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Foliar Spraying Alphatocopherol Enhances Salinity Stress Tolerance in Wheat Plant *Triticum Aestivum* L.

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Abstract

Alphatocopherol is a vitamin and antioxidant compound that plays a crucial role in amelioration of biotic and abiotic stresses and protects plants from stress – induced cellular oxidation .The experiment was carried out in a green garden of the Department of Biology, College of Education for Pure Sciences Ibn-AL-Haitham ,University of Baghdad during 2018 – 2019 to evaluate the potential effects of foliar application with (0 ,50 ,100 ,150 ,200 $mg.L^{-1}$) alphatocopherol on growth, yield components and the activity of antioxidant system of wheat plant under sodium chloride stress(0 ,75 , 150 ,225$mM.L^{-1}$) .Results indicated that sodium chloride caused reduction in shoot dry weight and some yield components (biological yield , no. spikelet .$spike^{-1}$ and 1000 grain weight)and a significant increase in the endogenous proline acid and alphatocopherol concentration and raised catalase activity. Foliar spray of alphatocopherol improved salt stress tolerance in wheat plant by increasing all the parameters.

Keywords: wheat plant, antioxidant defense, alphatocopherol, salinity tolerance

1.Introduction

Wheat (*Triticum aestivum* L.)is one of the most strategic crops in the world grains production, and is consumed as the main food by more than 36% of world population, wheat productivity is adversely affected by salinity stress[1]. Salinity is a major threat and global problems that negatively affect crops productivity. It impairs plant growth and development through water stress caused by an osmotic imbalance and ion toxicity due to uptake of ions such as sodium and chloride , and nutritional imbalance[2,3]. Plants are subjected to variety of abiotic and biotic stresses such as salinity which trigger oxidative stress within plant cells due to the high leakage of the electrons toward oxygen molecule during the metabolic processes of photosynthesis and respiration. As a result, there are over production of reactive oxygen species and these free radicals attack nucleic acids ,cell membranes , proteins, lipids, leading to cell death. Plants have developed a defense system that protects the cell and limits the oxidative stress. This system includes enzymatic and non- enzymatic antioxidants[4].

Vitamin E belongs to the chemical family of tocochromanols (tocopherols and tocotrienols) which are mostly produced by plants, algae and some cyanobacteria[5]. Alphatocopherol is one of the non-enzymatic antioxidants , it is considered as the most active form of vitamin E , it is amphipathic molecule in which the polar chromanol head group are exposed to the membrane surface ,while the hydrophobic prenyl tail associates with membrane lipids [6].

The research is taken from the second researcher's master thesis

The strong oxidative properties of alphatocopherol due to its ability to deactivate photosynthesis derived reactive oxygen species mainly single oxygen and interact with polyunsaturated acyl groups and scavenge lipid peroxyl radicals, thus protecting membrane fatty acid from lipid peroxidation [7]. Several studies recorded an increase in the antioxidant content in wheat plant grown under salt stress in response to alphatocopherol application [8,9].

In our study, we aim to evaluate the extent to which wheat plant AL-Baraka cultivar can overcome sodium chloride stress and determine the alphatocopherol concentration that minimizes the harmful effect of salinity stress .

2.Materials and Methods

The experiment was conducted in a green garden of the Department of Biology ,College of Education for Pure Sciences, Ibn-AL-Haitham, University of Baghdad, during the growth season 2018-2019 to a access the influence of alphatocopherol (0 ,50 ,100 ,150 ,200 mg.L^{-1})as a foliar spray on wheat plant c.v. ALBaraka irrigated with sodium chloride (0 ,75 ,150 ,225 mM.L^{-1}). The experiment was laid out in a completely randomized design (CRD) with three replications. Healthy grains were sown in pots (with capacity 11Kg soil) on November 21,2018, thinning of plants was done in two weeks after germination. Plants were irrigated with sodium chloride concentrations on December 30,2018 and continued until the end of the experiment. Plants were sprayed with alphatocopherol concentrations on January 9,2019 in the early morning and four randomly plants were harvest per pots on January 22,2019. Plants samples were oven dried (65C° to constant mass)and pulverized ,the dry mass of shoots and leaves were measured. Fully expand leaves for each treatment were used to quantified proline acid, using the ninhydrin reagent[10]and for determination endogenous alphatocopherol [11]and catalase enzyme activity[12]. Six plants were harvest on April 11, 2019 and some yield components of wheat plant were calculated (biological yield, number of spikelet.spike^{-1} ,weight of 1000 grain).Statistical analyses were carried out and the difference between the means were detected according to(< 0.05)using test [13].

3. Results and Discussion

Data presented in **(Table 1)** showed reduction in the shoot dry weight average at the concentration 225 mM.L^{-1} sodium chloride by 33.90% in comparison with control. In contrast, alphatocopherol foliar spray from 0- 200 mg.L^{-1} caused significant increase for shoot dry weight average and the best increase was at 150mg.L^{-1} by 52.62% .Regarding the combination between the two factors ,150 mg.L^{-1} alphatocopherol can minimize the adverse effects of 225 mM.L^{-1} sodium chloride on the shoot dry weight with the value 4.59 mg comparison with shoot dry weight value 2.70 gm. at the same sodium chloride concentration but without alphatocopherol spray and in comparison with 5.00 gm. at the control treatment .

Table 1: Effect of sodium chloride and alphatocopherol and their interactions on shoot dry weight (gm)of wheat plant

Sodium chloride concentrations (mM.L-1	Alphatocopherol concentrations (mg.L-1)					Sodium chloride average
	0	50	100	150	200	
0	5.00	5.22	6.18	6.69	6.40	5.90
75	3.83	4.35	5.11	5.79	5.44	4.90
150	3.01	4.07	4.89	5.10	5.20	4.45
225	2.70	3.71	4.18	4.59	4.30	3. 90
Alphatocopherol averag	3.63	4.34	5.09	5.54	5.34	
LSD(0.05)	Sodium chloride concentrations=0.10 Alphatocopherol concentrations=0.11 Interaction=0.22					

To determine whether proline acid and alphatocopherol accumulates in plants leaves in response to sodium chloride stress , the endogenous concentration for both of them was measured . Data in **(Table 2**) showed an increase in proline acid average by 77.04% with increasing sodium chloride concentration from to 225 $mM.L^{-1}$. Application of alphatocopherol up to 200$mg.L^{-1}$ as foliar spray increased proline acid average with the superiority to 150$mg.L^{-1}$ for giving the highest average with increase ratio 47.64%.The combination between the dual factors was significant, the value of proline acid at the treatment 225 $mM.^{L-1}$ sodium chloride and 200 $mg.L^{-1}$ alphatocopherol was 120.52 $\mu g.mg^{-1}$ in comparison with its value 115.45 $\mu g.gm^{-1}$ at 225 $mM.L^{-1}$ sodium chloride but without alphatocopherol spray and with the control value 33.93 $\mu g.gm^{-1}$.

Table 2: Effect of sodium chloride and alphatocopherol and their interactions on proline acid concentration ($\mu g.mg^{-1}$) of wheat plant leaves

Sodium chloride concentrations (mM.L-1)	Alphatocopherol concentrations (mg.L-1)					Sodium chloride average
	0	50	100	150	200	
0	33.93	40.28	57.23	102.33	99.67	65.69
75	63.98	98.31	105.23	115.37	101.21	96.82
150	95.44	106.23	116.32	118.61	116.92	110.70
225	115.45	116.97	117.82	119.60	120.52	118.07
Alphatocopherol average	77.20	90.45	99.15	113.98	109.58	
LSD(0.05)	Sodium chloride concentrations=2.47 Alphatocopherol concentrations=2.76 Interaction=5.5					

Data in**(Table 3)** showed that endogenous alphatocopherol average increased significantly due to sodium chloride concentrations from 0 to 225 $mM.L^{-1}$ by 38.99%, alphatocopherol foliar spray up to 200$mg.L^{-1}$ increased parameter average by 44.87% .The combination between both factors was demonstrated, 100 $mg.L^{1}$ alphatocopherol can reduce the adverse effect of 225 $mM.L^{-1}$ sodium chloride and raized the endogenous alphatocopherol concentration with the value 24.90 $\mu g.gm^{-1}$ in comparison with its value 21.79 $\mu g.gm^{-1}$ at the same concentration of sodium chloride but without adding alphatocopherol and with a control value 8.22 $\mu g.gm^{-1}$.

Table 3: Effect of sodium chloride and alphatocopherol and their interactions on endogenous alphatocopherol concentration($\mu g.mg^{-1}$) of wheat plant leaves

Sodium chloride concentrations (mM.L-1)	Alphatocopherol concentrations (mg.L-1)					Sodium chloride average
	0	50	100	150	200	
0	8.22	13.34	17.62	20.40	22.61	16.44
75	14.21	16.82	19.41	20.29	18.84	17.91
150	16.57	19.20	20.48	24.50	23.41	20.83
225	21.79	22.93	24.90	22.87	21.77	22.85
Alphatocopherol average	15.20	18.07	20.60	22.02	21.66	
LSD(0.05)	Sodium chloride concentrations=1.08 Alphatocopherol concentrations=1.21 Interaction=2.4					

Catalase is one of the most potent catalysts known, data in **(Table 4)** indicated that in response to sodium chloride concentrations up to 225 $mM.L^{-1}$ average of catalase activity enhances by about

50.75%. Alphatocopherol foliar spray up to 200 mg.L^{-1} improved average of enzyme activity by about 66.23% . The combination between the two factors was significant ,the value for enzyme activity at the treatment 225 mM.L^{-1} sodium chloride and 200 mg.L^{-1} alphatocopherol was 44.20 unit.ml^{-1} in comparison with the value 37.33 unit.ml^{-1} at the same sodium chloride concentration but without spray alphatocopherol and with the value 15.33 unit.ml^{-1} at control treatment.

Table 4: Effect of sodium chloride and alphatocopherol and their interactions on catalase enzyme activity (unit.ml^{-1}) of wheat plant leaves

Sodium chloride concentrations (mM.L^{-1})	Alphatocopherol concentrations (mg.L^{-1})					Sodium chloride average
	0	50	100	150	200	
0	15.33	20.67	23.00	38.34	39.33	27.33
75	18.67	22.02	25.22	39.40	42.67	29.60
150	32.67	35.67	40.00	42.67	46.67	39.54
225	37.33	39.33	41.33	43.80	44.20	41.20
Alphatocopherol average	26.00	29.42	32.39	41.05	43.22	
LSD(0.05)	Sodium chloride concentrations=2.44 Alphatocopherol concentrations=2.73 Interaction=5.4					

The yield components of wheat plant severely affected by sodium chloride treatment. Data in **(Table** 5, 6, 7) showed reduction in averages of biological yield, number of spikelet. Spike^{-1} and 1000 grain weight coincided with the increase in sodium chloride concentrations up to 225 mM.L^{-1} by (26.05 ,32.95 ,14.17)%,respectively. Adding alphatocopherol from 0 to 200 mg.L^{-1} foliar spray improved yield component averages and there is clear superiority to 150 mg.L^{-1} for giving the highest average for them with an increase ratio (45.82 ,50.05 ,34.49)% respectively .A significant interaction occurred between the two factors, the treatment 150 mg.L^{-1} alphatocopherol and 225 mM.L^{-1} sodium chloride gave the best values for biological yield (12.69 gm.), number of spikelet. Spik^{-1} (13.56)and 1000 grain weight (57. alphatocopherol(7.67 gm,7.80,44.76gm), and with their controls.

Table 5: Effect of sodium chloride and alphatocopherol and their interactions on biological yield(gm) of wheat plant .52gm)in comparison with their values at the same concentration of sodium chloride but without

Sodium chloride concentrations (mM.L^{-1})	Alphatocopherol concentrations (mg.L^{-1})					Sodium chloride average
	0	50	100	150	200	
0	11.95	13.40	14.88	15.67	15.72	14.32
75	10.63	12.55	13.03	14.65	13.78	12.93
150	8.50	10.42	12.33	13.52	12.25	11.41
225	7.67	9.73	11.28	12.69	11.56	10.59
Alphatocopherol average	9.69	11.53	12.88	14.13	13.33	
LSD(0.05)	Sodium chloride concentrations=0.34 Alphatocopherol concentrations=0.38 Interaction=0.7					

Table 6: Effect of sodium chloride and alphatocopherol and their interactions on number of spikelet. Spike^{-1} of wheat plant

Sodium chloride concentrations (mM.L^{-1})	Alphatocopherol concentrations (mg.L^{-1})					Sodium chloride average
	0	50	100	150	200	
0	13.43	16.00	18.45	19.80	18.80	17.30
75	11.80	14.42	15.60	16.55	15.22	14.72
150	9.66	11.43	13.68	14.12	13.32	12.44
225	7.80	10.84	12.26	13.56	13.52	11.60
Alphatocopherol average	10.67	13.17	15.00	16.01	15.22	
LSD(0.05)	Sodium chloride concentrations=0.34 Alphatocopherol concentrations=0.38 Interaction=0.7					

Table 7: Effect of sodium chloride and alphatocopherol and their interactions on1000 grain weight (gm) of wheat plant .

Sodium chloride concentrations (mM.L^{-1})	Alphatocopherol concentrations (mg.L^{-1})					Sodium chloride average
	0	50	100	150	200	

0	50.00	58.90	66.40	69.34	62.69	61.47
75	48.39	56.83	60.77	65.02	61.54	58.51
150	46.50	53.75	58.80	63.16	64.06	57.25
225	44.76	52.02	54.97	57.52	54.54	52.76
Alphatocopherol average	47.41	55.37	60.23	63.76	60.71	
LSD(0.05)	Sodium chloride concentrations=1.65 Alphatocopherol concentrations=1.84 Interaction=3.					

The reduction in wheat plant growth under the adverse effects of salt (sodium chloride) could be attributed to the osmotic effects, increases in growth inhibitors and reduction in growth promoters, disturbances in water balance of stress plants which they lead to stomata closure, ionic imbalance and reduction in photosynthesis [14]. Accumulation of toxic ions as (sodium and chloride)in plants tissue consequently caused inhibition in plants growth which was reflected in yield component [15].The negative effects of salinity occur by stimulating the overproduction of reactive oxygen species[16].To cope with oxidative stress plants have developed several protective mechanisms including the synthesis of antioxidant molecules (proline acid, alphatocopherol etc.)and several of anti-oxidative enzymes (superoxide dismutase catalase etc.) [17]. During salinity stress conditions, plants need to maintain internal water potential, cell turgor and water uptake. This requires to synthesis of compatible osmolytes such as proline acid that accumulates in cytosol where it contributes to cytoplasmic osmotic adjustment, it is a key signaling molecule , participates in protein membrane stabilization, elimination of free radicals, and balance of redox [18,19].Proline acid may act to avoid loading Na^+ in xylem and prevent it to transport to shoot so it is a key role in plant tolerance to salinity [20].Alphatocopherol is most powerful non-enzymatic antioxidants, that is synthesized in plants in response to stress ,it acts as a recyclable chain reaction terminators of polyunsaturated fatty acid free radicals which generated by lipid oxidation [21].Alphatocopherol is implicated in the maintenance of PSII function against the photo inhibition and control D_1 protein degradation by scavenging singlet oxygen and protect thylakoid membrane against photo oxidative stress by controlling lipid peroxidation and it is accumulation in plants leaves can eliminate the effects of reactive oxygen species induced by salinity stress [22]. Plant cells possess defense strategies against oxidative injury caused by salinity. Such strategies involve antioxidant enzymes as catalase, this enzyme is an important scavenger of reactive oxygen species induced by salinity stress that plays a vital role in oxidative protection by elimination H_2O_2 and producing H_2O and O_2 ,catalase can prevent hydroxyl radicals formation which are responsible for lipid peroxidation of cell membrane [23].

Application of alphatocopherol on plants has gained considerable attention and enabled wheat plant to tolerate salinity sterss ,it has a clear role in scavenging reactive oxygen species and minimize the adverse damage caused by oxidative processes through synergic function with several enzymatic and non-enzymatic antioxidant thus can alleviate the adverse effects of salinity stress on plant in term of plant growth and yield quantity and quality [24,25].

In our study, sodium chloride stress caused significant accumulation for proline acid, alphatocopherol and catalase in wheat leaves , alphatocopherol foliar spray had a clear function and can be minimized the adverse effects of salt stress through improving the activity of the antioxidant defense which was reflected on strong growth and satisfying yield.

4.Conclusion

It could be concluded that exogenous application of alphatocopherol 150 $mg.L^{-1}$ used as a foliar spray solution can remarkably increase the tolerance of wheat plant to survive under severe stress of sodium chloride 225 $mM.L^{-1}$ .Alphatocopherol improved the anti-oxidative defense system (i.e., non-enzymatic and enzymatic antioxidants) which reflects in improving plant growth and yield under sodium chloride stress.

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