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***Research Article***
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# The effect of supplemental irrigation and exogenous application of glycine betaine on chickpea performance in the semi-arid region

## Yarı kurak bölgede ilave sulama ve ekzojen glisin betain uygulamasının nohut performansına etkisi

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## ABSTRACT

**Objective:** The objective of this study was to evaluate the effect of foliar spraying of different concentrations of glycine betaine as an osmotic regulator and supplemental irrigation under rainfed conditions on chickpea growth and yield.

**Material and Methods:** Simultaneous effects of foliar spray of glycine betaine (0 mM: $GB_0$, 25Mm: $GB_{25}$, and 50 Mm: $GB_{50}$) and different levels of irrigation (RF: rainfed or no irrigation, $SI_1$: supplemental irrigation during flowering stage, $SI_2$: two supplemental irrigations during flowering and podding, and FI: full irrigation) were assessed on the morphophysiological characteristics of chickpeas under field condition in the western part of Iran.

**Results:** The effect of SI treatments on the growth was much more noticeable than GB foliar application. Utilization $SI_2$ + $GB_{25}$ significantly improved chlorophyll content, pod and seed number, hundred seed weight, and biological yield. $SI_1$ and $SI_2$ increased grain yield by 30% and 62%, respectively, compared to RF conditions.

**Conclusion:** Taken together, two *supplemental irrigation during flowering and podding* along with foliar spray of $GB_{25}$ as a reasonable management options increased seed yield and the water use efficiency.

**Keywords:** Chlorophyll, drought stress, evapotranspiration, pod number, rainfed, water productivity

## ÖZ

**Amaç:** Bu çalışmada, doğal yetiştirme koşulları altında ozmotik düzenleyici olarak farklı konsantrasyonlarda glisin betainin yapraklara uygulamasının ve ilave sulamanın nohutta büyüme ve verim üzerindeki etkisinin değerlendirilmesi amaçlanmıştır.

**Materyal ve Yöntem:** İran'ın batısında tarla koşullarında, yapraklara glisin betain uygulamasının (0 mM: $GB_0$, 25Mm: $GB_{25}$ ve 50 Mm: $GB_{50}$) ve farklı sulama düzeylerinin (RF: yağmurla sulama veya sulamasız, $SI_1$: çiçeklenme döneminde ek sulama, $SI_2$: çiçeklenme ve bakla bağlama döneminde iki ilave sulama ve FI: tam sulama), nohutun morfofizyolojik özellikleri üzerindeki etkileri değerlendirilmiştir.

**Araştırma Bulguları:** SI uygulamalarının büyüme üzerindeki etkisi, GB yaprak uygulamasına göre çok daha belirgin olmuştur. $SI_2$ + $GB_{25}$ kullanımı klorofil içeriğini, bakla ve tohum sayısını, yüz tane ağırlığını ve biyolojik verimi önemli ölçüde iyileştirmiştir. $SI_1$ ve $SI_2$, RF koşullarına kıyasla tane verimini sırasıyla %30 ve %62 artırmıştır.

**Sonuç:** Sonuçlar birlikte ele alındığında, uygun bir yönetim seçeneği olarak çiçeklenme ve bakla bağlama döneminde iki ilave sulama ile $GB_{25}$'in yaprağa uygulaması, tohum verimini ve su kullanım etkinliğini artırmıştır.

**Anahtar sözcükler:** Klorofil, kuraklık stresi, buharlaşma ve terleme, bakla sayısı, yağmurla beslenme, su verimliliği

## INTRODUCTION

The Kabuli chickpea (*Cicer arietinum* L.) is one of the valuable legume crop and provides protein needed by humans. The cultivated area of this plant in the world is around 15 million hectares and its annual global production is 15.8 million tons. The main chickpea producer are India, Australia, Myanmar, Pakistan, Turkey, and Iran. The amount of chickpea production in Iran is 168.142 tons and the cultivated area is 440 thousand hectares. In most of the countries that produce chickpea seed sown as rainfed crop in early spring, and the growth of the plant depends on the water stored in the soil during the fall and winter seasons. However, terminal water deficiency (during pod development phase) is one of the most important limitations for growing this crop. Most of the areas under chickpea cultivation in Iran are located in cold and semi-arid climates, which face extreme drought stress. This condition is aggravated in some years due to asymmetric distribution or a decrease in total precipitation and causes a significant decrease in chickpea yield or makes farming fail. In semi-arid areas due to shallow depth of agricultural soil, low soil organic matter, short high-intensity rainfall, high runoff, low permeability of the soil, low water retention capacity in the soil, inappropriate tillage, poor seedbed soil structure are some of the things that cause limited water storage are among the things that cause limited water storage in the soil and increase the possibility of terminal water stress (Doaei et al., 2020). In these areas, the intensity of drought stress varies from one season to another and depends on factors such as planting date, rainfall distribution during the growing season, air temperature, and the amount of moisture loss through evaporation and transpiration (Seleiman et al., 2021). Although inherent and genetic resistance to drought at the end of the season plays an important role in the amount of production in limiting conditions, the use of agricultural management to reduce stress in the mentioned conditions is of great importance in determining the seed yield (Korbu et al., 2020). Some agronomic management techniques such as planting dates, improving soil conditions and precise irrigation schedules are the most effective factors (Phiri et al., 2023). In the mentioned areas, the available water for irrigation is limited, and therefore, the use of full irrigation systems is not possible. This situation has become more complicated in recent decades due to climate change and global warming. Therefore, maximizing water productivity with accurate irrigation planning can be a suitable strategy for agricultural systems in semi-arid regions (Nikolaou et al., 2020). Supplementary irrigation is a strategic management in rainfed conditions, by providing a limited amount of water during the drought-sensitive period, causes the continuation of plant growth, and stabilizes production. This solution is done during dry spells and when the amount of moisture provided by rain is insufficient. Oweis et al. (2004) showed that the use of supplementary irrigation of around 200 mm during the growing season in Syria significantly improved chickpea yield, due to less moisture loss (ET) during the early growing season water use efficiency considerably increased as compared to full irrigated conditions and improved yield stability over years. However, water use efficiency decreased in supplementary irrigation conditions compared to rainfed conditions. The usefulness of supplementary irrigation is strongly influenced by the climate and the type of plant and needs to be evaluated for different regions. Singh et al. (2016) reported that the consumption of 75 mm of water under rainfed chickpea fields as supplementary irrigation before the start of reproductive growth and during the podding stages, respectively, increased the yield by 59% and 73% compared to the condition without irrigation. In addition, it seems that the external application of some growth stimulants and stress relievers in semi-arid areas can improve growth, reproductive characteristics, and final yield.

Glycine betaine (GB) is one of the derivatives of amino acids, it is soluble in water, non-toxic, and it is naturally synthesized in some plants, including sugar beet (Clendennen & Boaz, 2019). GB plays a role in processes such as osmotic regulation (as an osmotic regulator), and protection of photosystem II. Investigations indicate that the external application of GB can reduce the biosynthesis of reactive oxygen species (ROS) by stimulating the expression of antioxidant genes and preventing the occurrence of secondary oxidative stress (Giri, 2011). However, there have not been many studies on the consequences exogenous application of GB under supplementary irrigation in chickpea as a low-water crop. Therefore, a study was conducted and the objective of this study was to explore the effects of the external application of GB in rainfed conditions and supplementary irrigation on the growth characteristics and yield performance chickpeas.

## MATERIALS and METHODS

A field trial was designed and carried out during the year 2020-2021 in Razen Hamedan region in the west of Iran (35.39°N, 49.03°E, altitude of the area 1810 m) for investigate the effects of SI and GB foliar spraying under rainfed condition on chickpea growth and yield. Based on the Köppen-Geiger climate grouping, the region is cold and semi-arid in terms of climate and has predominant winter and spring rains (early and middle months). Some meteorological information during the growing period are tabulated in Table 1. Before the planting season, a suitable piece of land was selected that represented the conspicuous characteristics of rainfed areas in mentioned region. 10 t ha of rotted manure was integrated with the soil during the autumn season along with the initial chisel plowing. At the time of planting, 60 kg ha$^{-1}$ of ammonium phosphate was used. Each experimental unit was 3 × 3 m included 10 planting lines with 30 cm between rows and a 10 cm in-row spacing. To avoid the effects of moisture leakage, a distance of 2 meters was considered between the main plots and between the experimental blocks. Seed planting was done manually on March 16, 2021, at a depth of 5 cm. The subplots were assigned to different concentrations of glycine betaine (0, 25, and 50 mM).

Cultivar Filip 93-93 was obtained from Dryland Agricultural Research Institute (DARI), Maragheh, and used for this experiment. Compared to native cultivars, this variety has a higher yield is resistant to Ascochyta blight, and has a suitable growth pattern for mechanized harvesting. The field soil was clay loam and its chemical properties were pH 7.63, total nitrogen content 0.13%, calcium carbonate content 13.6%, electrical conductivity 1.23 dS m$^{-1}$, organic carbon content 0.39%, absorbable phosphorus 8.037 ppm, and absorbable potassium 198 ppm.

**Table 1**. Meteorological data recorded in the studied area during the chickpea growing season
***Çizelge 1.*** Çalışma alanında nohut yetiştirme mevsimi boyunca kaydedilen meteorolojik veriler

| | March | April | May | June | July |
|---|---|---|---|---|---|
| Relative humidity (%) | 59 | 61 | 48 | 39 | 41 |
| Average of minimum temperature (°C) | 5.6 | 7.4 | 13.2 | 15.8 | 19.2 |
| Average of maximum temperature (°C) | 11.4 | 18.3 | 24.6 | 29.7 | 31.4 |
| Average of temperature (°C) | 8.55 | 12.85 | 18.9 | 22.75 | 25.3 |
| Sunny hours per month | 227.3 | 245.8 | 308.9 | 342.5 | 337.8 |
| Total evapotranspiration (mm) | 65.2 | 114.5 | 184.3 | 257.6 | 286.2 |
| Total rainfall (mm) | 34.9 | 61.1 | 22.1 | 2.3 | 3.6 |

This study was conducted as a split-split plot arrangement based on a Randomized Complete Block Designs with five replications. Each block was divided into four main plots, and the irrigation treatment were randomized to them. The irrigation levels were FI: Full irrigation up to the field capacity (FC) and repetition of the irrigation with 30% moisture depletion, RF: rainfed farming or cultivation without irrigation and relying on rainfall, $SI_1$: application of supplemental irrigation in the early flowering stage of chickpeas, $SI_2$: application of supplemental irrigation in the flowering stage and during podding stage. Irrigation was done through polyethylene pipes and drip tape system. The foliar application of different concentrations of glycine betaine were then randomized to 3 m×3 m sub-plots in each main plot. Sub-plots were assigned to 0, 25, and 50 mM of glycine betaine. For 0 mM of glycine betaine concentration, distilled water was sprayed. Volumetric meters were used to measure the amount of water. Glycine betaine $(CH3)3N^+CH2COO^-$) was obtained from Sigma-Aldrich Company and was used externally by spraying on the shoot and leaves during the vegetative (V5) and reproductive stages (R1 and R5). The amount of foliar spraying was enough to wet the entire plant. The total water consumption for $SI_1$, which was done at the beginning of the bloom stage (fully open flower) in some plants, was 200 mm. However, $SI_2$ was performed at the stage of full bloom stage (R2) and early seed development (R5), and the total water consumption in this treatment was 300 mm. Under the conditions of full irrigation, the experimental plots were irrigated up to the field capacity, and the amount of water consumption was calculated as follows (Hasanuzzaman et al., 2016).

$$WR = (SFC \times SM) \times BD \times RD$$

Where; WR is the required water amount (mm) during the irrigation, SFC: is the selected field capacity percentage, SM: is soil moisture content before irrigation (through gravimetric method), BD: is soil bulk density g/cm3, RD: is the rooting depth (according to the investigations carried out in the region, a fixed number of 60 cm was considered for chickpea).

To estimate the total water consumption (TWC) during the growing season the equation soil moisture balance was used as below, which included the difference between inputs of water and losses of water (Reddy, 1983).

$$TWC = P + I - DR + (\Delta MC)$$

Where, P refers to the amount of rainfall during the five-month growing season, March through July (mm), I is the amount of water consumed during irrigation (mm), DR: water removed from roots environment due to gravity and called drainage water. ΔMC refers to the difference in soil moisture at the beginning and end of the season. According to the suggestion of Oweis et al. (2004) due to the relatively low amount of rainfall, the type of applied irrigation system, the absence of surface runoff, the type of field soil, and it is impossible to replace the lost moisture through rainfall and irrigation, the amount of drainage water was ignored.

To evaluate the content of chlorophyll as the most important photosynthetic pigment, a portable and non-destructive SPAD Chlorophyll Meter (SPAD-502Plus, Japan) was used non-destructively for the upper leaves during the R5 stage (pods had reached their final size).

To measure the height of the plant, and the number of pod and seeds per plant, in the physiological maturity stage, 10 plants were arbitrarily chosen, harvested and counted. The biological yield was measured for 2 $m^{-2}$ randomly selected using a quadrat and after harvesting, they were dried in an oven at the temperature of approximately 75°C for 24 h. Also, the weight of 100- seeds was randomly obtained in each experimental unit after counting and weighing. After separating the seeds, the yield of seeds per surface unit was calculated and then the yield per hectare was calculated. Seed protein percentage was determined using a near-infrared seed analyzer (zeltex, USA) in the physiological maturity stage. The grain or biomass water use efficiency was calculated through the ratio of grain (WUEg) or biomass (WUEb) to total water consumption (TWC).

The gathered data were subjected to analysis of variance with SAS 9.4 software. One-way analysis of variance was achieved and themodel using proc glm with split-plot design to test the effects of irrigation and foliar spray of glycine betaine on chickpea growth characteristics. Comparison of average data was done using LSD test at 5% level. Box plots were drawn with STATISTICA 10 software.

## RESULTS and DISCUSSION

The evaluation of plant height at the end of the growth period indicated that this trait was only affected by irrigation levels. The height of plants grown under RF conditions was 26% lower than the height of plants grown under FI conditions. Although the application of one time supplementary irrigation increased the height of plants compared to RF conditions, this increase was not significant (7%). However, applying twice supplementary irrigation ($SI_2$) increased plant height by 25% compared to RF conditions, and these plants were only 6% shorter than plants grown under IF conditions (Table 2).

Plant height is influenced by the interaction between hormones, especially auxin, and the process of cell elongation. Absence of water as the main driver of cell growth on the one hand and possibly the reduction of auxin biosynthesis under drought stress conditions (Pandey & Shukla, 2016) on the other hand are among the factors that caused the reduction of plant height under RF and $SI_1$ conditions. The soil moisture regime strongly affected the length of the development period and the number of days until physiological maturity. In IF conditions, the longest growth period was recorded, and the plants grown in RF conditions were 14% less than IF conditions.

**Table 1**. Growth characteristics and seed yield components of Kabuli chickpea (*Cicer arietinum* L.) under different supplementary irrigation and foliar application of glycine betaine

***Çizelge 1*** Kabuli nohutunun (*Cicer arietinum* L.) farklı ek sulama ve yapraktan glisin betain uygulaması altında büyüme özellikleri ve tohum verimi bileşenleri

| | | PH | DM | CHL | CW | SNP | BY | $WUE_b$ | SY | HI |
|---|---|---|---|---|---|---|---|---|---|---|
| FI | $GB_0$ | $32.6±2.32^a$ | $100.3±1.10^{cd}$ | $57.4±1.32^c$ | $30.9±3.14^d$ | $34.8±3.11^{bc}$ | $5824.0±87.2^c$ | $7.28±0.12^i$ | $1186.0±11.54^c$ | $20.3±0.31^b$ |
| | $GB_{25}$ | $32.56±1.46^a$ | $106.2±2.06^b$ | $64.0±2.03^a$ | $32.6±1.76^{ab}$ | $39.4±4.62^a$ | $6072.6±63.4^a$ | $7.77±0.16^h$ | $1229.0±24.50^{ab}$ | $20.2±0.46^{bc}$ |
| | $GB_{50}$ | $32.5±2.64^a$ | $110.0±1.33^a$ | $61.0±1.26^b$ | $33.8±1.02^a$ | $35.2±1.02^b$ | $5968.0±79.8^b$ | $7.77±0.12^h$ | $1250.0±18.92^a$ | $20.9±0.13^a$ |
| RF | $GB_0$ | $23.6±1.40^c$ | $89.2±1.06^g$ | $44.6±0.22^f$ | $21.3±3.51^h$ | $24.2±1.82^h$ | $3573.0±126.2^k$ | $13.75±0.35^c$ | $598.4±34.10^{ij}$ | $16.7±0.25^h$ |
| | $GB_{25}$ | $23.2±1.71^c$ | $87.8±0.56^{gh}$ | $41.3±1.66^g$ | $25.0±1.78^g$ | $24.3±2.53^h$ | $3760.4±45.7^j$ | $14.86±0.44^b$ | $647.4±26.6^{hi}$ | $17.2±0.17^{gh}$ |
| | $GB_{50}$ | $25.8±1.14^b$ | $95±0.89^{ef}$ | $48.7±3.46^e$ | $24.9±0.88^g$ | $23.0±1.81^{hi}$ | $3866.4±57.9^i$ | $15.37±0.49^a$ | $601.8±39.72^i$ | $15.5±0.42^i$ |
| $SI_1$ | $GB_0$ | $26.5±0.96^b$ | $99.4±1.02^c$ | $53.9±1.37^d$ | $27.0±1.03^{ef}$ | $27.5±2.21^{fg}$ | $4277.2±135.6^h$ | $9.82±0.22^f$ | $841.4±16.30^{fg}$ | $19.6±0.46^{de}$ |
| | $GB_{25}$ | $26.9±1.52^b$ | $96.2±1.13^{de}$ | $54.3±0.49^d$ | $27.8±1.68^e$ | $28.6±1.85^{ef}$ | $4513.0±92.4^g$ | $10.50±0.13^{de}$ | $858.4±11.365^f$ | $19.0±0.32^f$ |
| | $GB_{50}$ | $26.5±1.16^b$ | $94.0±0.73^f$ | $57.8±0.87^c$ | $26.3±1.35^{fg}$ | $28.1±1.71^f$ | $4728.8±67.91^f$ | $10.90±0.42^d$ | $835.2±21.34^{fg}$ | $17.6±0.37^g$ |
| $SI_2$ | $GB_0$ | $27.4±3.21^b$ | $97.0±0.95^d$ | $52.5±1.03^d$ | $31.3±1.58^{bcd}$ | $30.3±1.42^e$ | $5081.6±27.9^e$ | $9.03±0.16^g$ | $978.0±19.012^e$ | $19.2±0.14^{ef}$ |
| | $GB_{25}$ | $31.3±1.90^a$ | $86.4±0.87^h$ | $57.4±1.40^c$ | $32.4±0.45^{bc}$ | $32.4±0.62^{cd}$ | $5320.0±114.5^d$ | $10.08±0.21^{ef}$ | $1052.6±25.67^d$ | $19.7±0.22^{cd}$ |
| | $GB_{50}$ | $32.56±3.17^a$ | $106.2±1.53^b$ | $64.0±2.29^a$ | $31.12±1.78^{cd}$ | $30.1±0.33^e$ | $5157.6±75.2^e$ | $9.08±0.15^g$ | $1047.8±20.52^d$ | $20.3±0.35^b$ |
| Statistical significance | | | | | | | | | | |
| I | | ** | * | ** | ** | ** | ** | ** | ** | ** |
| GB | | *NS* | *NS* | ** | ** | ** | * | *NS* | ** | ** |
| I× GB | | *NS* | *NS* | ** | * | *NS* | * | ** | ** | ** |

FI: full irrigation, RF: rainfed condition without irrigation, $SI_1$: application of supplementary irrigation in the early flowering stage, $SI_2$: application of supplementary irrigation in the flowering stage and during podding stage, $GB_0$: $GB_0$: sprayed with distilled water (control), $GB_{25}$: foliar application of 25 Mm glycine betaine , $GB_{50}$: foliar application of 50 Mm glycine betaine, HP: the height of the plant (cm), CHL: chlorophyll content in upper leaves (SPAD unit), CW: canopy width (cm),SNP: seed number per plants, BY: biological yield (kg $ha^{-1}$), $WUE_b$: Water-use efficiency for biological yield (kg $ha^{-1}$ $mm^{-1}$), SY: seed yield (kg $ha^{-1}$), HI: harvest index (%).The numbers represent the mean ± standard error NS: statistically not significant, * and **: significant at 0.05 level and significant at 0.01 level, respectively. In each attribute, the means with the same letters do not have statistically significant differences.

It seems that under unfavorable conditions, plants try to speed up its growth process with internal planning to ensure survival, and this may be associated with the early onset of aging. This trend could be justified by the decrease in chlorophyll in IF and SI1 conditions. Chlorophyll as one of the important and key pigments of photosynthesis is sometimes referred to as the ability of the photosynthetic apparatus. The interactions of I×GB were significant for this pigment. Comparing the averages showed that foliar spraying of $GB_{25}$ under FI conditions and foliar spraying of $GB_{50}$ under SI2 caused a significant increase. The lowest amount of chlorophyll was observed under RF and $GB_{50}$. Despite the fact that the external application of $GB_{25}$ and $GB_{50}$ in the conditions of one-time supplementary irrigation caused an increase of 2% and 7% compared to no application, the greatest effect on the content of this pigment was recorded in the conditions of two supplementary irrigations with the application of $GB_{50}$, which compared to the condition without foliar application, it improved the amount of chlorophyll by 22%. The results about the chlorophyll content are in agreement with the ones obtained by Ibrahim et al. (2023) conclusions which indicated that drought stress significantly decreased chlorophyll content and GB foliar spry mitigated the effects of drought stress to a significant extent. The change in chlorophyll content by available oil soil moisture and its improvement by GB can reflect the photosynthetic capacity of leaves. These changes can be caused by increasing the biosynthesis of chlorophyll under foliar application conditions and reducing the degradation through increasing the scavenging capability of reactive oxygen species (Yang et al., 2023).

Canopy width was affected by GB foliar application and irrigation levels. The decrease in the amount of available water under RF conditions caused a significant decrease in lateral canopy growth, and plants grown under RF had 28% less canopy width than plants grown under FI conditions (Table 2). However, the application of GB showed its greatest effect on canopy width under RF conditions and the application of

$GB_{25}$ and $GB_{50}$ increased lateral growth by 17% on average compared to the control. The largest canopy was obtained under FI + $GB_{50}$ (33.89 cm). Foliar spraying under SI conditions did not have much effect on lateral growth. This verifies the results obtained by Salama (2022), who reported that foliar spray of GB increased canopy growth of Sweet pepper when compared with intact plants.

Examining the number of pods per plant showed that the interaction effects of FI×GB was significant for this trait ($P < 0.01$). The mean comparison showed that the highest number of pods was for plants grown in FI + $GB_{25}$ conditions. The lowest number of pods per plant was recorded under RF conditions (20.81), which was 53% less than plants grown under FI conditions. However, applying one and two supplemental irrigations improved the number of pods per plant by 18% and 28%, respectively. However, $GB_{25}$ foliar application could increase this component only under FI and $SI_2$ conditions (Figure 1).

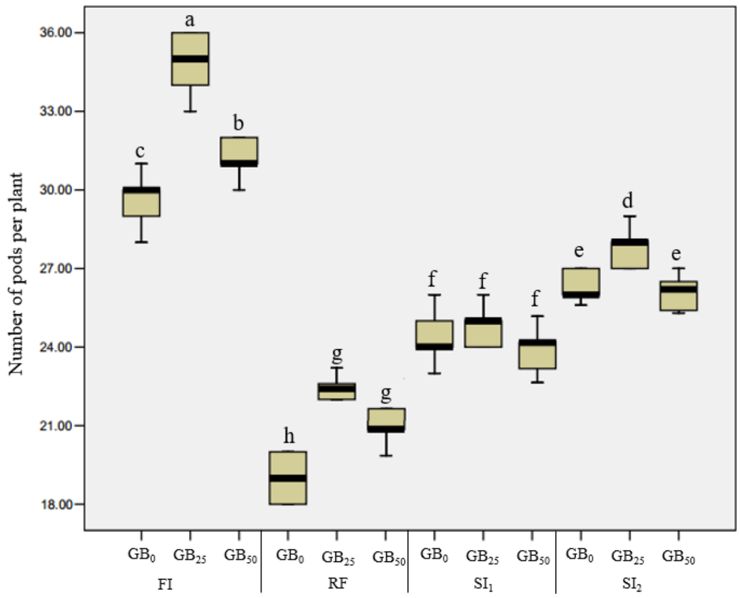


**Figure 1**. Evaluation of the number of pods in chickpea plants grown under different irrigation conditions and sprayed with different concentrations of glycine betaine. FI: full irrigation, RF: rainfed condition without irrigation, $SI_1$: application of supplementary irrigation in the early flowering stage, $SI_2$: application of supplementary irrigation in the flowering stage and during podding stage, $GB_0$: sprayed with distilled water (control), $GB_{25}$: foliar application of 25 Mm glycine betaine, $GB_{50}$: foliar application of 50 Mm glycine betaine. Boxes with the same letters do not have statistically significant differences ($P \leq 0.05$).

***Şekil 1.*** Farklı sulama koşullarında yetiştirilen ve farklı konsantrasyonlarda glisin betain uygulanan nohut bitkilerinde bakla sayısının değerlendirilmesi. FI: tam sulama, RF: sulamasız yağmur sularıyla yapılan yetiştiricilik koşulu, $SI_1$: erken çiçeklenme aşamasında ek sulama uygulaması, $SI_2$: çiçeklenme aşamasında ve bakla bağlama aşamasında ek sulama uygulaması, $GB_0$: distile su uygulama (kontrol), $GB_{25}$ : 25 Mm glisin betain'in yapraktan uygulanması, $GB_{50}$: 50 Mm glisin betain'in yapraktan uygulanması.

Seed number per plant also had a relatively similar response against the investigated treatments. Foliar application of $GB_{25}$ under FI conditions increased the number of seeds per plant by about 13% compared to control conditions (no foliar GB application). Also, foliar application of $GB_{25}$ under $SI_2$ conditions increased this component by 7% compared to the control. However, in other irrigation regimes, the application of GB could not have a positive effect. The findings showed that the investigated treatments have affected the source-sink Relationships. Number of sinks in chickpeas was highly dependent on moisture supply. It seems that the application of GB under optimal soil moisture conditions has shown its positive effect through increasing the supply of photoassimilates, increasing the ability to transform primordia into yield components and improving the *photoassimilate* partitioning to reproductive organs (Rani et al., 2020).The interaction effect was significant for the weight of 100-seeds ($P < 0.01$), the highest seed weight was recorded under FI+$GB_0$ and FI+$GB_{25}$ conditions. However, use of $GB_{50}$ in FI conditions reduced the seed weight by 6%. Under RF conditions foliar application of $GB_{50}$ increased seed weight by 4%. Furthermore, foliar application of $GB_{50}$ under $SI_1$ conditions did not result in any increase in seed weight, but foliar application of $GB_{25}$ caused a 3% increase in seed weight. Spray by $GB_{25}$ and $GB_{50}$ caused an increase of 5% and 3%, respectively, in the weight of 100-seeds (Figure 2). Seed weight in grain legumes is influenced by cell divisions in the cotyledons, grain filling rate and supply of current photoassimilates and remobilization and redistribution of stored carbohydrates from vegetative parts (Ayaz et al., 1999). Considering the greater contribution of the remobilization process in the chickpea grain filling, the effect growth regulators on seed weight can be attributed to the increase of translocation of stored photoassimilates to growing seeds (Sreenivasulu & Wobus, 2013). However, GB improved the chlorophyll content and greenness continuity under favorable moisture conditions and hence can increase the portion of current photosynthesis in seed filling.

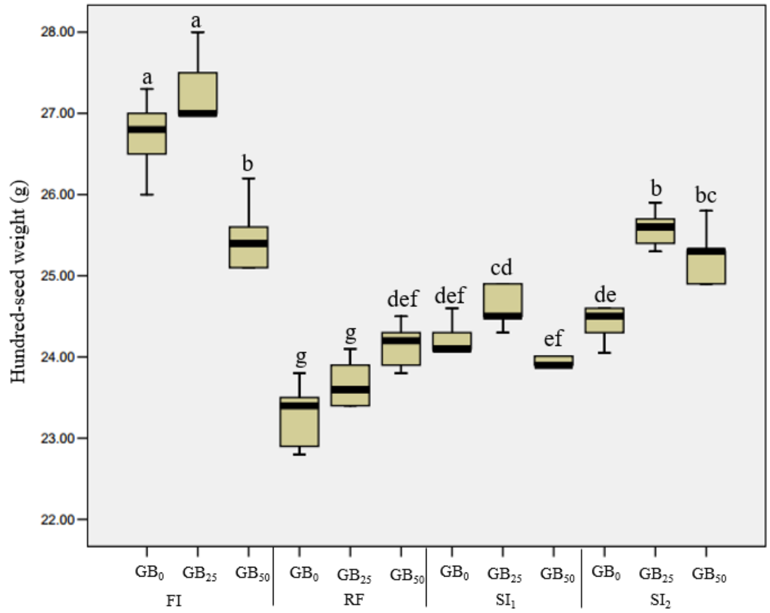


**Figure 2.** Mean comparisons of 100- seed weight of chickpea plants grown under different moisture conditions and foliar spraying with different concentrations of glycine betaine. FI: full irrigation, RF: rainfed condition without irrigation, $SI_1$: application of supplementary irrigation in the early flowering stage, $SI_2$: application of supplementary irrigation in the flowering stage and during podding stage, $GB_0$: sprayed with distilled water (control), $GB_{25}$: foliar application of 25 Mm glycine betaine, $GB_{50}$: foliar application of 50 Mm glycine betaine.

***Şekil 2.*** Farklı nem koşullarında yetiştirilen ve farklı konsantrasyonlarda glisin betain ile yaprağa uygulamasının nohut bitkilerinin 100 tohum ağırlığının ortalama karşılaştırmaları.

The highest amount of biological yield was recorded under + $GB_{50}$ conditions (6072 kg $ha^{-1}$) and the lowest amount under GB0 + RF conditions (3573 kg $ha^{-1}$). The spray of $GB_{25}$ and $GB_{50}$ under RF conditions increased the biological yield by 6% and 9%. Application of one and two supplementary irrigation in rainfed conditions increased the biological yield by 20% and 39%, respectively. A similar conclusion was reached by Pasandi et al. (2014). Assessment of water-use efficiency for biological yield ($WUE_b$) indicated that the highest $WUE_b$ was recorded under RF+$GB_{50}$ conditions with a value of 15.37 kg $ha^{-1}$ $mm^1$, while the lowest $WUE_b$ was obtained under FI conditions. Foliar application of $GB_{50}$ under $SI_1$ conditions increased $WUE_b$ by 11%. Foliar spraying with low concentrations of GB under $SI_2$ conditions increased $WUE_b$ by 12%. The effect of GB on the improvement of $WUE_b$ can be attributed to the improvement of photosynthesis and the increase of vegetative growth of the plant through mitigating the effects of water deficit. The outcomes of the present experiment are consistent with those of Oweis et al. (2004) who indicated that use of supplementary irrigation reduces the $WUE_b$. Although it also increases vegetative growth and biological yield. The evaluation of grain yield showed that the highest yield was obtained under FI+$GB_{50}$ and FI+ $GB_{25}$ conditions (1250 and 1229 kg $ha^{-1}$) and the lowest yield was recorded for plants grown under RF+$GB_0$ (598 kg $ha^{-1}$). Using 600 mm of irrigation water under FI conditions increased the yield by 100% compared to non-irrigated conditions.

Although foliar application of GB increased the grain yield, the effectiveness of $GB_{25}$ was more prominent than other levels. Using one and two times of supplemental irrigation in rainfed conditions improved the grain yield by 37% and 66%, respectively. These results emphasize the necessity of using supplemental irrigation for chickpea fields in the studied area. The highest water-use efficiency for grain yield (WUEg) was obtained under RF+$GB_{25}$ conditions (2.55), and the lowest WUEg was recorded for plants grown under FI+$GB_0$ conditions (1.48 kg $ha^{-1}$ $mm^{-1}$). Comparisons of WUEg between different levels of irrigation indicated that with one and two supplementary irrigations under rainfed conditions, the amount of water use efficiency decreased by 19% and 23% (Figure 3).

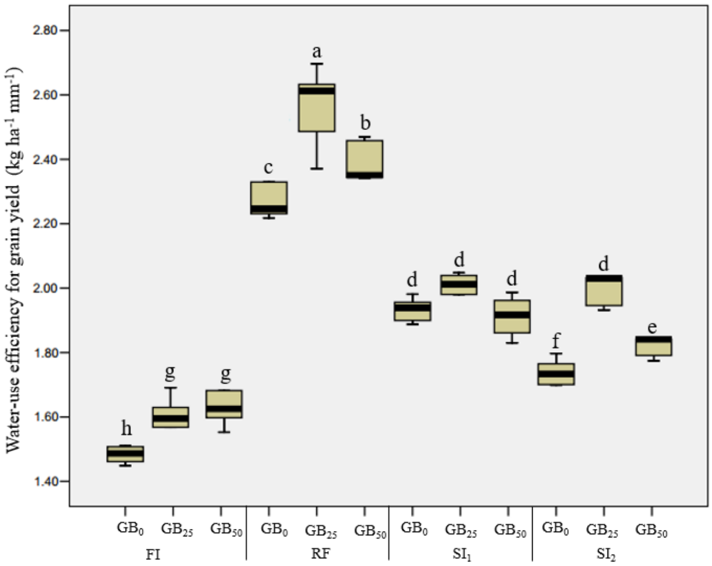


**Figure 3.** The means comparison of water-use efficiency for grain yield in chickpea plants grown at different levels of irrigation and sprayed with different concentrations of glycine betaine. FI: full irrigation, RF: rainfed condition without irrigation, $SI_1$: application of supplementary irrigation in the early flowering stage, $SI_2$: application of supplementary irrigation in the flowering stage and during podding stage, $GB_0$: sprayed with distilled water (control), $GB_{25}$: foliar application of 25 Mm glycine betaine, $GB_{50}$: foliar application of 50 Mm glycine betaine.

***Şekil 3.*** Farklı sulama seviyelerinde yetiştirilen ve farklı konsantrasyonlarda glisin betain uygulanan nohut bitkilerinde tane verimi için su kullanım verimliliğinin karşılaştırılması

Foliar spray of $GB_{25}$ under RF and $SI_2$ significantly improved WUEg. Similarly, the highest harvest index was also observed under FI + $GB_{50}$ conditions, and the plants grown in non-irrigated conditions had the lowest harvest index. The effect of irrigation on the harvest index was much noticeable than foliar spray of GB. The highest seed protein content was recorded under $GB_{50}$+ $SI_2$ conditions, and the plants grown under full irrigation conditions and without foliar application of GB showed the lowest amount of protein (Figure 4). It is interesting to note that under supplementary irrigation conditions the use of high concentrations of GB caused a significant increase in seed protein. These results further approve the idea of increasing or stimulating the allocation of nitrogenous compounds from the vegetative parts to the filling seeds under drought stress conditions increases the percentage of seed protein (Samineni et al., 2022). However, recently Benali et al. (2023) reported that severe drought stress can reduce seed protein content through disruption of seed growth processes and re-supply of nitrogenous compounds.

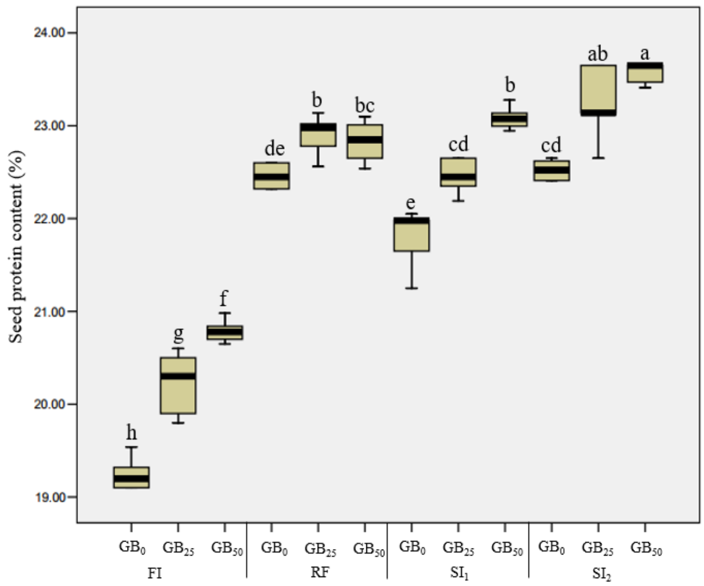


**Figure 4.** The effect of external application of glycine betaine in different supplementary irrigation conditions on chickpea seed protein percentage. FI: full irrigation, RF: rainfed condition without irrigation, $SI_1$: application of supplementary irrigation in the early flowering stage, $SI_2$: application of supplementary irrigation in the flowering stage and during podding stage, $GB_0$: sprayed with distilled water (control), $GB_{25}$: foliar application of 25 Mm glycine betaine, $GB_{50}$: foliar application of 50 Mm glycine betaine.

***Şekil 4.*** Farklı sulama koşullarında glisin betain harici uygulamasının nohut tohumu protein yüzdesine etkisi.

The principal component analysis related to the effects of the investigated treatments is depicted in Figure 5. The first component was able to distinguish the optimal moisture conditions (FI and $SI_2$) that had the best effect on growth and yield components. The second component was also able to separate the best effective combined treatment on growth characteristics such as FI+$GB_{50}$, $SI_2$+$GB_{50}$, $SI_1$+$GB_{25}$ from other treatments.

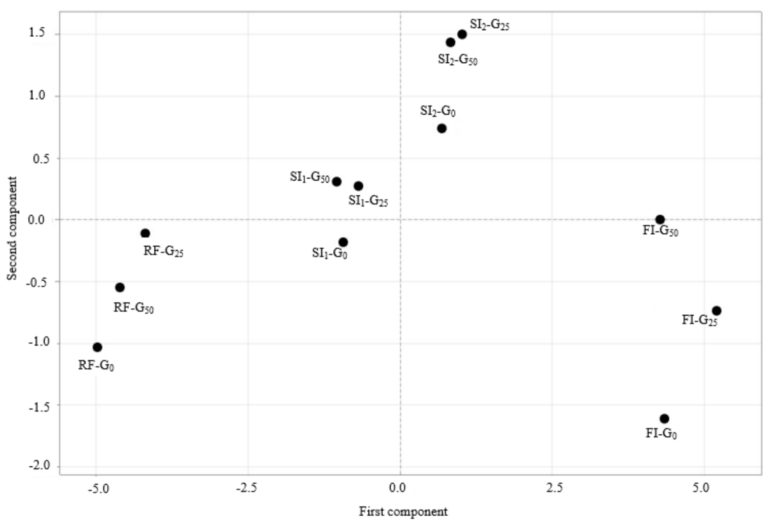


**Figure 5**.Principal component analysis (PCA) plot from the first 2 PCs. FI: full irrigation, RF: rainfed condition without irrigation, $SI_1$: application of supplementary irrigation in the early flowering stage, $SI_2$: application of supplementary irrigation in the flowering stage and during podding stage, $GB_0$: sprayed with distilled water (control), $GB_{25}$: foliar application of 25 Mm glycine betaine, $GB_{50}$: foliar application of 50 Mm glycine betaine.

***Şekil 4.*** İlk 2 PC'den temel bileşen analizi (PCA) grafiği.

## CONCLUSIONS

The results obtained from the study showed that due to the insignificant amount of rainfall during the season and insufficient soil moisture reserves, the rainfed cultivation of chickpeas resulted in a low and unacceptable yield. Applying two supplementary irrigation during flowering and podding stages increased grain yield by 60% compared to rainfed conditions. Although foliar application of GB compared to irrigation levels had a negligible effect on the studied traits, low concentrations of GB under moisture limited conditions stimulated vegetative growth and improved some yield components. The impact of GB was more evident under favorable soil moisture conditions than rainfed conditions. Application of two supplementary irrigation ($SI_2$) could significantly improve chlorophyll content, seed yield, water use efficiency and harvest index compared to rainfed conditions. Even though the amount of water consumed under the $SI_2$ condition was half of the full irrigation condition, an acceptable grain yield was produced and this irrigation program saved 300 mm of water. The amount of water saved is very important from the aspects of subsequent crop management, irrigation traffic, and limited available water resources for full irrigation systems. The current results emphasize the use of precise and reduced irrigation methods instead of full and conventional irrigations. It can be stated that considering the worsening of the problem of water shortage due to climate change, it seems necessary to investigate the effect of supplementary irrigation methods during different stages of development and with different irrigation volumes.

### Data availability

Data will be made available upon reasonable request.

### Author contributions

Conception and design of the study: MJ; sample collection: HK, NS; analysis and interpretation of data: MJ, HK; statistical analysis: NS; visualization: NS; writing manuscript: MJ.

### Competing interests

There is no conflict of interest between the authors in this study.

### Ethical statement

We declare that there is no need for an ethics committee for this research.

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