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# CHICKPEA (*CICER ARIETINUM* L.) RESPONSE TO SALINITY CONDITIONS THROUGH BIOCHEMICAL COMPOSITION

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## SUMMARY

The timely study aimed to evaluate the salt resistance in chickpea (*Cicer arietinum* L.) cultivars under the influence of salinity stress conditions. The research analyzed variations in antioxidant enzyme activity, osmoprotective metabolites and oxidative stress indicators, and protein content in grains to select the promising genotypes. The results revealed that under salinity stress conditions, the activities of superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD) displayed sharp increases in chickpea cultivars, with the highest index recorded in the chickpea cultivar Polvon (with 6 times SOD). In chickpea cultivars Darmon and Gulistan, the proline accumulation increased to 2–3 times as compared to the control cultivar, while it was a bit higher in the cultivar Polvon than the control. The hydrogen peroxide ($H_2O_2$) and malondialdehyde (MDA) content decreased in cultivars Polvon and Darmon, while the MDA increased in the cultivar Gulistan. Salinity caused a reduction in the grain protein content in all cultivars. In conclusion, antioxidant enzymes and osmoprotective metabolites proved important in chickpea genotypes' salt stress tolerance.

**Keywords:** Chickpea (*C. arietinum* L.), cultivars, salinity, antioxidant enzymes, proline, ascorbic acid, malondialdehyde, hydrogen peroxide, glutathione, protein

**Key findings:** The degree of chickpea (*C. arietinum* L.) genotypes' adaptation to salinity stress significantly varied. Cultivars Polvon and Darmon showed the highest salt-stress resistance through a considerable self-defense system.

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## INTRODUCTION

Legumes, including chickpeas (*Cicer arietinum* L.), are crucial for food security, especially in the developing countries in the Mediterranean region, as a relatively inexpensive source of protein, vitamins, and minerals. They occupy 12%–15% of the arable area worldwide and account for around 27% of total crop production. Abiotic stress factors, particularly salinity, are one of the most important environmental stresses negatively affecting the growth, development, and productivity of the legume crops (Bouzroud *et al.,* 2023).

Excessive accumulation of sodium chloride (NaCl), or salt, in the arable soil causes two types of negative processes in crop plants, i.e., osmotic and ionic stress conditions. With osmotic stress, the ability of plants to absorb water and mineral elements decreases (Khan *et al.,* 2012). Under such conditions, various inorganic and organic osmoprotective substances begin to accumulate in the plant cells. These bioactives are small and non-toxic molecules that perform a protective function by increasing the osmotic potential of the cells and ensuring the stability of cell membranes. These compounds comprise proline, ascorbate, and glutathione proteins (Kumar *et al.,* 2018). A sharp increase in plants' proline content is considerably the common physiological response to various abiotic stress factors (Hnilickova *et al.,* 2021).

Ascorbic acid (AsA) is a water-soluble vitamin, considered an important antioxidant found in large quantities in various crop plant tissues, especially in leaves. It is one of the most effective bioactive substances that reduce the negative impact of abiotic stress conditions (Krupa-Małkiewicz *et al.,* 2015). Excessive production of reactive oxygen species in crop plants severely damages the biomolecules, such as proteins, lipids, and nucleic acids, causing disruption in cell functions, viz., cell elongation and multiplication processes. As a result, plant growth and development slow down, which eventually affects plant productivity. In reducing the negative effects of reactive oxygen species, plants activate various defense mechanisms, including enzymatic and non-enzymatic antioxidant systems. The plant's self-activation system includes superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), glutathione reductase (GR), ascorbic acid (AsA), glutathione, and other antioxidants (You and Chan, 2015).

Superoxide dismutase (SOD) plays a primary role in plant detoxification and constitutes the first line of defense against the toxic effects of reactive oxygen species (Berwal and Ram, 2018). SOD converts superoxide radicals into $H_2O_2$ and $O_2$, then CAT and POXs decompose the derived hydrogen peroxide into water and oxygen molecules (Rangani *et al.,* 2016). Similarly, peroxidase has become one of the main enzymes that respond to stress conditions faced by crop plants. However, its activity reaches the highest level as a result of the influence of external stress factors on plants, in particular mechanical factors, and growth under arid and saline soil conditions (Amonova and Matniyazova, 2022). The presented study aimed to evaluate the salt resistance in chickpea *(C. arietinum* L.*)* genotypes under salinity stress conditions by analyzing variations in antioxidant enzyme activity, osmo-protective metabolites and oxidative stress indicators, and protein content in grains. The research also sought to select the promising genotypes.

## MATERIALS AND METHODS

### Experimental procedure

The research, conducted in 2024–2025, had two different agroecological conditions, i.e., a) the Tashkent Region with non-saline soils and b) the Navoi Region with moderately saline soils. The chickpea genotype plants grown in the Tashkent Region served as the control environment, with plants in the Navoi Region considered as saline conditions (Table 1). Some certain differences in climate and soil properties may exist between these two regions; however, in this research, the main focus centered on the salinity factor, with the observed differences interpreted mainly in terms of the salt-stress effects. The chickpea cultivars Gulistan, Darmon, and Polvon were

**Table 1.** Salinity type, level, and nutrient content of soil samples taken at different depths in Tashkent and Navoi regions (mean ± SD, n = 3).

| Layer | Depth (cm) | Cl | | $SO_4$ | | Type of salinity | Salinity level | pH | $K_2O$ mg/k (g) | $P_2O_5$ mg/k (g) | N-$NO_3$ (mg/100g) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | % | Miligram equivalent | % | Miligram equivalent | | | | | | |
| Tashkent region | | | | | | | | | | | |
| 1 | 0-30 | 0.022 | 0.063 | 0.094 | 0.21 | Chloride | Non-saline | 8.3 | 174 | 26 | 1.5 |
| 2 | 30-70 | 0.035 | 0.096 | 0.046 | 0.096 | Sulfate chloride | Non-saline | 7.8 | 120 | 20 | 1.36 |
| Navoiy region | | | | | | | | | | | |
| 1 | 0-30 | 0.026 | 0.52 | 0.51 | 1.06 | Sulfate | Medium saline | 8.6 | 237 | 24 | 1.1 |
| 2 | 30-70 | 0.058 | 0.16 | 0.45 | 0.93 | Sulfate | Medium saline | 8.2 | 193 | 18 | 0.5 |

samples used as breeding material in the study. The study took place during the flowering stage of the plants.

**Antioxidant enzymes' measurement**

Leaf tissues incurred homogenization in 0.1 M phosphate buffer (pH 7.5) containing 1% PVPP, 0.1 mM EDTA, and 0.1% Triton X-100 at (4 °C). The derived homogenate received centrifugation at 15,000 g for 20 min, afterward obtaining a free extract for enzymatic analysis.

The determination of SOD activity was by the method of inhibition of the photochemical reduction reaction with sodium riboflavin and nitroblue tetrazolium (NBT). The reaction medium contained 13 mM methionine, 75 µM NBT, 2 µM riboflavin, and 0.1 mM EDTA. After adding the sample, assessment of the mixture continued by the increase in absorbance at 560 nm. Enzyme activity expression was one unit of SOD = the amount (U/mg protein) that inhibits NBT photoreduction by 50%, quantifying the enzyme based on the percent inhibition it caused (Giannopolitis and Ries, 1977). The CAT enzyme activity determination was according to the method recommended by Sinha. Herein, dichromate reduction ensued to chromium acetate when heated in acetic acid with hydrogen peroxide ($H_2O_2$). In this process, generating an unstable intermediate substance, perchromatic acid, succeeded. Enzyme activity expression of CAT was µmol $H_2O_2$ decomposition/min mg protein (Hadwan, 2016).

In determining the POD activity, a method based on the oxidation of a guaiacol substrate proceeded. The reaction mixture contained 50 mM phosphate buffer (pH 6.0), 20 mM guaiacol solution, 10 mM $H_2O_2$ and the sample extract. The increase in absorbance (ΔA_470_) entailed recording at 470 nm for 1 min. Enzyme activity expression was the change in ΔA_470_ per minute (1 unit of optical density/min) (Boyarkin, 1951).

**Oxidative stress indicators' assessment**

The quantity of hydrogen peroxide ($H_2O_2$) detected underwent colorimetric determination using potassium iodide (KI) reagent according to the method of Velikova *et al.* (2000). The MDA quantity measurement by photometric analysis of the colored complex produced with thiobarbituric acid (TBA) (Rogojin, 2006).

**Metabolites' determination**

Proline content determination ran by the acid-ninhydrin reaction according to the method of Bates *et al.* (1973). The free reducing sugars content resulted in using the anthrone method developed by Yemm and Willis (1954). In the samples, obtaining the amount of total free sugars was in mg/g relative to the glucose standard. Ascorbic acid content (AsA)

determination employed the method of Mukherjee and Choudhuri (1983). The computation of the ratio of reduced and oxidized forms of glutathione (GSH/GSSG) followed the method of Griffith (1980).

**Total protein determination in grains**

The use of the Kjeldahl method determined the total protein content in the chickpea grains. The total protein content of plant samples, when determined, relied on the total nitrogen content, with the sample quantitatively carried out in a Kjeldahl flask. Then, the experiment continued according to the specific instructions (Rukovodstvo, 2004).

**Statistical analysis**

All experimental results' expression was the mean ± standard deviation (SD) on three replications. The data processing and analysis used the analysis of variance (ANOVA) in STAT VIEW software. The significant differences between the control and saline treatments succeeded in their determination by the LSD (least significant difference) test at three significance levels, i.e., $P \leq 0.05$ (*), $P \leq 0.01$ (**), and $P \leq 0.001$ (***).

**RESULTS AND DISCUSSION**

**Antioxidant enzymes**

According to the results, antioxidant enzyme activities sustained enhancements with varied responses, depending on the chickpea cultivars under salinity conditions. However, in control conditions, the SOD activity of the cultivar Polvon was slightly lower than the two other cultivars, Darmon and Gulistan (44.3 and 55.9 versus 63.1 U/mg proteins, respectively). With the influence of salinity, the SOD in cultivar Polvon increased sharply by about 6.4 times, reaching 283.1 U/mg protein. In cultivars Darmon and Gulistan, the SOD activity increased by 3.5 and about 1.5 times under saline conditions, respectively (Figure 1A). Salinity stress causes an increased accumulation of ROS, which leads to oxidative damage and disruption of redox homeostasis of tissues (Abd-Elgawad *et al.,* 2016). SOD, CAT, and POD play a vital role in the elimination of ROS; they become more active under stress conditions (Kusvuran *et al.,* 2016).

The same tendency was evident for CAT activity. The catalase activity increased by about three times in the cultivars Polvon and Gulistan under salinity conditions, while in the cultivar Darmon, this indicator rose about 1.5 times (from 284.8 to 419.1 μmol/min·mg protein, Figure 1B). Peroxidase (POD) activity determination revealed increases of 6 and 6.3 times in chickpea cultivars Polvon and Gulistan, respectively, and by about three times in the cultivar Darmon (Figure 1C). As shown in Figure 1, the SOD and POD activities of the cultivar Polvon under saline conditions were much higher than those of cultivars Darmon and Gulistan, indicating these chickpea genotypes have a considerable antioxidant defense response. In species belonging to the genus *Malus*, the activities of SOD, POD, and CAT enzymes had reports stating these increase markedly under salt stress conditions (Wang *et al.,* 2022).

**Oxidative stress indicators**

In this research, different patterns appeared in the amount of $H_2O_2$ and MDA depending on the antioxidant activity of the chickpea cultivars (Figure 2A). On the contrary, the $H_2O_2$ contents were significantly lower in the leaves of cultivars Polvon and Darmon under salt stress conditions (from 841 to 66.6 μmol/g and 863.5 to 277.1 μmol/g, respectively). In the cultivar Gulistan, the amount of $H_2O_2$ slightly decreased (from 482.3 to 83.0 μmol/g); however, under saline environments in Gulistan leaves, the $H_2O_2$ concentration was higher than that of the cultivar Polvon. Salinity stress conditions increased the oxidative stress in plants, and this was usually evident through an increase in MDA and $H_2O_2$ (Sachdev *et al.,* 2021).

The MDA content notably decreased twice in the cultivar Polvon under salinity conditions, compared to the control option (from 4.58 to 2.51 nmol/g). In the cultivar Darmon, the MDA decreased by 35% (from

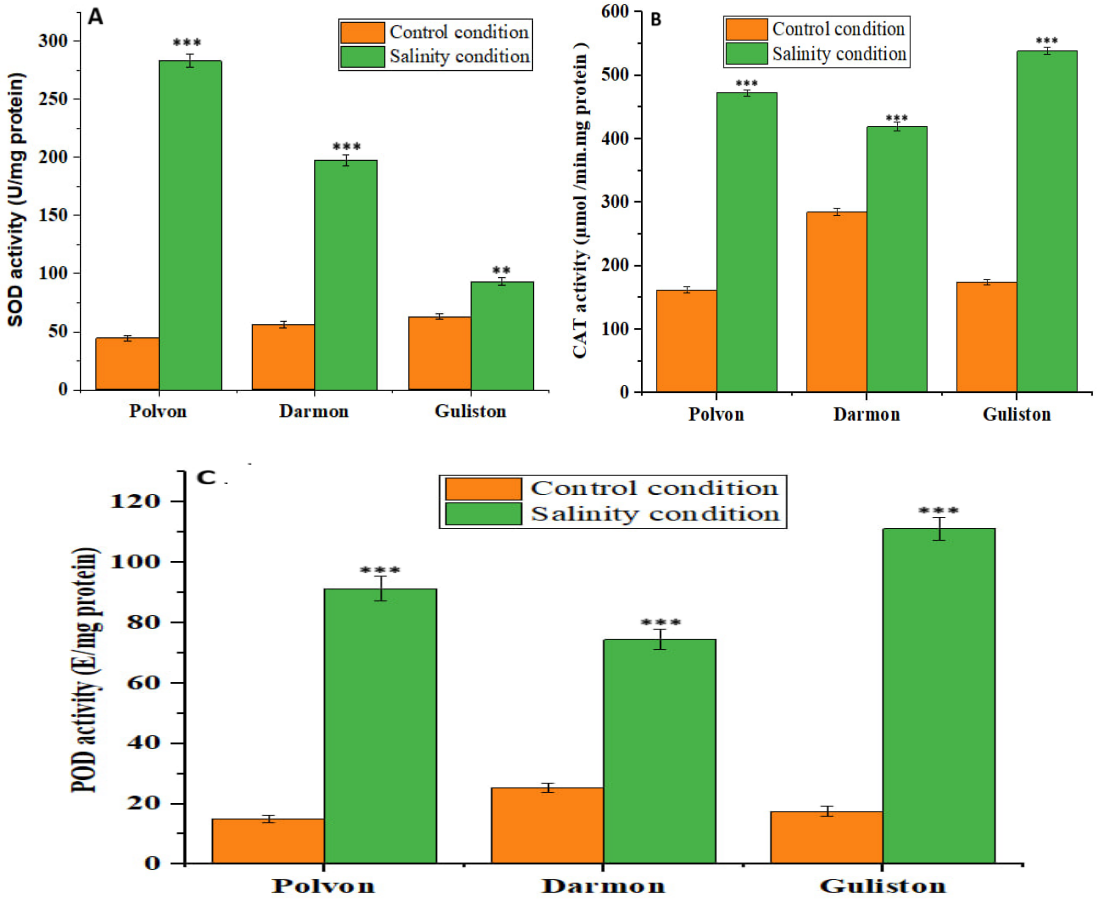


**Figure 1.** Variations in the activity of antioxidant enzymes in chickpea cultivar leaves under the influence of salinity conditions (activity mean ± SD, n = 3). A) superoxide dismutase (SOD), B) catalase (CAT), and C) peroxidase (POD). Significant differences compared with the control (*$P$ < 0.05, **$P$ < 0.01, ***$P$ < 0.001).

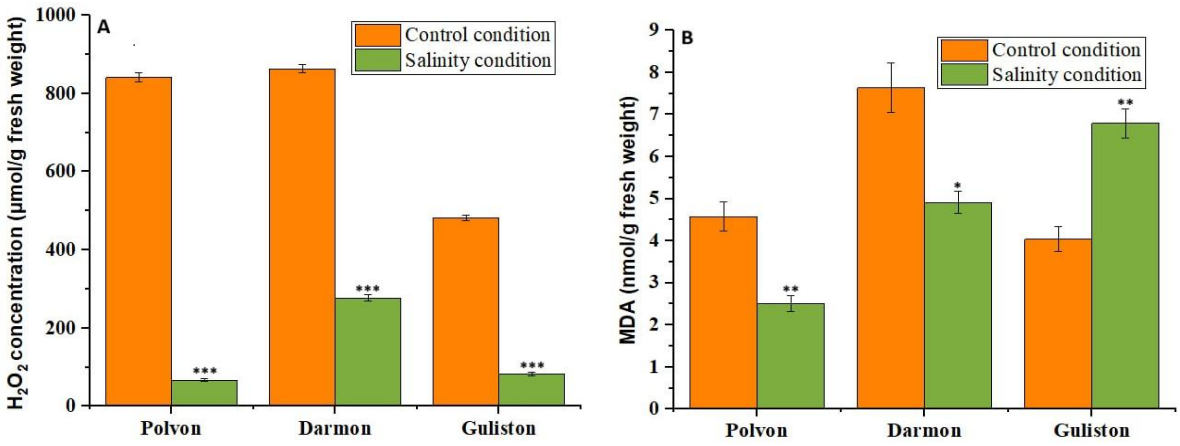


**Figure 2.** Oxidative stress indicators ($H_2O_2$ and MDA) with varied content in chickpea cultivar leaves under the influence of salinity conditions (mean ± SD). A) $H_2O_2$ and B) MDA. Significant differences compared with the control (*$P$ < 0.05, **$P$ < 0.01, ***$P$ < 0.001).

**Table 2.** Metabolites with varied values in chickpea cultivar leaves under the influence of salinity conditions (mean ± SD). Proline - µg/g fresh weight; free sugars mg/g, ascorbic acid (AsA) (mg/g), GSH/GSSG - ratio of reduced and oxidized forms of glutathione (the share of reduced glutathione in percents). Significant differences compared with the control (*p < 0.05, **p < 0.01, ***p < 0.001).

| Options | Chickpea cultivars | Proline (µg/g) | Free sugars (mg/g) | Ascorbic acid (mg/g) | GSH/GSSG ratio |
|---|---|---|---|---|---|
| Control condition | Polvon | 742±12.2 | 147.9±2.81 | 2.0±0.14 | 9.0±0.5 |
| | Darmon | 667±8,18 | 139.5±2.25 | 1.8±0.15 | 8.0±0.4 |
| | Gulistan | 510±7.53 | 138.8±2.28 | 1.9±0.17 | 8.5±0.5 |
| Saline condition | Polvon | 788±11.7 | 140.6±2.54 | 2.3±0.14 | 8.0±0.4 |
| | Darmon | 1454±17.7*** | 139.6±1.67 | 2.1±0.17 | 6.0±0.3* |
| | Gulistan | 1335±19.5*** | 145.0±3.56 | 1.5±0.12 | 4.0±0.2** |

7.63 to 4.91 nmol/g). In contrast, in the cultivar Gulistan, the MDA increased from 4.04 nmol/g in the control, reaching 6.79 nmol/g under salt stress conditions (Figure 2B). Thus, the level of lipid peroxidation decreased in two cultivars, Polvon and Darmon, under salinity conditions, while it increased in the cultivar Gulistan. The results further revealed cultivars Polvon and Darmon could limit oxidative damage at the expense of considerable antioxidant protection, while in the cultivar Gulistan, the harmful effects of ROS increased as the antioxidant system defense was insufficient.

In the chickpea cultivar Polvon, the sharp increase in activities of SOD, CAT, and POD and the decrease in $H_2O_2$ and MDA levels were noteworthy under the influence of salinity, indicating the robust antioxidant system (Figure 2). In cultivar Darmon, the activity of antioxidant enzymes also increased, and MDA and $H_2O_2$ decreased; however, this extent is unequal, as observed in the cultivar Polvon. Past studies also enunciated similar results in chickpea leaves, where a decrease in $H_2O_2$ and MDA and an increase in SOD activity were remarkable under the highest salinity stress conditions (Ozturk *et al.,* 2012).

In the cultivar Gulistan, the SOD activity almost showed no changes, though CAT, POD, and MDA activities increased, while $H_2O_2$ did not completely decrease. This indicates the said cultivar was highly sensitive to salt stress conditions. An increase in MDA and $H_2O_2$ content was evident under the influence of salinity stress in tomato (Aazami *et al.,* 2021) and maize crops (Zamani *et al.,* 2024).

**Proline, free sugars, ascorbic acid, and glutathione**

In the latest study, the proline content also differed based on the chickpea cultivars under salinity stress conditions. In cultivars Darmon and Gulistan, the proline content increased by 54% and 61%, respectively, as influenced by salinity stress, while in the cultivar Polvon, the proline was already high in the control condition and slightly increased (by 6%) in stress conditions (Table 2). This indicates that, given its highest basic level, the cultivar Polvon did not need to produce additional proline. However, a massive proline amount materialized in the cultivar Gulistan, but this enzymatic defense was insufficient to provide full resistance to salt stress conditions. Similar results have also come out from previous studies of Kaur *et al.* (2022) and Suleiman *et al.* (2023). Proline protects them from damage by scavenging ROS generated within stress conditions; besides, it is also important for stabilizing proteins, membranes, and subcellular structures (Hnilickova *et al.,* 2021).

Along with proline, ascorbic acid also increased in cultivars Polvon and Darmon by 13% and 14%, respectively, while in the cultivar Gulistan*,* it decreased by 21% under salinity stress conditions (Table 2). Significant differences were noticeable among the cultivars in the proportion of reduced form of glutathione (GSH/GSSG ratio) (Table 2).

Although the GSH/GSSG ratio in the leaves of cultivars Polvon and Darmon had slight decreases (from 9.0 to 8.0 and from 8.0 to 6.0, respectively) under the influence of salinity, a rather high proportion of reduced glutathione remained in these chickpea cultivars. In this research, the high retention of AsA and GSH in the cultivar Polvon indicated that this cycle worked efficiently (Table 2). Studies on *Arabidopsis* mutants also showed the importance of ascorbic acid and the reduced form of glutathione in neutralizing ROS activities (Rudenko *et al.,* 2023). According to these present results, the ascorbic acid increase in the cultivars Polvon and Darmon and the high retention of the GSH/GSSG ratio in the cultivar Polvon succeeded in their determination. Meanwhile, in the cultivar Gulistan, the said ratio dropped below 50%. A decrease in the GSH/GSSG ratio revealed an increased oxidative environment in a cell (Kaur *et al.,* 2023). Cultivars with a stable GSH/GSSG ratio usually tended to be salt tolerant, such as the cultivar Pokkali (Hasanuzzaman *et al.,* 2017).

In chickpea genotypes, the variations in the free-reducing sugars' content were not significant under salinity stress conditions (Table 2). Under the control condition, almost the same level of sugar accumulation resulted in the chickpea cultivar leaves (≈139–148 mg/g). Under salinity conditions, the sugar content in the cultivar Polvon gave a slight decrease (140.6 mg/g), while in the cultivar Gulistan, conversely, it slightly increased (145.0 mg/g). In the cultivar Darmon, however, these variations for free-reducing sugars were nonsignificant. Thus, salt stress had no significant effect on the free-sugar reserves for the studied chickpea cultivars. Wani and Hossain (2015) and Gammoudi *et al*. (2016) also reported similar findings on free-reducing sugars in crop plants under salt stress conditions.

**Total protein**

Under salinity stress conditions, the reduction in protein synthesis has had wide reports in legumes, with this phenomenon explained by several factors. According to this study's present results, salinity stress had a significant negative effect on the accumulation of protein in the chickpea grains. The chickpea genotypes grown under control conditions emerged with the highest protein content, with the highest value recorded in the cultivar Darmon (26.2%), followed by cultivars Gulistan (22.6%) and Polvon (23.9%) (Figure 3). The same can refer to more active mechanisms of nitrogen and ammonia assimilation and its conversion to protein biosynthesis in the cultivar Darmon. Firstly, the excess of $Na^+$ and $Cl^-$ ions disrupts the cellular ionic balance and inhibits the activity of biosynthetic enzymes (Bouzroud *et al.,* 2023). Secondly, salt stress

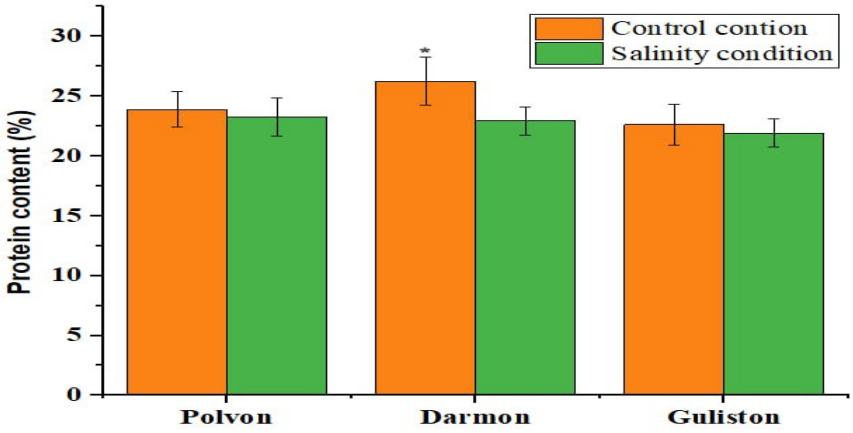


**Figure 3.** Protein with varied content in the chickpea cultivar grains under the influence of salinity conditions. Significant differences compared with the control (*$P$ < 0.05, **$P$ < 0.01, ***$P$ < 0.001).

conditions limit photosynthesis, and due to decay of stomatal closure and chloroplast activity, it reduces the carbohydrate and energy sources (Khan *et al.,* 2022). Thirdly, salinity stress reduces the nitrogen ($NO_3^-$, $NH_4^+$) uptake and nitrate reductase activity and, eventually, limits the amino acid and protein biosynthesis (Zayed *et al.,* 2023).

Under saline stress conditions, a sharp decrease occurred in the protein accumulation in chickpea cultivar grains. This can be because of the disruption of ionic balance in plants by the highest level of sodium and chlorine ions in the soil, restricting nitrogen metabolism processes. As a result, this reduced the activity of enzymes involved in protein synthesis. Nevertheless, the cultivar Polvon (23.9%) maintained a higher protein content even under salinity conditions, which may refer to its robust stress resistance mechanisms. On the contrary, the cultivar Gulistan showed the lowest protein content (21.9%) in saline conditions. This may be ascribable to its weaker system of nitrogen assimilation due to ion toxicity and its fixation in the form of protein in this cultivar. Karimi *et al.* (2025) also reported a decrease in protein in kidney beans under salinity stress conditions.

## CONCLUSIONS

Under salinity stress, chickpea cultivars Polvon and Darmon exhibited the highest adaptability associated with enhanced activities of SOD, CAT, and POD, which reduced ROS accumulation and lipid peroxidation, thereby protecting cell membranes from oxidative damage. The accumulation of osmoprotective compounds, such as proline, ascorbic acid, and glutathione, also contributed to stress tolerance. In contrast, the cultivar Gulistan showed weaker antioxidant and osmoprotective responses, resulting in pronounced salt stress symptoms. Overall, antioxidant enzyme activity and osmoprotectant levels can serve as reliable biochemical indicators for evaluating and selecting salt-tolerant chickpea cultivars.

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