



Glutathione Mediates Growth Regulation of Chickpea Plant *Cicer arietinum* and Mitigates Salinity Stress

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Abstract

Glutathione is water-soluble with a low molecular weight and is commonly spread in plants. It is a co-factor in several biochemical reactions and acts together with signaling molecules and hormones, and its redox state activates signal transduction. The experiment was conducted in the botanical garden, Department of Biology, College of Education for Pure Sciences, Ibn Al-Haitham, University of Baghdad, during the growing season 2020-2021 to evaluate the potential effects of foliar spraying with (25, 50, 75 mg.L⁻¹) glutathione in addition to the control (0) on the growth of chickpea plants subjected to sodium chloride salt (100, 200 mM.L⁻¹) addition to the control (0). The results point out that salinity clearly decreased, as did plant height, branch number, shoot dry weight, nitrogen, phosphorus, potassium concentration, protein percentage, and increased sodium concentration in chickpea plants. A foliar spray of Glutathione, notably 50 and 75 mg.L⁻¹, enhanced the tolerance of chickpea plants by improving growth traits.

Keywords: Salinity stress, Chickpea, Glutathione, Growth regulation.

1. Introduction

The chickpea plant (*Cicer arietinum*) is one of the main crops grown in arid and semiarid regions [1]. Chickpea is a primarily salinity-sensitive plant [2]. With high nutritional value and being an essential source of protein for human nutrition [3]. Environmental stresses are significantly related to plant productivity. Abiotic stresses, particularly salinity, have been expected to decrease yields by 50% [4, 5]. It caused water toxicity resulting from an osmotic and nutritional imbalance and ion toxicity, driven by the increase in the absorption of ions such as Na and Cl [6]. Salinity causes differences in many biochemical reactions and plant metabolic processes, and the severity depends upon stress duration [7].



Generally, abiotic stresses lead to the overproduction of reactive oxygen species (ROS) like hydrogen peroxide, singlet oxygen, hydroxyl radicals, and superoxide. These ROS cause lipid peroxidation, protein oxidation, enzyme inhibition, and DNA/RNA damage [8]. The severity of damages caused by the increased levels of ROS depends on the imbalance between the production and its removal by the use of antioxidant scavenging [9]. Antioxidants are the key to plants protection from the damages caused by oxidative stresses. Glutathione is the most popular and powerful non-enzymatic antioxidant. It is a low-molecular-weight thiol that accumulates in plant cells, plays a critical role in controlling the growth of plants, and enhances their tolerance to abiotic stress. GSH has vital roles in numerous metabolic processes, including protein synthesis, amino acid transference, DNA repair, control of cell division, and programmed cell death [10]. [11] described the acts of salt-sensitive T44 and salt-tolerant Pusa Vishal, mung bean cultivars. The tolerant one indicated higher accumulation of GSH and limited the content of sodium and chloride in plant leaves, more significant activities of cycle components (AsA-GSH), better effectiveness of PSII, higher efficiency of photosynthetic nitrogen use, and enhanced water relations when compared to the salt-sensitive cultivar. The study of [12] on onion epidermal cell membranes declared the mitigation of GSH alterations induced by salinity, referred to the importance of Glutathione scavenging ROS and improving plant tolerance against salinity stress, and regulated other antioxidant components and compatible organic solutes or osmo-protectants. Foliar spray of Glutathione with different concentrations (0, 50, 100, and 150 mg/L) was clearly effective in improving chickpea effectiveness by decreasing H₂O₂ and increasing compatible osmolytes, the stability of membranes, and the activities of antioxidant enzymes, either in salinity-stressed conditions or with regular irrigation [13].

The present study is an effort to enhance the tolerance of chickpea plants under salinity stress conditions by improving some of the morpho-physiological parameters with the non-enzymatic antioxidant Glutathione foliar application.

2. Materials and Methods

The experiment was carried out in the botanical garden, Department of Biology, College of Education for Pure Sciences, Ibn Al-Haitham, University of Baghdad, through the growing season 2020-2021 to study the impact of glutathione foliar spraying (0, 25, 50, 75 mg.L⁻¹) and sodium chloride irrigation (0, 100, 200 mM.L⁻¹) on chickpea plants. The experiment was arranged in a Completely Randomized Design (CRD) with three replicates. The seeds were planted in pots with an 11-kg soil capacity on October 30, 2020. The plants have been thinned 15 days after germination. Plants were watered with sodium chloride on December 30, 2020. Foliar spraying with Glutathione was done on 1/1/2021. Four plants were harvested for each pot on January 27, 2021. Plant height and branch no. plant⁻¹ were measured. Plant samples were oven dried (65C^o) and crushed; the dry weight of them was measured, and a known weight was digested according to [14]. Mineral composition, nitrogen [15], Phosphorus [16], potassium, and sodium [17] were determined. Protein percentages were measured using [18]. Statistical analyses were done, and differences between the means were identified depending on (<5%) using SPSS program.

3. Results

Tables (1, 2, 3) showed that saline irrigation water reduced the averages of plant height, branch no. plant⁻¹ and shoot dry weight, increasing salinity to 200 mM.L⁻¹ by 22.46, 23.79, and 48.05%, respectively. In contrast, Glutathione foliar application up to 75 mg.L⁻¹ increased the averages of the parameters by 21.70, 40.24, and 36.50%, respectively. About the combination of the two factors, 75 mg.L⁻¹ Glutathione minimized the harmful effects of 200 mM.L⁻¹ sodium chloride, the values for those treatments were 44.50 cm, 5.00 cm, and 1.67 gm, compared to their values at 200 mM.L⁻¹ sodium chloride, but without Glutathione spraying, and compared to their controls treatment.

Table 1. Effect of Glutathione on plant height (cm) under sodium chloride stress.

Sodium chloride (mM.L ⁻¹)	Glutathione (mg.L ⁻¹)				Sodium chloride average
	0	25	50	75	
0	47.50	48.00	50.00	52.50	49.50
100	37.17	40.00	42.50	45.00	41.17
200	32.00	37.50	39.50	44.50	38.38
Glutathione average	38.89	41.83	44.00	47.33	
LSD 0.05	Sodium chloride	Glutathione	Sodium chloride x Glutathione		
	0.67	0.77	1.34		

Table 2. Effect of Glutathione on branches no.plant⁻¹ under sodium chloride stress.

Sodium chloride (mM.L ⁻¹)	Glutathione (mg.L ⁻¹)				Sodium chloride average
	0	25	50	75	
0	5.00	4.50	5.00	4.50	4.75
100	3.00	4.00	3.50	4.50	3.75
200	2.00	3.00	4.50	5.00	3.62
Glutathione average	3.33	3.83	4.33	4.67	
LSD 0.05	Sodium chloride	Glutathione	Sodium chloride x Glutathione		
	0.53	0.61	1.06		

Table 3. Effect of Glutathione on shoot dry weight (gm) under sodium chloride stress.

Sodium chloride (mM.L ⁻¹)	Glutathione (mg.L ⁻¹)				Sodium chloride average
	0	25	50	75	
0	2.27	2.30	2.34	2.32	2.31
100	1.32	1.40	1.49	1.61	1.46
200	0.51	1.10	1.50	1.67	1.20
Glutathione average	1.37	1.60	1.78	1.87	
LSD 0.05	Sodium chloride	Glutathione	Sodium chloride x Glutathione		
	0.024	0.027	0.047		

The results of **Tables (4, 5, 6, 7)** indicated that salinity significantly increased the average sodium concentration by 79.11 % and reduced the average of nitrogen, phosphorus, and potassium by 12.46, 12.12, and 16.72%, respectively, in chickpea plants. In contrast, 75 mg.L⁻¹ Glutathione foliar application increased the average of phosphorus by 30.77% and reduced the average of sodium by 46.25% and 50 mg.L⁻¹ Glutathione increased the average of nitrogen and potassium by

19.08 and 19.12%, respectively. Salinity stress plants accumulated significantly less sodium concentration and more nitrogen and potassium upon foliar application with 50 mg.L⁻¹ Glutathione, the values for nitrogen were 3.58%, potassium was 2.86%, and sodium was 1.95% compared with their values at 200 mM.L⁻¹ sodium chloride, but without glutathione spraying, and compared to their control treatment.

Table 4. Effect of Glutathione on nitrogen concentration (%) under sodium chloride stress.

Sodium chloride (mM.L ⁻¹)	Glutathione (mg.L ⁻¹)				Sodium chloride average
	0	25	50	75	
0	3.48	3.54	3.68	3.42	3.35
100	3.18	3.35	3.59	3.50	3.41
200	2.45	2.86	3.58	3.45	3.09
Glutathione average	3.04	3.25	3.62	3.46	
LSD 0.05	Sodium chloride	Glutathione	Sodium chloride x Glutathione		
	0.015	0.017	0.030		

Table 5. Effect of Glutathione on phosphorus concentration (%) under sodium chloride stress.

Sodium chloride (mM.L ⁻¹)	Glutathione (mg.L ⁻¹)				Sodium chloride average
	0	25	50	75	
0	0.30	0.33	0.35	0.34	0.33
100	0.26	0.29	0.32	0.33	0.30
200	0.23	0.27	0.30	0.34	0.29
Glutathione average	0.26	0.30	0.32	0.34	
LSD 0.05	Sodium chloride	Glutathione	Sodium chloride x Glutathione		
	0.012	0.014	0.025		

Table 6. Effect of Glutathione on potassium concentration (%) under sodium chloride stress.

Sodium chloride (mM.L ⁻¹)	Glutathione (mg.L ⁻¹)				Sodium chloride average
	0	25	50	75	
0	2.90	3.10	3.37	2.82	3.05
100	2.36	2.55	2.73	2.62	2.57
200	2.27	2.45	2.86	2.57	2.54
Glutathione average	2.51	2.70	2.99	2.67	
LSD 0.05	Sodium chloride	Glutathione	Sodium chloride x Glutathione		
	0.016	0.018	0.032		

Table 7. Effect of Glutathione on sodium concentration (%) under sodium chloride stress.

Sodium chloride (mM.L ⁻¹)	Glutathione (mg.L ⁻¹)				Sodium chloride average
	0	25	50	75	
0	1.80	1.80	1.46	1.25	1.58
100	3.05	2.55	2.05	1.65	2.33
200	4.35	2.95	1.95	2.05	2.83
Glutathione average	3.07	2.43	1.82	1.65	
LSD 0.05	Sodium chloride	Glutathione	Sodium chloride x Glutathione		
	0.016	0.019	0.032		

Table 8 showed that salinity stress reduced the average protein percentage by 12.84%. Glutathione application increased the average of the parameter by 18.69%. The combination of the two factors indicated that 50 mg.L⁻¹ Glutathione able to reduce the harmful effects of 200 mM.L⁻¹ sodium chloride on protein percentage, and the value was 22.38% compared to their values at 200 mM.L⁻¹ chloride but without Glutathione spraying and compared to their control treatment.

Table 8. Effect of Glutathione on protein percentage (%) under sodium chloride stress.

Sodium chloride (mM.L ⁻¹)	Glutathione (mg.L ⁻¹)				Sodium chloride average
	0	25	50	75	
0	21.96	22.13	23.01	21.38	22.12
100	19.88	20.94	22.44	21.88	21.28
200	15.32	17.88	22.38	21.57	19.28
Glutathione average	19.05	20.31	22.61	21.61	
LSD 0.05	Sodium chloride	Glutathione	Sodium chloride x Glutathione		
	0.090	0.104	0.180		

4. Discussion

Salinity affects plant growth in three ways. The first of them is a deficiency of water through reducing the potential of water in plant roots, so the osmotic stress limits plant growth, followed by the ion toxicity of Na and Cl, and the third is nutrient imbalances resulting from their inappropriate uptake [7]. The competition between sodium ions and potassium ions for the specialized binding sites essential for biological and chemical reactions leads to disorders in Na accumulation in plant organs and a reduction in mineral ions contents, thus causing inhibition in plant growth and decreased yield components [19]. The decrease in growth of chickpea with the brutal effects of salinity stress, due to the reduction in growth promoters and increases in growth inhibitors, and the disorders in water imbalance of stress plants all lead to the closure of stomata, an imbalance of ions, and a drop in photosynthesis efficiency [20]. The negative effects of salinity arise by inducing the production of reactive oxygen species [21].

The most potent non-enzymatic antioxidant is Glutathione. It acts as a substrate for Glutathione-transferases and Glutathione peroxidase. It controls many metabolic activities and avoids protein denaturation under different stresses by maintaining their thiol groups. It also regulates the plasma membrane by keeping zeaxanthin and α -tocopherol in a reduced state [22]. Glutathione can improve plant performance under salinity stress. It regulates seedling growth in media containing salt or Glutathione-dependent enzymes and regulates enzyme activities [23]. Glutathione treatment causes the accumulation of proline acid in plants; the expansion of these osmolytes leads to osmotic regulation under salinity stress by increasing the main enzymes of proline biosynthesis [13]. Glutathione foliar application might relieve the harmful effects of salinity stress on ion content by improving the contents of nitrogen, phosphorus, and potassium [24; 25]. Thus, the helpful effects of glutathione, might have been achieved by enhancing osmotic tolerance and also because of its influence in maintaining membrane permeability and avoiding the loss of photosynthetic pigments. These responses improved plant growth under salinity stress [26; 27; 28]. Glutathione reduces lipid peroxidation, protects plasma membrane stabilization by lowering the passive Na influx, and enhances plant salt tolerance [29; 30].

5. Conclusions

The present study could conclude that Glutathione foliar application with concentrations of 50–75 mg.L⁻¹) could alleviate the harmful stress created by irrigation of chickpea plants with sodium chloride concentrations of 200 mM.L⁻¹, via enhancing mineral ion contents, which is reflected in healthy growth and suitable yield components.

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Conflict of Interest

There are no conflicts of interest.

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