Role of exogenous application of proline and cystiene on growth, yields and antioxidant enzymes activities of cabbage plants (*Brassica oleracea* var. *capitata* L.) grown under salt stress

Jameel H. Hiji **Awatif Neamah Jerry**
Jameelhassan813@gmail.com a_n_jerry@yahoo.com
Department of Horticulture and Landscape design, College of Agriculture, University of Basrah, Iraq

Abstract:

Salinity decreases yield in arid and semi-arid areas. in the present study, the effect of treatment amino acid on cabbage plants grown on a saline soil (dS m^{-1}) was investigated. This study was conducted during season of 2019/2020 at at Abu Al-Khaseeb district in Basrah/Iraq to study the effect of two amino acids proline at three concentration (0, 50,100) mg l^{-1} and cysteine at three concentration (0, 25, 50) mg l^{-1} and interaction between them on cabbage plants grown on saline soil at EC18.50 (dS.m^{-1}). The results indicated that proline and cysteine significantly increased growth characteristics, physiological attributes and yields compared to the control. The interaction between proline at 100 mg l-1 and cysteine at 50 mg l-1 led to the highest levels of plant growth, yield. Proline and cystiene increased the endogenous proline and the activity of (catalase (CAT) and peroxidase (POD) enzymes) under salinity stress.The use of amino acids such as proline at 100 mg.l^{-1} and cysteine at 50 mg.l^{-1} had an important effect on cabbage plants to mitigate the adverse effects of salinity stress.

Key Words :amino acids, proline, cysteine, salt stress, cabbage.

Introduction:

Salinity stress is one of the severe abiotic stresses which adversely affect plant growth and productivity in various regions, particularly in arid and semi-arid areas. Cabbage is moderately sensitive to salinity and the salinity has caused negative effects on production[1]. Plants have evolved many mechanisms for inducing salt tolerance, including antioxidant accumulation to prevent cells and tissues from oxidative damage [2]. The exogenous application of the amino acids are important for stimulating cell growth, and also a well-known as biostimulant which has positive effects on plant growth, yield and significantly alleviates abiotic stress injuries. Amino acids could act as a part of co-enzymes and precursors of certain plant bio-regulators which promote plant growth by enhancing photosynthesis [3].

Proline is an important amino acid which plays different roles in plants. In addition, to its role in protein synthesis, It can also protect plants from environmental stresses as well as singlet oxygen and free radical, due to its role in protein synthesis [4]. It function as an intracellular structure which protects enzymes as well as membranes. moreover, it promotes the stability of some enzymes and conserve enzymes as well as membranes [5]. [6] showed that exogenous application of optimized dose of proline improved the performance of rice by improving, plant water-relations, chlorophyll pigments, morphological, yield and improved the efficiency of salt tolerance.

Cysteine is an α-amino acid that differs from other amino acids, because it contains the thiol chain (- SH) that is involved in enzymatic reactions. Cysteine is the precursor molecule of glutathione (antioxidant), the predominant non-protein thiol that plays a major role which plays an important role in plant stress responses [7]. Cysteine plays a structural main function in proteins. As well as its function as a precursor to essential biomolecules such as vitamins and certain protection compounds, such as glucosinolates and thionins [8]. Cysteine treatments had a beneficial role in alleviating the adverse effect of salinity stress on growth and yield through increasing photosynthetic pigments, proline content and antioxidant enzymes activities [9].

The objective of this study is to evaluate the role of cysteine or proline in alleviating the

 ISSN 2072-3875

harmful effect of salinity stress on growth and yield of cabbage Plants.

Materials and Methods

The experiment was carried out during 2019/2020 season at Abu Al-Khaseeb district in Basrah/ Iraq in sandy loam soil to study the effect of different levels of artificial cysteine and proline and interaction between them on physiological parameters and the activity of antioxidant enzymes Catalase and Peroxides and Proline on cabbage cv hybrid Galaxy of Paracid company Holland. The chemical and physical characteristics of field soil and irrigation water used in the field reported in (table, 1).

The experiment had consists of 9 treatments, including of proline (0, 50, 100) mg l^{-1} and cysteine (0, 25, 50 mg l^{-1} after 20, 35 and 50 days from transplanting as foliar application.

The soil field had prepared and manures fertilizer used at the rate 10 $M^3.D^{-1}$ and divided into ridge in 3.2 m of length and distance between them 0.75 m. The cultivation was on both sides of the ridge, 40 cm distance between plants 14 plants in every experimental unit. The plants fertilizered 20 days after transplanting and 20 days after the first fertilizing with urea 40 Kg D^{-1}.

The experiment was immpllminted in a randomized complete block design in a factorial experiment with three replicates. Treatments means were compared by using Least Significant Differences (L.S.D.) at the probability of 0.05.

Head cabbage was harvested at the marketable stage at 25/2/2019

A random sample of 10 plants was assigned for investigation in each plot, to study the morphological character estimate the following characteristics: Plant height (cm), shoot fresh weight (g), head weight (g), head diameter (cm), dry matter (%), stem diameter (cm) chlorophyll (mg g^{-1}) in fresh leaves according to [10], Proline (mg g^{-1} DW) was determined spectrophotometrically according to the ninhydrin method described, by [11], catalase activity was measured by hydrogen peroxide assay based on formation of its stable complex with ammonium molybdate [12], Peroxidase activity was measured by using a guaiacol assay[13].

Table 1. The Soil physic-chemical proprieties and irrigation water in the field

Soil characteristics	
Character	Value
E.C. (dS.m^{-1})	18.50
pH	7.59
Organic matter (%)	1.71
Total nitrogen (mg Kg^{-1})	1.75
Total phosphor (mg Kg^{-1})	0.143
available potassium (mgL^{-1})	0.918
Organic matter (%)	1.71
Na+ (mg g^{-1})	2.16
Ca^{++} (mg g^{-1})	6.0
Mg^{++} (mg g^{-1}	5.8
SO4 (mg g^{-1})	3.37
Cl^- (mg g^{-1})	7,45
$HCO3^-$ (mg g^{-1})	1.45
Soil structure	
Sand (%)	11.8
Silt (%)	67
Clay (%)	21.2
Texture Class	Sandy Loam
E.C. (ds m-1) of Irrigation water	5.64

Water analysising lab./Marin Science Center / Basra University

ISSN 2072-3875

Result and Discussion

Data presented in (Table 2) showed that, proline levels 100 mg l/ significantly increased plant growth more than other treatment plant height stem weight and diameter, head weight and diameter, dry matter, compared to the controls. Maximum values of growth traits were obtained with the proline level of 100 mg l^{-1} as the most effective concentration. proline might have protected cell membranes against ion toxicity and salt-induced oxidative stress, increased cellular growth [14], and thus increased the growth of cabbage plants.

Foliar applied cysteine levels (i.e.,25 50) mg l^{-1} significantly increased the plant height, stem weight and diameter, shoot fresh weight head weight and diameter, dry matter and head diameter compared to the controls (Table, 2). Foliar applications of 50 mg l^{-1} cystiene had more effective for achieving the best yield under saline conditions. The enhancement effect of cysteine amino acid on growth criteria of cabbage plant might be attributed to GSH synthesis from cysteine [15]. and cysteine produce energy which needed to reduction sulfate due to its contains the organic sulfur that use for direct growth of plant[16] Data presented in (Table 3) showed that foliar application of proline at different at (100) mg l^{-1} significantly increased leaf photosynthetic pigment concentrations such as chlorophyll compared to the control plants, these increases in chlorophyll concentrations could be attributed to the more efficient scavenging of ROS by proline and other antioxidant compounds [17]. The positive effect of proline on chlorophyll concentrations under salinity stress might also because of stabilizing photosynthetic reactions [18].

Foliar application of cysteine on cabbage plants at 25 mg l^{-1} induced significant increase in chlorophyll contents of leaves (Table 3) compared with untreated control. The role of cysteine in increasing total chlorophyll could be due to cysteine had reduced sulfur donor molecule involved in the synthetic of essential biomolecules such as chlorophyll [19].

The interaction between proline at 100mg.l^{-1} and cysteine 50mg.l^{-1} levels increase the vegetative growth and chlorophyll in the study.

Application of proline induced significant increase of proline contents. Results showed that salinity stress or the application of proline, increased the accumulation of proline in the cabbage plants (Table 3). It has been suggested that the accumulation of proline in control plants may be caused by the increase in proteolysis or by the reduction in protein synthesis or an increase in the content of the precursors of proline. [20; 21].]. Interaction between Proline at 100mg.l-1 and cysteine at 50 mg.l-1 led to increase vegetative growth.

Application of proline increased the activity of POD and CAT antioxidative enzymes under salt stress. Because Proline protected cabbage plant from damaging of ROS in addition to protect and activate the POD and CAT enzymes [22]. It agree with [15; 5].

Cysteine treatments at 25 mg l and 50 mg l^{-1} caused significant increase in proline and POD and CAT activity in plants grown in saline soils as compared with controls. It is agree with [8].

ISSN 2072-3875

Table (2): Effect of proline, cysteine and their interactions on vegetative growth characters and yield of cabbage

Proline (mg l^{-1})	Cysteine (mg l^{-1})	Plant height (cm)	Stem weight (g)	Shoot fresh weight (g)	head weight (g)	head diameter (cm)	Dry matter (%)	Stem diameter (cm)
0	0	20.413	80.570	1686.0	1285.000	14.533	8.450	2.817
	25	21.417	90.320	1763.33	1388.333	15.067	9.193	3.217
	50	21.753	97.953	1963.33	1586.667	15.320	9.83	3.443
50	0	21.607	113.150	1825.0	1685.000	15.447	10.220	3.550
	25	21.657	118.467	1942.67	1660.000	15.230	10.393	3.693
	50	22.843	120.570	2080.0	1708.667	15.647	10/370	3.750
100	0	23.427	97.953	2135.0	1740.667	15.730	10.577	3.620
	25	23.933	121.683	2103.67	1785.000	15.807	10.597	3.637
	50	22.517	126.417	2340.0	1810.333	16.037	11.052	3.860
LSD 0.05		0.155	0.968	17.214	12.497	0.083	0,354	0.163
Proline mean	0	21.194	89.164	1804.222	1420.000	14.973	9.158	3.159
	50	22.036	117.396	1949.223	1657.889	15.441	10.328	3.664
	100	23.959	123.123	2192.889	1785.000	15.858	10.742	3.706
LSD 0.05		0.089	0.559	9.938	7.215	0.0481	0.205	0.094
cysteine Mean	0	21.816	104.997	1882.000	1543.556	15.237	9.749	3.329
	25	22.226	110.157	1936.556	1611.111	15.368	10.061	3.516
	50	23.036	114.980	2127.778	1701.889	15.668	10.418	3.684
LSD 0.05		0.088	0.559	9.939	7.126	0.0482	0.204	0.093

ISSN 2072-3875

Table (3): Effect of proline, cysteine and their interactions on chlorophyll, proline, and catalase and peroxidase activity of cabbage leaves.

Proline mg l^{-1}	cysteine mg l^{-1}	chlorophyll (mg g^{-1} FW)	proline (mg g^{-1} DW)	catalase (U mg FW)	Peroxidase **(U mg FW)**
0	0	48.993	1.296	0.543	11.457
	25	56.693	1.708	0.621	22.360
	50	57.243	1.923	0.677	31.990
50	0	58.280	2.143	0.714	42.223
	25	60.650	2.180	0.714	49.437
	50	57.603	2.278	0.797	66.637
100	0	56.440	2.253	0.790	87.627
	25	57.527	2.520	0.755	90.600
	50	60.903	3.245	0.788	95.587
LSD 0.05		0.475	0.081	0.160	1.537
Proline mean	0	54.310	1.643	0.614	21.936
	50	58.844	2.200	0.760	52.766
	100	58.290	2.706	0.780	91,271
LSD 0.05		0.274	0.046	0.0093	0.887
cysteine Mean	0	54.571	2.997	0.674	65.670
	25	58,.290	2.395	0.596	55.819
	50	58.583	2.020	0.581	52.754
LSD 0.05		0.273	0.0385	0.009	0.215

Conclusion

Treatments with proline and cysteine had played a beneficial role in alleviating the adverse effect on the cabbage plant of salinity. The most successful treatment for improving salinity tolerance of cabbage plants was 100 mg l^{-1} proline and 50 mg l^{-1} cysteine. . The results would support the efforts of mitigating the soil salinity and the sustainable agriculture concept.

References

1-Ayers, R. S. and Westcot, D.W. (1985). Water quality for agriculture. FAO Irrigation and Drainage, Paper No.29. FAO, Roma., 174pp.

2-Munns, R.; James, R.; Läuchli, A. (2006). Approaches to increasing the salt tolerance of wheat and other cereals. Journal of Experimental Botany, 53:39–47

3-Bakhoum GS, Badr EA, Sadak MS, Kabesh MO, Amin GA (2019) Improving growth, some biochemical aspects and yield of three cultivars of soybean plant by methionine treatment under sandy soil condition. Int J Enviro Res 13(1):35–43

4-Alia, A.; Mohanty, P. & Matysik, J. (2001). Effect of proline on the production of singlet oxygen. Amino Acids, 21: 195–200.

5-Ashraf, M. & Foolad, M.R. (2007). Roles of glycinebetaine and proline in improving plant abiotic stress tolerance. Environ. Exp. Bot., 59:206–216.

6-Tabssum, F., Zaman, Q.; Chen, Y.; Riaz, U.; Ashraf, W.; Aslam, A.; Ehsan, N.; Nawaz, R.; Aziz, H. & Shah. S.S. (2018). Exogenous application of proline improved salt tolerance in rice through modulation of antioxidant activities. Pakistan Journal of Agricultural Research, 32(1): 140-151.

 ISSN 2072-3875

7-Azarakhsh, M.; Asrar, R.Z. & Mansouri, H. (2015). Effects of seed and vegetative stage cysteine treatments on oxidative stress response molecules and enzymes in *Ocimum basilicum* L. under cobalt stress. J. Soil. Sci. Plant. Nutr., 15 (3): 651–662.

8-Alvarez, C.; Bermudez, A.; Romero, M.; Gotor, LC. & Garcia, CI. (2012). Cysteine homeostasis plays an essential role in plant immunity. New Phytologist, 193:165–177.

9-Sadak, M.; Abd El-Hameid, A.R.; Zaki, F.S.A.; Dawood, M.G. & El-Awadi, M.E. (2020). Physiological and biochemical responses of soybean (*Glycine max* L.) to cysteine application under sea salt stress. Bulletin of the National Research Centre, 44 (1):

10-Zaehringer, M. V.; Davis, K. R. and Dean, L. L. (1974). Persisten green color snap beans *Phaseolus vulgaris* color-related constituents and quality of cooked fresh beans. J. Amer. Soc. Hort.Sci., 99 (1) :89-92.

11-Bates, L.S.; Waldren, R.P. & Teare, I.D. (1973). Rapid determination of free proline for water-stress studies. Plant Soil, 39: 205–207.

12-Goth, L. (1991). A simple method for determination of serum catalase activity and revision of reference range. Clinica Chimica Acta., 196: 143 - 152.

13-Angelini, R.; Manes, F. & Federico, R. (1990). Spatial and functional correlation between diamine-oxidase and peroxidase activities and their dependence upon de-etiolation and wounding in chick-pea stems. J. Planta, 182: 89 - 96.

14-Banu, M. N. A.; Hoque, M. A.; Watanabe-Sugimoto, M.; Matsuoka, K.; Nakamura, Y. & Shimoishi, Y. (2009). Proline and glycinebetaine induce antioxidant defense gene expression and suppress cell death in cultured tobacco cells under salt stress. J. Plant Physiol., 166 :146–156

15- AL-Marjani, A.H.(2011). Effect of application some amino acid with irrigation water and spray on growth and yield of tomato (Lycopersion esculentum Mill) in desert zubair soil, thesis, Baghdad University/Iraq.

16-Nasibi, F.; Kalantari, KM.; Zanganeh, R.; Mohammadinejad, G. & Oloumi, H. (2016). Seed priming with cysteine modulates the growth and metabolic activity of wheat plants under salinity and osmotic stresses at early stages of growth. Ind. J. Plant Physiol., 21(3):279–286

17-Abdelhamid, M.T.; Rady, M.M.; Osman, A.S.h. & Abdalla, M.A. (2013). Exogenous application of proline alleviates salt-induced oxidative stress in *Phaseolus vulgaris* L. Plants. The Journal of Horticultural Science and Biotechnology, 88: 439–446.

18-Romero L. C., Aroca M. A., Laureano-Marin A. M., Moreno I., Garcia I., Gotor C. (2014). Cysteine and cysteine-related signaling pathways in *Arabidopsis thaliana*. *Mol. Plant* 7 264–276

19- Anderson, J.W.(1978). Sulphur in biology . The Camelot Press Ltd., Southamption.Great Britiain.

20-Lin, C.C.; Hsu, Y.T. and Kao, C.H. (2000). The effect of NaCl on proline accumulation in rice leaves. Plant Growth Regulation, 36: 275–285.

21-Borgo, L.; Marur, C.J. & Vieira, L.G.E. (2015). Effects of high proline accumulation on chloroplast and mitochondrial ultrastructure and on osmotic adjustment in tobacco plants. Acta Sci., Agron., 37 (2): 191-199.

22- Oudah, H. K.; Mokdad, M. and Jawad, E.H.A. (2017). Role of enzymes catalase, peroxidase and amino acid (proline) in raphunus sativus and lepidium sativus in exposure levels different water pollution of ion lead. Iraq Hournal of Science, 58(2): 592-599.

 ISSN 2072-3875