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Effect of Drought on Various Agro-physical Parameters of Chickpea (*Cicer arietinum* L.) Genotypes in a Field Experiment

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Abstract: Drought stress is one of the major factors limiting the growth and development of legumes. In order to improve water deficit tolerance of this crop, several techniques have been put into practice such as seed priming or the selection of tolerant genotypes to water stress. In 2020, field experiment was conducted to assess the drought tolerance of thirty-six chickpea genotypes (*C. arietinum*) by analyzing the behaviour of certain physiological and biochemical parameters of plants harvested in a randomized field experiment. The genotypes analysed presented a diversity of behavior concerning the accumulation of mineral elements under drought. The results showed an accumulation of inorganic ions, especially calcium and potassium (1.8 and 2 $mg.g^{-1}$, respectively) and increased proline and protein content (3.4 and 1.7 $mg.g^{-1}$, respectively) has been observed in drought tolerant chickpea genotypes. Also, the results obtained showed that the P contents in the aerial parts are generally higher for plants with a high biomass, such as the case of genotypes V36 and V32. This tends to prove the positive effect of P on plant growth. After analysis of the various parameters, the results obtained allowed us to classify the tolerant genotypes:, V36, V38 andV41, intermediates: V40 and V4 and sensitive: V17 and V28.

Keywords: Chickpea, Physiological and Biochemical Parameters, Tolerance

1. Introduction

Chickpea (*Cicer arietinum* L.) is a good contributor to the supply of energy, vegetable proteins, carbohydrates in the form of starch, fiber, several minerals (phosphorus, copper, manganese...) and vitamin B9 [1]. Chickpea contains polyphenols including flavonoids with anti-oxidant properties, as well as phytosterols and saponins, which participate in the prevention of diseases such as cardiovascular disease or even certain cancers [2, 3]. One hundred grams of mature boiled chickpeas contain little fat (3 g), composed mainly of polyunsaturated fatty acids (1.59 g) in the form of omega 6 and of monounsaturated fatty acids in the form of oleic acid. Like all plants, it does not contain cholesterol. Chickpeas are a good source of vegetable protein (8.31 g/100g). It provides on average 40% more than cereals. It also contains phosphorus (340 mg/100 g) and manganese (0.86 mg/100 g) which contributes to normal energy metabolism, protect cells against oxidative stress and contributes to a normal functioning of cell membranes [4, 5].

Chickpea (*Cicer arietinum* L) is a widely cultivated crop species throughout the world and is one of the most nutritionally, agronomically and economically important grain legumes in Morocco [6]. It is a species mainly cultivated under rainfed conditions and generally in areas with a semi-arid climate. Frequent droughts and poor distribution of rains constitute the major abiotic constraint on production. Yield losses can be the result of intermittent droughts during the vegetative phase, development or during the reproduction

phase [7]. On the other hand, Chickpea occupies the second position after the bean in terms of area and the fourth after the lentil as regards the yield. The total area under chickpea cultivation in Morocco is estimated at around 60200 ha with a yield of 718 kg ha^{-1} and a production of around 42600t [8]. Despite this importance, the yield of this legume remains unstable and very modest due to many stresses related to nutrient deficiencies (such as phosphorus deficiency) and water (mainly, osmotic stress), temperature variations, acidity soil, toxicity due to elements such as manganese and aluminum, and salinity [9-11].

Drought is one of the most abiotic stress affecting crop growth and productivity [12]. Moreover, the poor distribution of rains and osmotic stress constitute the major abiotic constraint in chickpea production [7]. Drought affects Chickpea growth, reduces grain yield and quality, and causes morphological, physiological, biochemical and molecular alterations [13]. Drought causes nutrient disturbances such as decreased rate of diffusion of nutrients (sodium (Na) / calcium (Ca), potassium (K)) in the soil and restricted transpiration rates in plants. ionic deficiency can occur in expanding leaves in drought [14, 15]. P available in soil is influenced by a number of factors, including soil type and environmental conditions, as well as land use and management practices. In natural ecosystems, plant growth is often limited by the availability of P [16]. The responses of chickpeas to water deficit are essentially molecular and involve several compounds such as osmoregulators (proline, proteins and sugars) aimed at to adapt the plant to the imposed water stress [6-17].

Thus, the objective of the present work is to analyze certain agronomic, physiological and biochemical responses of thirty-six genotypes of chickpea (*C. arietinum*) in a field experiment under drought.

2. Materiels and Methodes

The study was carried out at Jemâat Shaim station, which is located in the agricultural area of Abda. It is located at an altitude of 180 m, its geographical location is (08°.00'E, 32°.00'N). It is a site with a dry climate and an average annual rainfall of 320 mm and well-developed deep clay soils. However, the rainfall balance for 2019 was 60% deficit compared to the usual rainfall in the Abda region.

Sampling of plants: A randomized field experiment was carried out to determine the effect of drought to various agro-physical parameters on chickpea genotypes in INRA Settat fields. In order to assess 36 genotypes for their tolerance to water deficit, 3 plants per block were chosen at random. Each plant was taken with its rhizospheric soil and was put in an individual plastic bag and brought back to the laboratory to perform the various analyzes.

Analysis of the plants sampled: various agronomic parameters were determined for the plants harvested: the length of the aerial and root parts, the number of pods per plant, the fresh root and aerial weight as well as the dry weight obtained after drying for 48 hours in the oven at 80°C.

Mineral analysis of the plants: 0.5g of the aerial and root dry matter was crushed and calcined at 600°C in an oven for 6 hours. The ashes were collected in 3mL of hydrochloric acid (10N), the suspensions were diluted in distilled water and filtered. These filtrates were used for the determination of phosphorus and various mineral elements (sodium, potassium and calcium).

Determination of total phosphorus in plants and roots: it was determined in the different parts of the plant. 4mL of distilled water and 5mL of reagent AB were added to 1mL of the filtrate, the whole was placed in a water bath at 95°C for 10min. The optical density was measured with a spectrophotometer at 825nm.

Determination of mineral elements (K, Na and Ca): the potassium, sodium and calcium contents in the two parts of the plant samples were determined using a Jenway flame spectrophotometer.

Protein assay: it was carried out according to the method of [18]. 50 mg of the fresh material was cold ground in 2 mL of 0.1M Tris-HCl buffer at pH 7.5. Then the ground material was collected in tubes and then centrifuged at 16000xg for 15min. The supernatant was collected, after centrifugation, in tubes to which 4mL of distilled water and 2mL of Bradford's reagent were added, with stirring to homogenize the whole. Subsequently the optical densities were read at 595nm. A standard range was established by solutions of bovine serum albumin (BSA).

Proline assay: it was performed according to the method of [19]. A 0.2 to 1mL aliquot was taken from the upper phase, 10mL of distilled water was added followed 5mL of ninhydrin and 5mL of glacial acetic acid. After stirring and heating in a water bath at 100°C. for 45 min, the mixture was cooled and added with 2 mL of toluene. The extraction of the proline-ninhydrin complex formed was carried out by adding 2mL of toluene to the various tubes after cooling. After stirring and standing for 30 min, the optical density of the upper phase was read by spectrophotometry at 520 nm. The concentration of the proline content was determined using a standard range produced under the same conditions from the different concentrations of proline.

Statistical Analysis: the results were expressed as the mean ± the standard error. Statistical comparisons were made using SPSS software (version 20) with Tukey's test. The differences were found to be significant at $p < 0.05$.

3. Results and Discussion

3.1. Comparison of Some Growth Parameters

The results of the principal component analysis of the correlation between the 36 genotypes of chickpea (*C.arietinum*) and certain physiological parameters (number of pods, stem length, Fresh weight stem, dry weight stem, fresh weight root and dry weight root), show that the first two axes of the PCA represent 72.51% of the variability between samples.

Chickpea genotypes showed significant genotypic variability for the agronomic and biochemical parameters

studied. In fact our results are in the same direction with [20] who ensured that the empirical selection of genotypes is based on grain yield and the importance of physiological indicators in the screening of drought tolerant genotypes. [21] demonstrated the sensitivity of chickpeas to deficit conditions during the reproductive phase and especially during the seed filling and maturity phases. Moreover, the same study specified that the exposure of a chickpea crop to temperatures above 30°C induces the sterility of 50% of the flowers and a gradual reduction in grain yield. The results of [22] showed that a water deficit induced a decrease in the yield of seeds of (*Medicago sativa* L.) as well as a decrease in biomass and plant vigor. This confirms our results for certain genotypes such as V16, V22, V28, V8 and V19 which presented a small number of pods and a low biomass (Figure 1). In fact, the results of [23] on the analysis of the tolerance to water deficit of five different species indicated that the morpho-physiological parameters of plants were negatively affected by water restriction, except some species such as Acacia senegal which have shown their performance due to their low sensitivity to water stress. The most tolerants varieties in our study are V2, V7, V9, V24, V32, V35 and V36 (Figure 1).

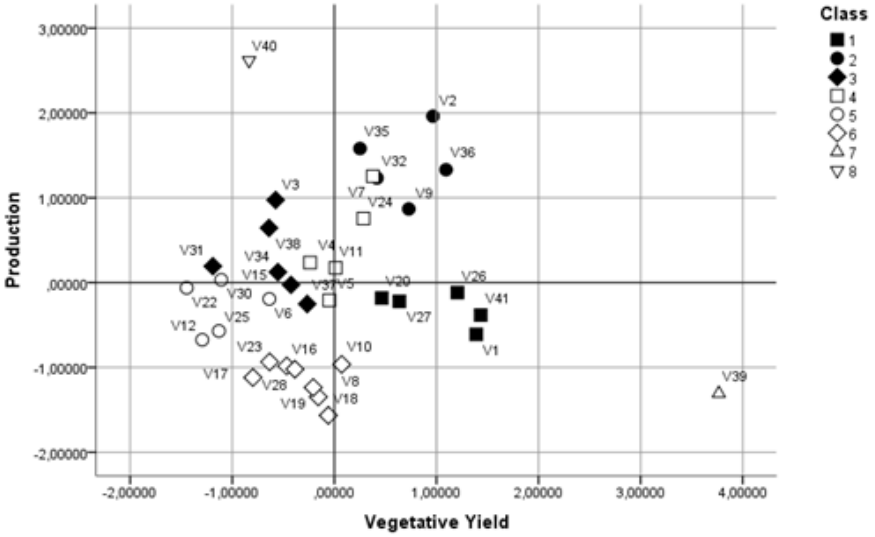

Figure 1. *The relationship of vegetative yield (biomass) and production (pods) for 36 chickpea genotypes.*

3.2. Comparison at the Biochemical Level

3.2.1. Mineral Elements in the Aerial Part

The results of the principal component analysis of the correlation between the 36 chickpea genotypes and certain mineral elements in the aerial part, show that the first two axes of the PCA represent 62.13% of the total variability between samples. Figure 2 shows that the K Plant is significantly and positively correlated (r=0.93) while characterizing component 1. Likewise the parameters P Plant and Ca Pant, which characterize component 2, are positively and significantly correlated with each other (r=0.75 and r=0.7, respectively).

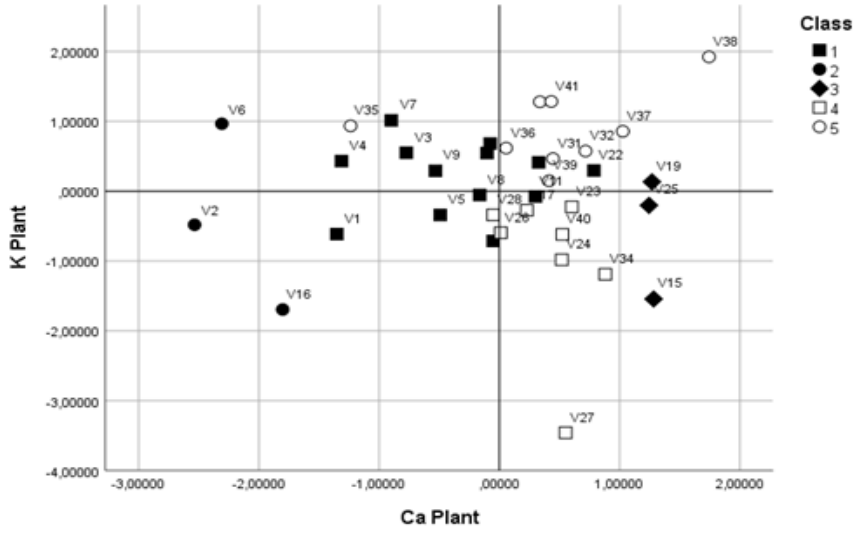

Figure 2. *The mineral elements in the aerial part (K and Ca) for 36 chickpea genotypes.*

3.2.2. Mineral Elements in the Root Part

The results of the principal component analysis of the correlation between the 36 chickpea genotypes and certain mineral elements in the root part, show that the first two axes of the PCA represent 76.03% of the total variability between samples. Figure 3 shows that the root parameters Na, K and Ca are correlated with each other significantly and positively while characterizing component 1. Likewise, the P root which characterizes component 2 is positively and significantly correlated (r=0.92). The varieties containing the highest levels of calcium and phosphorus present in the roots are V3, V4, V18, V24 and V26 (Figure 3).

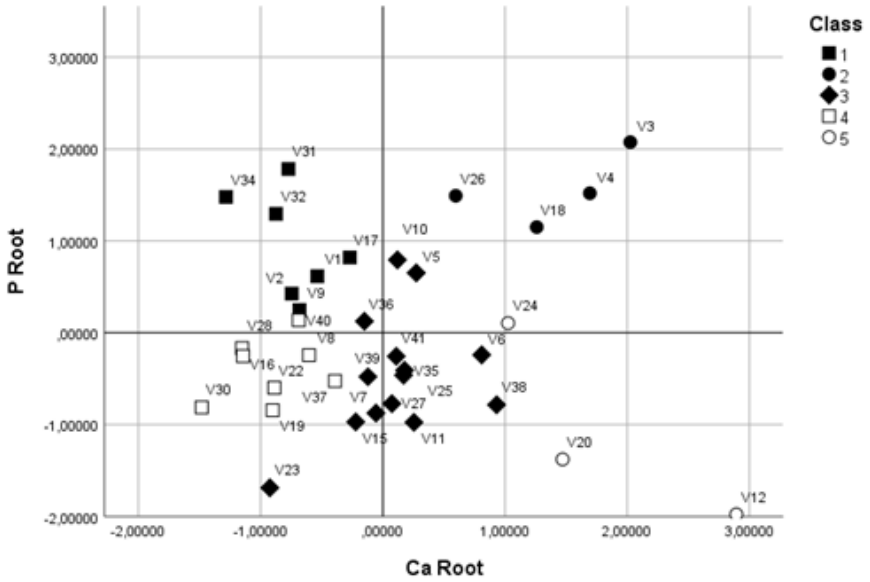

Figure 3. *The mineral elements in the root part (P and Ca) for 36 chickpea genotypes.*

3.2.3. Proteins and Proline

The results of the principal component analysis of the correlation between the 36 chickpea genotypes and certain mineral elements in the root part, show that the first two axes of the PCA represent 73.68% of the total variability between samples. Figure 4 shows that proline, polyphenols and flavonoids are correlated, with each other, significantly while characterizing component 1. Likewise, the level of proteins and proline which characterizes component 2 is positively and significantly correlated (r=0.98 and r=0.87).

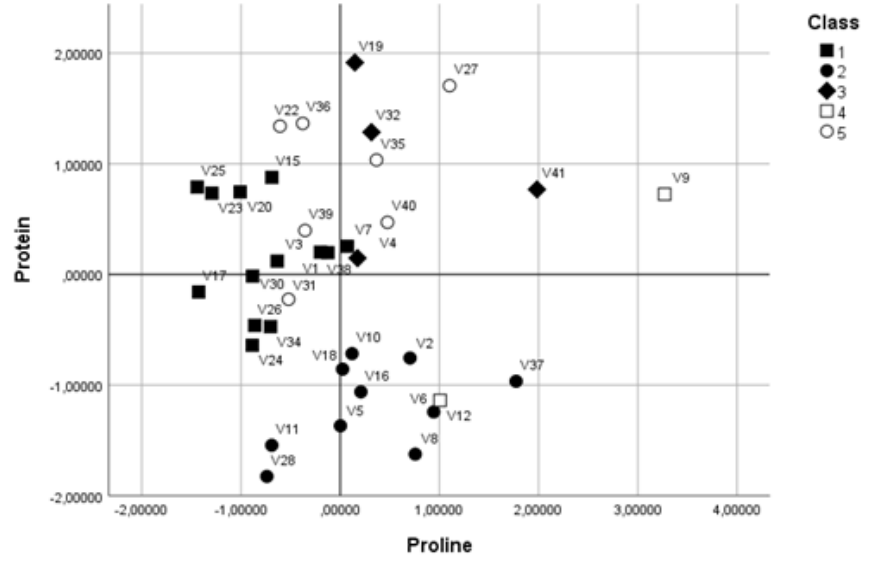

Figure 4. *Dosage of protein and proline in the root part for 36 chickpea genotypes.*

Based on the results of statistical analysis, tolerance to applied water stress differs from genotype to genotype. Moreover, different genotypes have been revealed: the genotypes which showed the best tolerance towards the growth parameters (biomass and production) are: V2, V7, V9, V11, V24, V32, V35 and V36 in addition to the genotypes: V1,

V10, V20, V27, V26, V39 and V41. The genotypes which showed the best tolerance towards the biochemical parameters (contents of mineral elements (P, Na, K and Ca) of the aerial and root parts, and contents of proteins, of proline) are: V3, V19, V20, V22, V30, V32, V36, V37, V39, and V38, V41 in addition to genotypes V4, V5, V7, V10, V18, V24, V26 and V35.

The results obtained showed that the P contents in the aerial parts are generally higher for plants with a high biomass, such as the case of genotypes V36 and V32. This tends to prove the positive effect of P on plant growth. Likewise, [24] showed that the accumulation of P was significantly correlated with biomass of cultivated plants. Indeed, from the results, we observed a positive correlation between the potassium and sodium contents of plants with the richness of the soil in assimilable phosphorus, which provides information on the tolerance of the genotypes under water stress. Moreover, the genotypes studied presented a diversity of behavior concerning the accumulation of mineral elements under drought, as well as differences between the absorption organs and the photosynthetic organs (roots and leaves), which is in agreement with the work of [25] on genotypes of wheat (*Triticum durum*). Indeed, the drought restricts the supply of plants with essential nutrients, stressed genotypes accumulate Na^+, K^+ and Ca^{++} in their organs (leaves and roots) by severely limiting the supply of these elements [26]. Our results were able to reflect the behavior of genotypes tolerant to drought: a strong accumulation of Na^+, Ca^{++} and K^+, the case of genotypes V38, V20 and V41. In fact, plants accumulate substances with an osmoprotective effect such as inorganic ions such as K^+ and Na^+, thus reflecting one of the defense strategies, which has been shown in our results. The results of [27] showed the same tendency, in alfalfa plants under water deficit, to increase the concentration of Na^+ and K^+ in both the aerial and root parts. On the other hand, Amede and Schubert, observed in 2003 a strong accumulation of K^+ in bean plants under water stress. The increase in these inorganic ions constitutes an important role in maintaining the turgor of plant cells, and, consequently, a greater water potential. The accumulation of inorganic ions, more precisely K^+, which plays a very important role in tolerance to abiotic stress, through its activity in stomatal and enzymatic regulation [28].

On the other hand, it was determined that the effect of drought on the chickpea genotypes caused an increase in the foliar content of proline, proteins and total soluble carbohydrates, which constitutes a criterion of tolerance of the genotypes. in question, which agrees with the research results of [6] on chickpeas and those of [29], the latter showed that the rapeseed variety most resistant to water stress was characterized by a significant accumulation of proline. In fact the accumulation of proline would be involved in the protection of the cell membrane and would participate in osmotic adjustment thus it will constitute reduced carbon reserves and nitrogen, used by the plant after the stress period [30, 31]. Regarding proline and protein accumulation, a set of genotypes (V41, V32, V35..) showed a higher rate than others, these results are in the same direction as those of [6] who concluded that water stress tolerant chickpea plants tend to accumulate strongly total soluble sugars and total free amino acids. This increase is an adaptation reaction of the plant to stress, and can be explained by its osmotic adjustment effect (osmoticum), to balance the osmotic potential of the soil [32].

4. Conclusion

Water stress induced changes in all the parameters considered in the comparison between 36 genotypes of chickpea cultivated under water deficiency. The principal component analysis of the characteristics of the genotypes allowed a classification of the genotypes which is in agreement with their level of tolerance. This study therefore shows that the selection of plants for tolerance to water deficit in chickpeas can be guided by the identification of convincing biochemical and physiological criteria (biomass, yield, mineral content, etc.).

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