



Original Research Article

The effect of supplemental irrigation, nitrogen levels and inoculation with Rhizobium bacteria on seed quality of chickpea (*Cicer arietinum* L.) under rainfed conditions

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A B S T R A C T

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In order to study the effects of supplemental irrigation, different level of nitrogen fertilizer and inoculate with rhizobium bacteria on the yield of two varieties of chickpea, an experiment was performed in during 2009-2010 cropping season in Zanzan agricultural research station, Iran. The factors of experiment consisted irrigation (no irrigation (I1), irrigation at flowering (I2), irrigation at flowering and grain filling and full irrigation (I3)) and levels of nitrogen (without using of nitrogen fertilizer (N0), 75 kg/ha (N75), 150kg/ha (N150) and inoculation with rhizobium bacteria (N4) . The results of the analysis of variance showed that the effects of irrigation, nitrogen fertilizer levels and bacterial inoculation, were significant on number of pods per plant, number grains per plant, grain weight, grain yield, biological yield, harvest index at 1 % probability level. Also Results showed that the grain yield in full irrigation treatment and inoculated with rhizobium bacteria was significantly higher than the other treatments. Analysis of variance showed that the effects of irrigation and nitrogen were significant on absorption of nitrogen, phosphorus, potassium, iron, copper, manganese, and zinc. In all levels of irrigation, the lowest value of the nitrogen absorption to achieved in non nitrogen condition. In non irrigation condition and one irrigation at flowering was obtained maximum nitrogen uptake with 150 kg/ha N. By applying irrigation at flowering, grain filling and normal irrigation, highest N uptake was obtained with the use of 75 kg/ha N. In general, normal irrigation and use of nitrogen fertilizer and inoculation with bacteria, improves the value of seed element absorption.

Introduction

Currently, the population growth and low protein content of cereals, has attracted the attention of people to legumes

consumption as an important source of required protein of people (Batani 1983). The legumes because of presence the

nitrogen fixing bacteria in their roots, are effective on soil fertility and after harvest of these products, large amounts of nitrogen will be added to the soil (Majnoon hosseini 1993). Among legumes, chickpeas with cultivated area about 11 million ha and production of 650 kg/ha (kochaki et al., 2004), has more adaptations than the climatic conditions of the country, especially with the most dry farming areas of Iran compared to other legumes (Bagheri et al., 2007). In general, drought stress is the second a biotic stress, that has negative effects on chickpea grain function. That with applying of supplemental irrigation in stage that plant has maximum requirement water can be significantly increases the yield compared to in drought conditions and rainfed. On the other hand considering with nitrogen is a key element for the plant growth, nitrogen fixation by legumes including chickpea, could obviate plant requirement to this element and it replaces by chemical fertilizers. Such replacement in terms of economically and environmentally has been welcomed in sustainable agricultural programs. Pawar et al (1992) in investigate the irrigation effect at critical stages growth of chickpea reported that highest grain yield be obtained with three irrigations at branching, flowering and pods stages. Sing (1995) expressed that because the lack of water, vegetative and reproductive growth of chickpea accelerated and in result decreases the duration of these stages. Bagheri et al (2007), introduced the initial growth stage of pea pods as the most sensitive to drought stress. Sksina (1980) about response of Chickpea to supplemental irrigation in different regions expressed that supplemental irrigation has serious effects on increasing yield. Providing the humidity is one of important factors of nitrogen accumulation in grain and nitrate

of soil is one of the most limiting factors of legume rhizobium symbiosis. In experiments Sksyna (1984), Ghasem-Zadeh et al (2003) and Majnoon Hosseini (2003) rhizobium inoculation, significantly increased yield and nitrogen content in grain of chickpea. Unfortunately, in Iran farmers use excessive of nitrogen fertilizers regardless of good potential of fields for nitrogen fixation and because of the very low prices of nitrogen fertilizers. For this purpose can have opposed with water scarcity crisis and environmental pollution (by reducing the use of chemical fertilizers) by useful programs and also products propose with suitable quality to agricultural. Thus, the present study were carried out to enhance the yield and optimize the use of nitrogen fertilizers in the area. The aim of this study was evaluate to absorb of nitrogen, phosphorus, potassium, iron, manganese, zinc and copper in pea seed after applied treatments.

Materials and Methods

The present experiment was carried out at the Agricultural Research Station of Zanzan province, Iran in during 2009-2010 cropping season. Properties of the soil in studied region are shown in Table 1 and 2. According to the results obtained of soil analysis, 100 kg /ha triple super phosphate was added to the experiment field .The experiment was done as split plot base on factorial in randomized complete blocks design with three replications. The treatments were consisted of different levels of irrigation (no irrigation (I1), irrigation at flowering (I2), irrigation at flowering and grain filling and full irrigation (I4) and different levels of nitrogen fertilizer (no fertilizer N (N0), 75 kg/ha (N75) 150 kg/ha (N150) and

inoculation with rhizobium bacteria. All seeds were disinfected using pesticide Benomyl except seeds were coated with bacteria. For treatments were inoculated with bacteria, the sugar solution 20% was prepared due to sticking bacteria to seeds and then seeds soaked with sugar solution and bacteria in three replications. Then seeds immediately planted after become dry in shade. Nitrogen required for each treatment was calculated and before planting and added to the ground. In treatment I4 (full irrigation) soil moisture and its required water were calculated using A Class evaporation pan and the relevant formulas and then plants was irrigated. The total consumed water in each of the treatments I4, I3, I2 were measured by the contour. Traits measured consisted the rate of uptake of nitrogen, phosphorus, potassium, iron, manganese and copper seed. In mature stage, seed samples for analysis were taken, to determine the amount of NPK and micronutrients in plants. The harvested seed samples were washed with distilled water and then they were dried in an oven at 55-60 degrees centigrade for 72 hours. After drying and grinding off seed samples, total nitrogen using of Kjeldahl device and phosphorus with using of spectrophotometer, potassium with flame photometer device, and iron by atomic absorption spectrometry were measured. At the end, analysis of variance (ANOVA) was done using SAS 9.1 statistical software and means were compared using Duncan's test.

Results and Discussion

Nitrogen

Analysis of variance showed that the effect of irrigation and nitrogen fertilizer levels and interaction between these two

factors was significant at 1% level on seed nitrogen concentration (Table 3). The highest concentration of seeds nitrogen (0.79/3 %) was for irrigation at flowering time and grain filling and lowest seed nitrogen concentration (0.499/2 present) obtained for treatment without irrigation. Between the irrigation and treatment without irrigation at flowering time and grain filing, there were no significant differences (Table 4). Among the fertilizer treatments, treatment with nitrogen 150 kg/ha had the maximum concentration of seed nitrogen (3.14 %) and treatment without the nitrogen fertilizer (2.65 %) had the lowest concentration of seed nitrogen (Table 4). Study of the interaction of different levels of irrigation and nitrogen fertilizer showed the highest concentration of seed's nitrogen was at flowering and grain filing and consumption of 150 kg/ha nitrogen (Table 4) .

Phosphorus

Analysis of variance showed that the effect of irrigation and nitrogen fertilizer levels and the interaction of these two factors were significant at 1% level on phosphorus concentration of seeds. The highest (0.32) and lowest concentration of seed's phosphorus (0.18) obtained under irrigation condition and no irrigation .there was not any significant difference between the without irrigation and the irrigation during the flowering period (Table 5). Study of the interaction of different levels of irrigation and nitrogen showed that the highest phosphorous concentration of seed (1.44 %) was related to treatment 75kg/ha nitrogen and in full irrigation. The lowest phosphorus concentration of seed (0.166) related to treatment without irrigation with consumes 75 kg/ha nitrogen along with three other treatment of irrigation with the same amount of fertilizer in class f.

Potassium

Analysis of variance showed that irrigation and nitrogen fertilizer levels had significant effect on K concentration of seed (Table 3). The highest and lowest seed K concentration obtained under full irrigation condition and without irrigation (Table 6). There was no significant difference between limited irrigation and no irrigation treatment. In among nitrogen fertilizer treatments, the highest concentration of seed's potassium achieved of 75kg/ha nitrogen and the lowest concentration achieved of the treatment without nitrogen fertilizer. There were not any significant differences between fertilizer treatments. Interaction of irrigation and nitrogen levels was significant at the 5% probability level (Table 6). The highest concentration of seed's potassium (1.44 %) obtained under full irrigation condition and 75kg/ha nitrogen

Iron

Analysis of variance results showed there were significant effects of different levels of nitrogen in 1% level and irrigation in 5% level on Iron content of seed (Table 3). Among the irrigation treatments, maximum concentration of iron obtained of the full irrigation (81.5). Comparisons of means revealed that there is not any significant difference between full and limited irrigation treatments and also between the limited irrigation treatment and treatment without irrigation. Between different levels of nitrogen fertilizer and inoculation, the highest concentration (83.58 mg/kg) related to inoculation of seeds and the lowest concentration (78.8) related to treatment without use nitrogen (Table 7). The interaction of different levels of irrigation treatment and nitrogen

fertilization do not had significant effect on iron level of leaf.

Zinc

Analysis of variance showed that the effect of irrigation and nitrogen fertilizer treatments on seed's zinc was significant at 1% probability level (Table 3). The highest (34.25 mg/kg) and lowest (27.88 mg/kg) concentration of seed's zinc related to full irrigation treatment and the irrigation in flowering time respectively (Table 8). Among levels of nitrogen, the lowest zinc concentration of seed obtained with consumption 150 kg/ha nitrogen (Table 8). The interaction of irrigation and nitrogen fertilizer levels was not significant (Table 8).

Manganese

Analysis of variance showed that the effect of irrigation, nitrogen fertilizer levels and the interaction of these two factors was significant at 1% probability level (Table 3). Among the irrigation treatments, full irrigation and without irrigation causes the accumulation the maximum (40.91 mg/kg) and lowest concentration (33.76 mg/kg) of seed's manganese. Among nitrogen fertilizer treatments, the highest concentrations manganese (37.4937 mg/kg) in seed achieved with use 75kg/ha nitrogen fertilizer. There were not any significant between nitrogen fertilizer treatments (Table 9). Study of interaction effect of irrigation and nitrogen fertilizer levels on seed manganese concentration showed the highest concentration of seed manganese (42.21 mg/kg) was achieved under full irrigation condition and with use 75kg/ha nitrogen.

Table.1 Results of analysis of soil experiment before planting (0-30 cm depth)

| (Absorbable)) Mg/ kg soil | | | | | | | organic carbon (%) | material neutralize (%) | Response of saturated soil | electrical conductivity | saturation (%) |
|-----------------------------|-----|------|-----|-----|------|----|--------------------|-------------------------|----------------------------|-------------------------|----------------|
| Zn | Mg | Mn | Fe | K | P | N | | | | | |
| 2.8 | 228 | 10.1 | 8.9 | 850 | 23.5 | 10 | 0.54 | 3.2 | 7.8 | 0.68 | 34.05 |

Table.2 Characteristics of Water location experiment (chemical analysis of water properties)

| MEq per liter | | | | | | PH | EC × 10 ⁶ μ mho/cm |
|---------------|------|---------|-----|-------------|-----------|------|-------------------------------|
| Mg + Ca | Na | Sulfate | Cl | Bicarbonate | Carbonate | | |
| 1.76 | 1.11 | 0.31 | 0.4 | 2 | 0.16 | 8.08 | 360 |

Table.3 Variance analysis of different irrigation levels of nitrogen fertilizer and inoculation on nutrient uptake

| | | | | | MS | | | |
|-----------------------|-----|---------|-----------|---------|----------------------|----------------------|---------|--------|
| S.O.V | d.f | N | P | K | Fe | Zn | Mn | Cu |
| replication | 2 | 0.001 | 0.00 | 0.00 | 4.39 | 2.62 | 0.23 | 1.618 |
| Irrigation | 3 | 0.733** | 0.036** | 0.013** | 2.159 ^{n.s} | 64.18* | 102.10* | 13.26* |
| Error | 6 | 0.002 | 0.000166 | 0.001 | 11.179 | 9.05 | 12.46 | 1.92 |
| nitrogen | 3 | 0.658** | 0.005** | 0.085** | 59.908** | 52.491** | 14.10** | 9.93** |
| Irrigation × nitrogen | 9 | 0.027** | 0.001** | 0.002* | 2.545* | 3.965 ^{n.s} | 30.83** | 2.23* |
| Error | 24 | 0.003 | 0.0000625 | 0.0101 | 0.810 | 1.66 | 0.85 | 0.57 |
| CV (%) | - | 1.86 | 3.57 | 1.87 | 1.1 | 4.16 | 2.51 | 11.9 |

ns, * and **: Non – significant at 5% and 1% probability levels, respectively

Table.4 The simple and interaction effects of irrigations different levels and nitrogen fertilizer and inoculation on nitrogen content of the grain (%)

| Irrigation Nitrogen | No irrigation | one irrigation at flowering | irrigation at flowering and grain filling | full irrigation | Mean |
|---------------------|---------------|-----------------------------|---|-----------------|--------|
| No nitrogen | 2.267i | 2.68g | 2.817fg | 2.86ef | 2.656c |
| 75 kg/ha | 2.487h | 2.787efg | 2.993cd | 3.053c | 2.830b |
| 150 kg/ha | 2.743fg | 2.903de | 3.487a | 3.323b | 3.114a |
| Mean | 2.499c | 2.79b | 3.099a | 3.079a | |

Table.5 The simple and interaction effects of irrigations different levels and nitrogen fertilizer and inoculation on Phosphorus content of the grain (%)

| Irrigation Nitrogen | No irrigation | one irrigation at flowering | irrigation at flowering and grain filling | full irrigation | Mean |
|---------------------|---------------|-----------------------------|---|-----------------|---------|
| No nitrogen | 0.1767f | 0.1833f | 0.2167de | 0.2733c | 0.2125c |
| 75 kg/ha | 0.16667f | 0.1767f | 0.2133e | 0.3233b | 0.2225b |
| 150 kg/ha | 0.2033e | 0.2133e | 0.2333d | 0.3533a | 0.2508a |
| Mean | 0.1822c | 0.1911c | 0.2211b | 0.32a | |

Table.6 The simple and interaction effects of irrigations different levels and nitrogen fertilizer and inoculation on The Potassium content of the grain (%)

| Irrigation Nitrogen | No irrigation | one irrigation at flowering | irrigation at flowering and grain filling | full irrigation | Mean |
|---------------------|---------------|-----------------------------|---|-----------------|--------|
| No nitrogen | 1.18d | 1.213cd | 1.263c | 1.24c | 1.264b |
| 75 kg/ha | 1.323b | 1.357b | 1.263b | 1.44a | 1.37a |
| 150 kg/ha | 1.337b | 1.357b | 1.350b | 1.433a | 1.36a |
| Mean | 1.28b | 1.309b | 1.326ab | 1.371a | |

Table.7 The simple and interaction effects of irrigations different levels and nitrogen fertilizer and inoculation on The Iron content of the grain (%)

| Irrigation Nitrogen | No irrigation | one irrigation at flowering | irrigation at flowering and grain filling | full irrigation | Mean |
|---------------------|---------------|-----------------------------|---|-----------------|--------|
| No nitrogen | 74.41gh | 75.53gh | 76.64fgh | 76.64fgh | 75.8d |
| 75 kg/ha | 76.08fgh | 79.97cde | 77.75efg | 80.52bcde | 78.58c |
| 150 kg/ha | 78.86def | 75.53gh | 80.52bcde | 82.74bc | 80.94b |
| Mean | 77.89b | 79.41ab | 80.11ab | 81.5a | |

Table.8 The simple and interaction effects of irrigations different levels and nitrogen fertilizer and inoculation on the zinc content of the grain (%)

| Irrigation Nitrogen | No irrigation | one irrigation at flowering | irrigation at flowering and grain filling | full irrigation | Mean |
|---------------------|---------------|-----------------------------|---|-----------------|--------|
| No nitrogen | 32.76abc | 31.65bc | 32.76abc | 35.54a | 33.18a |
| 75 kg/ha | 29.99cd | 26.99de | 32.21abc | 33.88ab | 30.77b |
| 150 kg/ha | 27.77de | 24.99e | 29.99cd | 33.32abc | 29.02c |
| Mean | 30.17bc | 27.88c | 31.65ab | 34.25a | |

Table.9 The simple and interaction effects of irrigations different levels and nitrogen fertilizer and inoculation on the Manganese content of the grain (%)

| Irrigation Nitrogen | No irrigation | one irrigation at flowering | irrigation at flowering and grain filling | full irrigation | Mean |
|---------------------|---------------|-----------------------------|---|-----------------|--------|
| No nitrogen | 39.99h | 36.65fg | 34.43def | 31.1abc | 35.54b |
| 75 kg/ha | 42.21gh | 38.87ef | 36.10bcd | 32.76a | 37.49a |
| 150 kg/ha | 40.54fg | 36.65cde | 37.76def | 34.43ab | 37.35a |
| Mean | 32.76c | 36.1bc | 37.37ab | 40.91a | |

Table.10 The simple and interaction effects of irrigations different levels and nitrogen fertilizer and inoculation on The Copper content of the grain (%)

| Irrigation Nitrogen | No irrigation | one irrigation at flowering | irrigation at flowering and grain filling | full irrigation | Mean |
|---------------------|---------------|-----------------------------|---|-----------------|--------|
| No nitrogen | 39.99h | 36.65fg | 34.43def | 31.1abc | 35.54b |
| 75 kg/ha | 42.21gh | 38.87ef | 36.10bcd | 32.76a | 37.49a |
| 150 kg/ha | 40.54fg | 36.65cde | 37.76def | 34.43ab | 37.35a |
| Mean | 32.76c | 36.1bc | 37.37ab | 40.91a | |

Copper

The results of the analysis of variance showed that effect of irrigation treatments and nitrogen fertilizer was significant on seed Cu concentration at 1% level (Table 3). The highest seed Cu (7.589 mg/kg) related to full irrigation treatment and lowest seed Cu (4.81 mg/kg) was associated with no irrigation treatment. There was not any significant different between limited irrigation and treatment without irrigation (Table 10). Among the nitrogen fertilizer treatments, the highest concentration of seed cooper (7.21 mg/kg) related to use 150kg/ha nitrogen and the lowest concentration of seed nitrogen (5.415 mg/kg) related to treatment without nitrogen fertilizer. Interaction of irrigation treatments and nitrogen fertilizer different on Cu seed concentration were not significant (Table 3). The highest concentration of seed Cu (8.33 mg/kg)

obtained of complete irrigation and irrigation at flowering and grain filling with consuming 150 kg/ha N.

The results showed that the main effect of irrigation and nitrogen on absorption of nitrogen, phosphorus, potassium, iron, copper, manganese and zinc were significant. In all modes of irrigation, the lowest percentage of nitrogen uptake was achieved under without use of nitrogen condition. In the absence of irrigation condition, irrigation at flowering time, and use 150 kg/ha nitrogen was achieved absorption maximum of nitrogen. Under irrigation at flowering and grain filling and normal irrigation, was obtained the highest rate of nitrogen absorption with use of 75kg/ha nitrogen. In general, normal irrigation and nitrogen fertilizer and inoculation with bacteria, improved the percentage uptake of seed element. It is recommended that research about deficit

irrigation was done for all regions and Critical stages of plant growth to water deficit and also yield reduction products for every different degrees of deficit irrigation should be determined. To optimize water use, water use efficiency increases. Given the potential for nitrogen fixation in legumes, this property can play an important role in providing of plant's nitrogen and replacement of chemical fertilizers. Such replacement of economically and environmentally has many advantages in achieving their goals of sustainable agriculture.

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