under Dryland Conditions

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In order to evaluate the effect of cytokinin and iron on biological nitrogen fixation in chickpea cultivars, experiment was conducted as a factorial experiment in randomized complete block design in four replications. The treatments consisted of three cultivars: (Hashem, Azad and local mass),four levels of iron [control (lack of consumption), 5, 10 and 15 ppm] and four levels of cytokinin (6-Benzyl aminopurin) [control (lack of consumption), 5, 10 and 15 ppm]. Local mass with 3.69, 6.1 and 3.78 percent, had the highest nitrogen percent in the above ground, noude and grain. By increasing concentrations of iron, increased nitrogen percent in aboveground and grain, but in noude increased up to 10 ppm and then decreased. However, by increasing concentrations of cytokinin , the nitrogen percent of grain increased, but nitrogen percent of aboveground and noude increased up to 10 ppm and then decreased. Local mass with 129.8 kg/ha had the highest nitrogen fixation. Effects of iron and cytokinin on nitrogen fixation was positive, and by increase their concentration, nitrogen fixation increased and in concentration of 15 ppm content of fixed nitrogen were 130.9 and 129.8kg/ha respectively. From of all fixed nitrogen in plant, Contribution of aboveground, grain and noude were 46.19, 46.41, and 7.4 percent.

Keywords: Chickpea, biological nitrogen fixation, cytokinin, iron, aboveground, noude and grain.

Nitrogen is the main nutrient in chemical compounds, metabolism and protein synthesis[18,25]. Although the plant nitrogen is about 1 to 2 and the maximum 4 to 6 percent, however, nitrogen is the limiting factor for crop production in the world[25]. Biological nitrogen fixation in legumes can be done effectively, therefore, plant can grow by consuming less of nitrogen fertilizer[13]. The importance of biological nitrogen fixation are to replace the nitrogen lost by culture, leaching, volatilization, runoff, erosion and denitrification[9].Chickpea is an annual plant that by providing the environmental conditions, through symbiosis with Rhizobium bacteria causing nitrogen fixation and don't need to nitrogen fertilizer and in desired rotations, will provide the part of required nitrogen for next crop[10]. The amount of nitrogen fixation in chickpea are different from zero to 248 kg/ha in the growing season[17,25]. Islam *et al.*, [15] estimated the amount of nitrogen fixation 52 to 55 percent from of all nitrogen plant. Evers *et al.*,[9] in a study on clover cultivars, estimated up to 296 kg/ha nitrogen fixation.

Cytokinins, generally adenine derivatives, are plant hormones that promote growth and cell division and essential for plant growth and development[4,11] and reducing their amount in the root causes limiting of protein synthesis and reducing of legheamoglobin,

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restriction of node respiration, change in node metabolism or effect on nitrogenase enzyme activity and finally reduced nitrogen fixation[12]. Cytokinins play an important role in forming of plant mutualism with bacteria and mycorhiza through symbiont. The hormone to stimulating of symbiosis, increase activity of nodulation genes and facilitate transfer of nutrients between plant and symbiotic[24].

Iron is a key factor to activity of many enzymes and often is very important for catabolism activity. The low solubility of iron in water, limites biochemical processes such as photosynthesis and nitrogen fixation[22].

genotype like environment and growth period are important factors that affect on plant growth[16,18].Species and genotypes differ in susceptibility to iron deficiency and traits that affects by genotypes ability on the use of iron, are dry matter and grain yield[28]. Mansour *et al.*,[1] showed that there was significant difference between genotypes of chickpea in nitrogen uptake.

MATERIALS AND METHODS

This study was performed at Grit Agricultural Services Center (48° 18' long. E; 33° 32' lat. N, and 1175 m above sea level) located in 35 kilometers Eastern-North of Khorramabad of lorestan province in 2011 and 2012 .According to meteorological long term, mean annual precipitation is 425 mm.

Experiment was conducted as a factorial experiment in randomized complete block design in four replications. The treatments consisted of three cultivars: (Hashem, Azad and and local mass),four levels of iron [control (lack of consumption), 5, 10 and 15 ppm] and four levels of cytokinin (6-Benzyl aminopurin) [control (lack of consumption), 5, 10 and 15 ppm]. Each plot consisted of five rows with 6 m length that space between rows and plants space on rows were considered 25 and 15 cm, respectively. Planting was done according to current planting date in area and atmospheric conditions in March 23, 2011 and March 13 , 2012.

Before planting, soil sample prepared from the 0-30 cm depth and were measured the physical and chemical properties of soil (Table 1). In this study, neither macro and nor micro fertilizers were applied. Iron($FeSo_4.7H_2O$) was sprayed in 3-4 leaf stage(45 DAP[1]) and cytokinin(6-Benzil aminopurine) sprayed 10 days later.

Traits and their measurements

At 50% flowering stage, from each plot, were eliminated two lateral rows and a half meters from start of middle rows as border effects. from three middle rows, selected three plants with 30 cm^3 of soil their around and after washing the soil around the roots, the nodules on the roots were collected, then, were dried for 72 hours in air and weighed with a digital scale with an accuracy of 0.001. At this stage, from each plot, cut six plants and was prepared composite sample. as well as, in harvest stage, 20 seeds selected randomly from each plot. in laboratory were measured nitrogen percent of nodes, aboveground and grain by Kjeldahl method.

Nitrogen fixation

Based on nitrogen percent of aboveground, noude and grain, fixed Nitrogen in any part of plant and total nitrogen fixation in plant were calculated by use of following formula:

$$NF_g = DW_g \times \%N_g$$
$$NF_n = DW_n \times \%N_S$$
$$NF_a = DW_a \times \%N_n$$
$$TNF = NF_g + NF_S + NF_n$$

that:

NF_g: Nitrogen fixation in grain(kg/ha)

NF_n:Nitrogen fixation in nodule (kg/ha)

NF_a: Nitrogen fixation in above ground plant(kg/ha)

TNF Total nitrogen fixation in plant(kg/ha)

DW:Dry weight of plants(kg/ha)

N%: Nitrogen percen.

Statistical analysis

Data were combined analyzed using MSTAT-C software and graphs were drawn using EXCEL2010. Means were compared using Duncan's Multiple Range Test.

RESULTS AND DISCUSSION

Dry weight of aboveground, grain and node

The results showed that local mass with 8.164, 6.15 and 7.156 gr/m^2 had the highest dry weight of aboveground, grain and node, respectively. (Table 2). This result were confirmed by James *et al.*,[15], Seyfi[28], Biabani and Carpenter[5] and Khan *et al.*,[17].

Genotype like environment and growth period are important factors that affect plant growth[16,18]. James *et al.*,[16] showed that dry weight of aboveground was significantly affected by cultivar. In a study by Khazaei *et al.*,[19], genotypes in respect of noude number, noude weight and dry weight of aboveground were significantly different, Mcc480 genotype had more number of nodes with higher specific weight to Mcc876 genotype.

With increasing iron concentration up to 10 ppm, dry weight of aboveground and nodes increased then decreased, while with increasing concentration of iron, grain dry weight had increased (Table 3).

Species and genotypes differ in susceptibility to iron deficiency and traits that affects by genotypes ability on the use of iron, are dry matter and grain yield[28]. In study by Khan *et al.*,[17], response of chickpea cultivars to iron use was positive and obtained highest dry weight of shoot and grain by spray of 2 kg/ha ferrous sulfate. Saifi[28] to study of iron on grain yield of chickpea cultivars, reported that iron were effective on grain

Table 1. Physical and chemical properties of soil test site

Feature year	%N	P(ppm)	K(ppm)	Fe(ppm)	Mn(ppm)	Zn(ppm)	S(ppm)	%humus	Texture	pH
2010-2011	0.085	14.8	342.2	8.88	8.68	1.58	80.62	1.1	Silty loam	7.6
2011-2012	0.069	11.4	367.3	7.42	6.65	0.95	92.45	0.9	Silty loam	7.3

Table 2. The effect of cultivar on dry weight, nitrogen percent and fixed nitrogen in aboveground, grain and node

Cultivar	Dry weight (g / m²)			Nitrogen percent			Ffixed nitrogen (kg / ha)			Total fixed nitrogen
	aboveground	grain	node	aboveground	grain	node	aboveground	grain	node	(kg/ha)
Hashem	161.5b	152.1b	12.6b	3.63c	3.69b	6.06b	58.6b	56.2b	7.6b	122.4b
Azad	161.1b	152.0b	12.1c	3.65b	3.65b	6.05b	58.7b	55.6b	7.3c	121.7b
Local mass	164.8a	156.7a	15.6a	3.69a	3.78a	6.10a	60.8a	59.3a	9.5	129.8a

Table 3. The effect of iron on dry weight, nitrogen percent and fixed nitrogen in aboveground, grain and node

Iron	Dry weight (g / m²)			Nitrogen percent			Ffixed nitrogen (kg / ha)			Total fixed nitrogen
ppm	aboveground	grain	node	aboveground	grain	node	aboveground	grain	node	(kg/ha)
0	160.7c	139.1c	12.4d	3.64b	3.69b	5.91d	58.5b	51.3c	7.4d	117.2d
5	161.2b	153.4b	13.5c	3.64b	3.7b	6.08c	58.7b	56.7b	8.2c	123.6c
10	164.3a	155.8b	14.1a	3.67a	3.7b	6.18a	60.3a	57.6b	8.7a	126.6b
15	164.4a	166.2a	13.7b	3.67a	3.76a	6.15b	59.9a	62.5a	8.4b	130.8a

Table 4. The effect of cytokinin on dry weight, nitrogen percent and fixed nitrogen in aboveground, grain and node

Cytokinin	Dry weight (g / m²)			Nitrogen percent			Ffixed nitrogen (kg / ha)			Total fixed nitrogen
ppm	aboveground	grain	node	aboveground	grain	node	aboveground	grain	node	(kg/ha)
0	160.1d	140.6d	12.7c	3.56c	3.61c	5.88d	56.9c	50.7d	7.5d	115.1d
5	161.7c	149.6c	13.4b	3.64b	3.69b	6.00c	58.8b	55.2c	8.0c	122.0c
10	163.2b	159.4b	13.8a	3.71a	3.74a	6.24a	60.5a	59.7b	8.6a	128.8b
15	164.9a	164.8a	13.8a	3.70a	3.79a	6.19b	61.1a	62.5a	8.5b	132.1a

yield and Arman cultivar in 15 kg/ha iron treatment had highest yield (692.56 kg / ha)

Grain weight is one of the important yield components that will be influenced by genetic characteristics such as grain potential, grains competition, grain filling period and environmental conditions[29]. Amini dehoghy and dadkhah[2] reported that iron caused significantly different in number and weight of nudes. Welch[34] stated that iron with participation in metabolism of hydrocarbon material and protein and their transfer, and effects on reproductive process, had significant role in increasing the number and weight of grains. Rai *et al.*,[26] reported that iron deficiency reduced number and weight of noudes in chickpea. With increasing concentrations of cytokinin , dry weight of aboveground, seed and node significantly increased and highest dry weight was obtained in 15 ppm (Table 4). Hormone use has a great impact in achieving high-yield. Cytokinins are plant hormones that as regulate growth and development has an important role in preventing the aging of organs and nutrient mobility[3]. Zulkarnyan *et al.*,[36] studied the effect of cytokinin on rice, the results showed that use of cytokinin increased the number of tillers, spikes and finally yield. Najel *et al.*,[23] examined the effects of cytokinin on soybean yield, results showed that consumption 5×10^{-6}µ increased aboveground dry weight, number and weight of seeds, In this study, the doses more than 5×10^{-6}µ decreased 30% number of grains and 39% grain weight compared with control, In this study, the reason of produce more grain, effect of cytokinin in reducing stimulate production of flowers and prevent of flower abortion were expressed. Fatima *et al.*,[10] examined effects of IAA, ABA and kinetin on growth, yield and yield components of chickpea in spray and seed soaking in solution of growth hormones, results indicated an increase in aboveground dry weight in both spray and seed soaking by Kinetin, and increasing grain weight by seed soaking in Kinetin, the maximum grain weight in kinetin were attributed to transfer of assimilates from vegetative parts to reproductive parts. Based on research conducted by Iqbal *et al.*,[14], cytokinin had a positive effect on seed size, this is due to the effect of cytokinin on the processes of accumulation of nutrients in the grain, continuation of grain filling period and increase transfer nutrients from roots to grains. kinetin as growth-stimulating hormone increases the activity of nitrogenase enzyme in chickpea root nodes[7], and by increasing the amount of legheamoglobin influences efficiency and viability of the nodes[33]. Fatima *et al.*,[10] examined effects of IAA,ABA and Kinetin and two strains of Rhizobium on growth, yield and nitrogen fixation in chickpea under natural conditions, the results showed that maximum weight of nodes obtained in seeds treated with kinetin and Tal 620 rhizobium race.

Nitrogen percent of aboveground, node and grain

Local mass had highest percent of nitrogen in abovegrond, noude and grain (Table2). N concentration in plant depends on factors such as amount of nitrogen in soil, plant type, plant organs and plant growth stage[20]. Mansour *et al.*,[21] showed that there was significant difference between genotypes of chickpea in terms of nitrogen uptake.

With increasing concentrations of iron and cytokinin up to 10 ppm, increased nitrogen percent of aboveground and node, while the highest percent of grain nitrogen obtained in 15 ppm (Tables 3 and 4). Singh *et al.*,[31] to study of effect of iron concentration on chickpea found that with increasing iron concentration, content and nitrogen uptake were significantly increased, in this study, iron caused more nitrogen absorption and transfer it between different parts of plant. the positive reaction of nitrogen percent to iron is due to its undeniable role in symbiotic bacteria and plant roots[2] and Iron involvement in the building of enzymes such as nitrogenase, nitrate and nitrite reductase and legheamoglobin[32]. Singh *et al.*,[30] found a positive correlation between nitrogen and cytokinin in the plant species.

Nitrogen fixation

The results showed that the effects of iron and cytokinin on the amount of nitrogen fixation in different parts of plant and total nitrogen fixation were significant. Local mass with 60.87 kg/ha had the hieghest content of nitrogen fixation, while Hashem and Azad with 58.71 and 58.59 kg/ha were analyzed in a group, respectively (Table 2). Nitrogen fixation by chickpea is determined by two factors:

1. Amount of nitrogen accumulation in plant during growth period
2. Amount of nitrogen absorbed by plants from of all fixed nitrogen[8].

Biabani *et al.*,[5] studied nitrogen fixation of 40 cultivars that were collected from Iran, Afghanistan, Iraq, Ethiopia, Mexico, India, Pakistan, Turkey and United States.they reproted significant differences between cultivars and Genotype 254549 from Iraq to 0/084 g/plant had the highest nitrogen fixation.

The effect of iron and cytokinin were significant on content of fixed nitrogen in aboveground, nodes and grain and total fixed nitrogen. except node, with increasing iron and cytokinin concentrations, increased amount of nitrogen fixation (Tables 3 and 4).Khan *et al.*,[17] reported that iron and cultivar were affected on nitrogen fixation and most fixed nitrogen obtained by karek cultivar (Kabuli type) that treated with 2 lit/ha iron. Calsikan *et al.*,[6] reported that iron deficiency had negative effect on nitrogenase enzyme activity and nitrogen fixation in soybean. Amini Dehoghi and Dadkhah[2] by investigation effects of iron and zinc fertilizers on nitrogen fixation found that in salinity condition, iron fertilizer moderated salinity effects and increased nitrogen fixation.

Fatima *et al.*,[10] to survey of effect of growth regulators on nitrogen fixation in chickpea found that kinetin caused an increase in total fixed nitrogen. kinetin as growth-stimulating, increased activity of nitrogenase enzyme in nodes[7]. Rao[27] reported irritant effect of kinetin on nitrogen fixation efficiency. Overall, local mass in concentrations of 15 ppm iron and cytokinin with 142.1 kg/ha had highest nitrogen fixation. Of all fixed nitrogen in plant, the share of aboveground, grain and node were 46.19, 46.41 and 7.4 percent, respectively.

CONCLUSION

Cultivars had different responses to nitrogen fixation in different parts of plant. It seems that local mass due to the long term adaptation to environmental conditions and possibility of better strains of Rhizobium bacteria in soil, had been able to fix a higher content of nitrogen. Cytokinin as growth-stimulating hormone, and iron as an essential component of legheamoglobin, increased nitrogenase enzyme activity in root nodes and caused more nitrogen fixation efficiency. It is recommended to increase fixed nitrogen, increases harvest index, till to increasing the grain yield, increases total amount of fixed nitrogen.

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