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Research Article

Positive Effects of Chickpea Flour Amendment on Chickpea (*Cicer Arietinum* L.) Growth Under Water Deficit Conditions

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Abstract

Plant growth and germination can be affected by a number of factors, the most important of which is water stress. The aim of our work is to study the effect of amendment by chickpea flour on the germination of seeds of the Douyet variety and to monitor plant growth under water deficit. After germination of the seeds of the Douyet variety, the plants were separated into two batches and then placed in pots under glass. Different amendments (2, 5 and 10% of chickpea flour) were applied to the plants. Water deficit was applied with irrigation at 40% and 80% of field capacity (FC). The parameters analyzed were the final percentage of seed germination (FPG), and the Germination energy (GE). The final germination percentage of the Douyet variety was FGP 90% and the germination energy of the seeds was GE 58%. The results showed also that plants amended with a concentration of 10% chickpea flour showed an improvement over control plants in the physiological parameters analyzed, in particular chlorophyll (65%), total soluble sugars (33%) and proline levels compared with plants in the control group. The growth of plants amended with chickpea flour powder increased significantly compared with control plants. At 10% amendment, growth increased by 26.7% underwater deficit conditions. In conclusion, treatment with chickpea flour improved the growth of chickpea plants underwater deficit.

Keywords

Amendment, Chickpea, Flour, Germination, Growth, Water Deficit

1. Introduction

Food legumes play an important role in the human diet for many developing countries [1]. Chickpea is the third most important grain legume produced, after common beans (21.5 million tons/year) and peas (10.4 million tons/year) [2]. Chickpea is a low-cost crop and currently a counts for more than 12 million hectares worldwide [3]. India, Australia and Mexico are the main exporters [4]. Among legumes, chickpea ranks 2nd after bean and is one of the most important legumes in Morocco in nutritional, agronomic and economic terms [5, 6]. Chickpea is a real source of protein, amino acids, dietary fiber and micro-elements, as well as several other fatty acids. It is used in the prevention of several diseases such as diabetes, and also has anti-inflammatory properties [7, 8]. The wide-spread use of chemical fertilizers such as nitrogen, potassium and phosphate destroys the microorganisms and non-pathogenic insects that protect plants, making crops more susceptible to disease [9]. Their harmful effects on humans and the environment have been observed, causing serious diseases and imbalances in terrestrial ecosystems [10]. The restrictive use of non-harmful chemical fertilizers, respecting

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the prescribed dosage, and the development of organic farming wherever possible could contribute to sustainable agriculture [11]. Climate change has also a negative effect on soil fertility and agricultural productivity in developing nations [12]. As plants are constrained to immobility, they continually adjust their growth and development according to variations in the environment. A lack of water, for example, has a negative impact on growth and yield. Plants have therefore developed numerous adaptation strategies, ranging from morphology to physiological and biochemical reactions, in order to preserve their water status [13, 14]. Plants can react to this deficit by developing new roots to absorb more water, but this also makes plants more sensitive to other stresses. Chickpea, with its resilience to water stress and its well-developed root system, can be grown in arid regions and dry soils [15].

The physiological changes linked to water deficit include a change in stomatal regulation, which affects photosynthesis and respiration. In addition, the physiological activity of the leaf is strongly affected and linked to the reduction in leaf water potential. This reduction is linked to the closure of the stomata, resulting in a decrease in CO_2 diffusion conductance and biochemical limitation of the chloroplast for CO_2 fixation [16]. According to [17], this stress can lead to oxidative stress through the production of reactive oxygen species (ROS), particularly the superoxide radical and hydrogen peroxide. The damage caused by a water deficit will also have serious structural and metabolic consequences by affecting the integrity of membranes and proteins, leading to metabolic dysfunctions [18].

Cell osmotic adjustment and membrane protection are among the plant responses to abiotic stresses. Plants accumulate organic solutes such as sugars, amino acids and other secondary metabolites [19, 20]. Several authors have reported that glutarate, proline, glutamate and tryptophan are the most important metabolites involved in drought stress tolerance [13].

Soil amendments are one of the most important methods of improving soil quality and crop productivity [21]. Soil nutrients are the key to soil fertility. They have become the main constraint to the sustainability of agriculture worldwide. The use of soil amendments from different organic sources is an effective solution for improving soil fertility and rehabilitating degraded soils [22]. Biological nitrogen fixation by rhizobia in legumes can also be enhanced by soil amendment, due to the improved pH and micronutrients provided [21, 23]. Soil amendments, such as compost or manure, have a positive impact on the physical, chemical and biological characteristics of the soil, by increasing the stock of organic matter [24]. These amendments can improve water retention, nutrient availability, soil biological activity and disease resistance. Some mineral amendments, such as phosphate or potassium, can also increase yield, seed quality and tolerance to water stress [25]. The aim of our study is to examine the effect of chickpea flour-based amendments on the growth of chickpea underwater deficit conditions.

2. Materials and Methods

2.1. Plant Material and Growing Conditions

The Douyet variety used is the most widely grown in Morocco. The seeds were sterilized with 2.5% sodium hypochlorite for 20 minutes, then washed with sterile water and left to germinate in petri dishes were placed in an oven at 25 ℃. The number of germinated seeds was recorded for seven days. Seedlings at uniform stages of development were selected and placed in pots filled with a mixture of sterilized sand and peat (4:1). The experiment was conducted in a greenhouse (approximate temperature 26/20 ℃ (day/night), 50-80% relative humidity and 16-hour photoperiod) at the Faculty of Science and Technology in Marrakech. After 20 days, water deficit was applied by maintaining soil moisture at 40% of field capacity for stressed plants, and at 80% of field capacity for control plants. Finally, we measured the length (in cm) of the aerial part. Five replicates per treatment were used.

2.2. Germination Parameters

2.1.1. Final Germination Percentage

Calculated using the formula:

$$(FGP) = (n/N) \times 100,$$

Where n is the number of germinated seeds and N is the total number of seeds.

2.1.2. Germination Energy of the Seeds

(GE) =nj/N x 100, Where nj is the number of seeds germinated during half the germination time and N is the total number of seeds.

2.3. Determination of Total Chlorophyll (Chl) Content

The method of the previous study was used to extract chlorophyll from chickpea leaves [26]. 50 mg of fresh leaf material were ground in acetone (80%, v/v). Chl content was measured after centrifugation at 5000 rpm for 10 minutes. Finally, the optical density (OD) was read using a spectrophotometer at 663 and 645 nm. Three replicates per treatment were performed.

The chlorophyll content was determined according to the formula:

$$\text{Total Chl} = 8{,}02\ (\text{OD at } 663) + 20{,}2\ (\text{OD at } 645)\ \text{mg mL}^{-1}$$

2.4. Determination of Proline Content

The determination of proline was carried out using the method of [27]. A 0.5 mL aliquot of the upper phase was taken, 2.5 mL of ninhydrin and 2.5 mL of glacial acetic acid were added after the addition of 5 mL of distilled water followed by stirring. The mixture was stirred and then heated in a water bath at 100 ℃ for 45 minutes. 2 mL of toluene was added after cooling the mixture (toluene triggers the reaction and extraction of the proline-ninhydrin complex). After stirring and standing for 30 min, the optical density of the upper phase was read by spectrophotometer at 520 nm. To obtain the concentration of the proline content, a standard range was carried out using known proline concentrations under the same conditions.

2.5. Total Soluble Sugars Contents

The total soluble sugars contents were carried out using the method of [28]. Approximately 100 mg of fresh leaves were ground in 80% ethanol. After centrifugation for 10 minutes at, 5000 g, the supernatant was recovered and added to a 5% phenol solution and concentrated sulfuric acid. After cooling, the optical density was measured at 485 nm. The concentration of total soluble sugars contents was calculated using glucose in the standard curve and expressed in mg/g Fresh matter (FM).

3. Results and Discussion

3.1. Germination Parameters

The final germination percentage of the Douyet variety was FGP 90% ±1,5 and the germination energy of the seeds was GE 58% ±0.6, which is in line with the work of [17].

3.2. Effect of Amendment on Chlorophyll (Chl) Content

The chlorophyll assay revealed that, under an irrigation regime of 40% FC, the control plants showed a content of 0.12 mg/g FM, while those treated with 2%, 5% and 10% chickpea flour recorded contents of 0.28, 0.24 and 0.34 mg/g FM, respectively. This indicates that, even underwater deficit, amendment with chickpea flour resulted in an increase in chlorophyll in the aerial part compared with control plants. Under less stressful conditions, with an irrigation regime of 80% FC, the control plants showed a chlorophyll content of 0.42 mg/g FM. Plants amended with pea flour at concentrations of 2%, 5% and 10% showed higher levels, with values of 0.5, 0.44 and 0.56 mg/g FM, respectively (Figure 1). Water stress limits the vegetative growth of various legumes, notably chickpea, by causing a multitude of effects on the integrity of the plant's cellular functions, such as reduced cell elongation and cell division, leading to a reduction in photosynthetic leaf area [16, 17]. It also reduces tissue water content and decreases the rate of gas exchange [29]. It also causes a reduction in stomatal conductance, which increases leaf temperature and leads to wilting [29, 30]. Furthermore, drought causes membrane damage and disrupts chlorophyll synthesis [17, 31], which has a negative impact on the photosynthesis process.

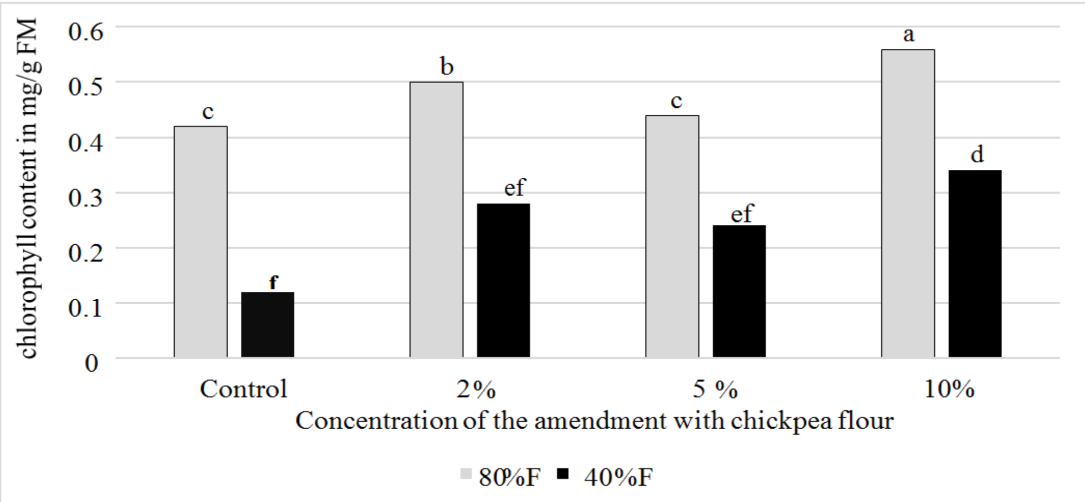

Figure 1. Chlorophyll content in chickpea plants amended with chickpea flour under two irrigation regimes (80 and 40%).

3.3. Effect of Amendment on Total Soluble Sugar Content

The results of the soluble sugar assay showed a difference between stressed and non-stressed plants. In the control plants, the concentration was 3 mg/g MF underwater stress (40% FC) and 4.9 mg/g MF in the plants irrigated at 80% FC. Plants amended with 2% chickpea flour showed a slight increase, with concentrations of 3.6 mg/g MF (40% FC) and 4.8 mg/g

MF (80% FC). At 5% concentration, the soluble sugar content was 3.1 mg/g MF (40% FC) and 8.2 mg/g MF at 80% FC. Plants amended with 10% chickpea flour showed values of 4.5 mg/g MF (40% FC) and 7.9 mg/g MF (80% FC), reflecting an increase in the concentration of soluble sugars compared with the control underwater deficit (Figure 2). The increase in sugar concentration improved drought tolerance. Indeed, sugars are thought to be involved in osmotic adjustment mechanisms [32]. They also enable membrane stability, protein protection, enzyme synthesis processes and energy production [33]. This osmoregulation phenomenon enables the maintenance of numerous physiological functions such as photosynthesis and growth, and can occur at all stages of development [34].

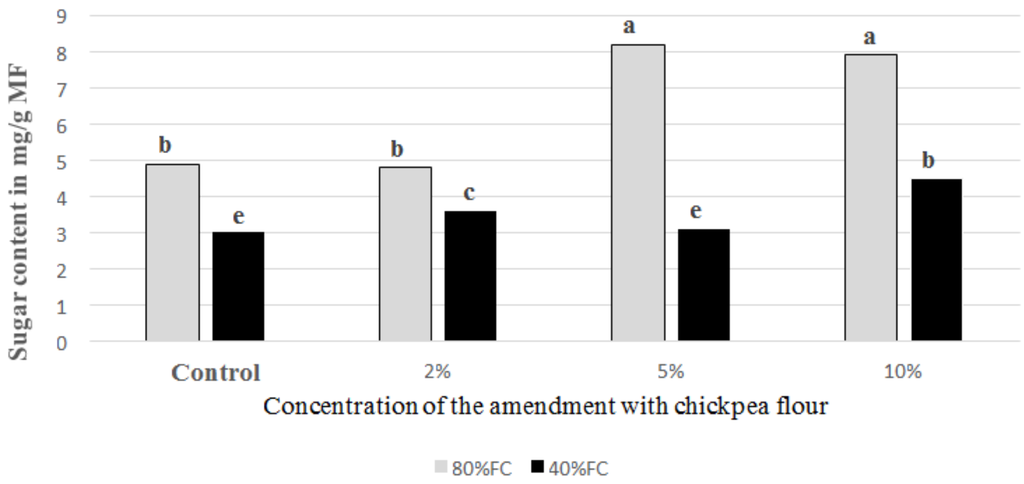

Figure 2. sugar content in chickpea plants amended with chickpea flour under two irrigation regimes (80 and 40%).

3.4. Effect of Amendment on Proline Accumulation

Proline content increased with the intensity of the chickpea flour amendment, showing greater resilience to water stress. In control plants, the proline content was 0.3 mg/g MF underwater stress and 0.7 mg/g MF under 80% FC. The proline content was 0.4 mg/g MF at 40% and 0.9 mg/g MF at 80% with the amendment of 2% chickpea flour. At 5%, the proline concentration was 0.6 mg/g MF and 0.88 mg/g MF respectively for 40 and 80% FC. At 10%, the concentration was 0.7 mg/g MF and 1.01 mg/g MF at 40% and 80% respectively (Figure 3). These results suggest that plants treated with chickpea flour can better tolerate water stress, as evidenced by the higher proline content. Our results are consistent with those of other authors, who mentioned that the increase in proline content is a protective response of plants against all factors leading to a decrease in water in the cytoplasm [35]. It has also been found that proline accumulation occurs in plant tissues in response to stresses resulting in a reduction in water content [36]. In conclusion, proline accumulation plays a defensive role in various plant stress conditions.

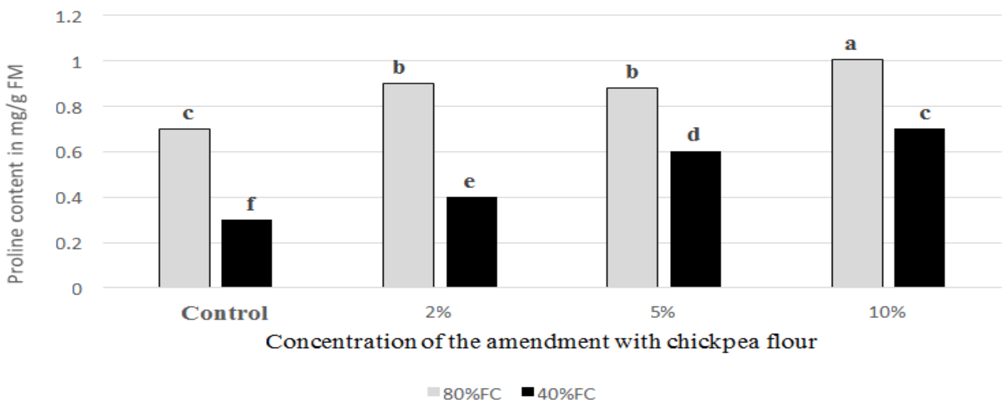

Figure 3. Proline content in chickpea plants amended with chickpea flour under two irrigation regimes (80 and 40%).

3.5. Effect of Amendment on Plant Growth Under Water Deficit

Growth of plants amended with chickpea flour showed a difference between the length of the aerial part of stressed and unstressed plants. In fact, amendment with 10% chickpea flour gave the greatest length of aerial part compared with the other concentrations, and this for both irrigation regimes (under an irrigation regime of 40%, the length increased by 26.67% compared with the control, which was 11 cm, and under an irrigation regime of 80%, the improvement was even more marked with an increase of 36%, the length going from 16 cm for the control to 25 cm for the treated plants). These results show that amendment with chickpea flour has a positive effect on the growth of chickpea plants underwater stress (Figure 4). Plant growth depends on soil quality, in fact it is a rich natural environment composed of minerals, organic matter, water and organisms with symbiotic interactions [37, 38]. Healthy soil is the basis of crop yields and the amendment improves biological properties but also physical and chemical properties of soils [38]. Organic amendments promoting plant growth in the event of water stress. Indeed, they play a key role in reducing damage due to water deficit by improving morphological and physiological characteristics [39].

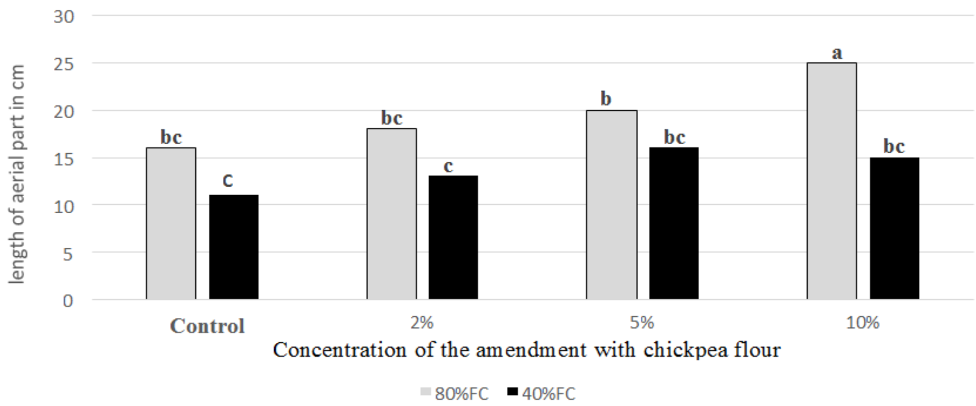

Figure 4. *Growth of chickpea plants amended with chickpea flour under two irrigation regimes (80 and 40%).*

4. Conclusion

The study showed that amendment with different concentrations of chickpea flour improved growth underwater stress. The concentration 10% amendment of chickpea flour showed a significant increase in the levels of chlorophyll, soluble sugars and proline, which are plant responses to underwater stress. These results suggest that chickpea flour could be used as an amendment to improve plant resistance under drought conditions.

Abbreviations

Chl	Chlorophyll
FC	Field Capacity
FGP	Final Germination Percentage
GE	Germination Energy
FM	Fresh Matter

Statistical Analysis

The statistical analysis was performed using SPSS Ver.21 software (IBM Corporation and Others, Ar-monk, NY, USA) and a Two-way analysis of variance (ANOVA II). The data were expressed as the mean ±standard error. Means were statistically compared using Student-Newman-Keuls's multiple-range test at the level of $p < 0.05$.

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Author Contributions

Ahmed Khadraji: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing

Ahmed Qaddoury: ResourcesVisualization

Cherki Ghoulam: ResourcesVisualization

Conflicts of Interest

The authors declare no conflicts of interest.

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