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# Effect of Pyridoxine (Vitamin B6) and Saline Stress on the Growth and Antioxidant Enzymes of Wheat Triticum Aestivum L.

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**Abstract:** This study was carried out in the green house of the fields of the College of Agriculture and marshes -Thi-qar university- south of Iraq in the year 2018, to determine the impact of pyridoxine (vitamin B6) on the growth and antioxidant enzymes of the wheat plant cultivated under the salinity stress (9.23) milimos. $cm^{-1}$. The wheat seeds were socked in seven concentrations of pyridoxine (0, 250, 1000, 1750, 2500, 3250, 4000) mg. $L^{-1}$ , growth traits and antioxidant enzymes (DHAR, GPX and POD) were studied at flowering stage. The output showed significant differences, by increasing the concentration of pyridoxine gradually to 1750 mg. $L^{-1}$ most of growth traits increased also, then decreased after this concentration.The increasing of pyridoxine (vitamin B6) concentrations to a certain extent (1750) mg. $L^{-1}$ can protect the plant and increases its resistance to salinity stresses.
**Key words:** Pyridoxine, Saline stress, Growth, Antioxidant enzymes, Wheat.

## INTRODUCTION

Ascorbic acid (AsA), the water-soluble antioxidant molecule is an essential compound for plants and act as a modulator of plant development through regulation of photosynthesis, hormone biosynthesis and regeneration of other antioxidants, salt taken up by plants (Pastori et al., 2003). The salt in the soil solution the "osmotic stress" reduces leaf growth and to a lesser extent root growth, and decreases stomatal conductance and thereby photosynthesis (Munns, 2000). Sodium decreases soil permeability but increases compactness, which reduces the flow of water to the plants (Sadeghian and Yavari, 2004). AsA is additionally involved within the management of organic process, cell growth, growth and development, together with flowering, senescence and root development and conjointly regulates defense response and survival of plants under abiotic and biotic stress. AsA, its oxidoreduction couple also/DHA and connected enzymes (MDHAR, DHAR associated APX) along type an AsA oxidoreduction system to with efficiency shield plants from aerobic stress caused by exogenous and endogenously generated reactive chemical element species (ROS) and its merchandise (Akram et al., 2017). Various studies have reported that salt stress significantly affected the growth resulting in growth retardation and consequently yield (Mohammad et al., 2014; Tatatabaei and Larijani, 2016). Sort of wheat plants (Afzal et al., 2006), bean (Azooz and Al-Fredan, 2009), the pea plant (Burguieres et al., 2007), tomato plant (Barh et al., 2008) and sorghum plant (Arafa et al., 2009). Ahmed (1995) observed a definite benefit from immersing barley grains in the solution of Pyridoxine before planting for most of the characteristics of vegetative and root growth, which led to an increase in grain yield and raise

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the nutritional value. In Iraq, a study was conducted on the barley yield through soaking the grains in Aqueous solution of Pyridoxine for 24 hours before planting using concentrations, there was a high significant response to most of the growth characteristics and yield components and grain yield. There are very few studies regarding the effective application of use on wheat plants grown in both normal and saline condition and their effect on the growth and antioxidant activities. The study aimed was carried to know the effect of pyridoxine (vitamin B6) in counteracting salt stress.

**PLANT MATERIAL AND TREATMENTS**

This study was conducted during the autumn season of the year 2018 in the agricultural fields of the College of Agriculture and Marshes / University of Thiqar, using pots to grow the wheat crop and with (84) pots, The soil physico - chemical properties are presented in table (1). Wheat seeds were soaked in different concentrations of pyridoxine solution As follows:

1- First concentration (comparative treatment) (0) mg. $L^{-1}$ pyridoxine: As distilled water only.

2- The second concentration (250) mg. $L^{-1}$:It was prepared by dissolving (250) mg of pyridoxine in a liter of distilled water.

3 - The third concentration (1000) mg. $L^{-1}$: It was prepared by dissolving (1000) mg of pyridoxine in a liter of distilled water.

4 - The fourth concentration (1750) mg.$L^{-1}$: It was prepared by dissolving (1750) mg of pyridoxine in liters of distilled water.

5- The fifth concentration (2500) mg. $L^{-1}$: It was prepared by dissolving (2500) mg of pyridoxine in a liter of distilled water.

6 - The sixth concentration (3250) mg. $L^{-1}$: It was prepared by dissolving (3250) mg of pyridoxine in a liter of distilled water.

7 - The seventh concentration (4000) mg.$L^{-1}$: It was prepared by dissolving (4000) mg of pyridoxine in liters of distilled water.

Urea as a source of nitrogen has been added at a rate of 50 kg/ h urea fertilizer (N% 46) in the form of batches, the first one at the planting stage and the second at the elongation phase (a month after the first adding) and the third at the expulsion of spikes. Phosphate fertilizer was added at a rate of (160) kg / ha as one adding during the field tillage. Pots were planted with soaked seeds (50 seeds) /pot. The pots were irrigated cautiously until full germination.

Table (1): Physico-chemical properties of soil at the experimental site

| **Physico-chemical properties of soil** | **pH** | **O.M %** | **E.C milimos. $cm^{-1}$** | **C.E.C meq/100 g soil** | **N%** | **$Po_4$** | | **Soil texture** |
|---|---|---|---|---|---|---|---|---|
| | **7.3** | **1.43** | **9.23** | **1.27** | **0.87** | **0.03** | | **Silty loam** |

**Growth parameters:**

The growth traits such as (dry root weight, dry leaves' weight, dry plant weight, percentage of

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leaves/plant and enzyme activities were estimated through flowering stage.

**Measurements of chlorophyll content:**

The chlorophyll content was determined using a Minolta SPAD-502 Chlorophyll Meter.

**Enzyme assays**

Leaf samples (0.5 g) were homogenized in ice cold 0.1 M phosphate buffer (pH 7.5), the protein content was determined according to Bradford 64 (1976) using Bovine Serum Albumin V as a standard.

**Dehydroascorbate reductase (DHAR):**

DHAR activity was determined at 295nm according to (Nakano and Asada, 1981).

**Guaiacol peroxidase ( GPX):**

GPX activity was assayed spectrophotometrically by following the oxidation of NADPH at 340 nm (Veal et al., 2002).

**Peroxidase (POD):**

Peroxidase POD (EC 1.11.1.7) activity was estimated according to Hemeda and Klein (1990).

**Statistical analysis:**

Statistical analysis was based on one way analysis of variance (ANOVA) using SPSS (version 20) $p \leq 0.05$.

**RESULTS AND DISCUSSION:**

The results of ANOVA for all traits are summarized in table (2), all of these traits were influenced by the different levels of pyridoxine (vitamin B6), the results showed significant differences ($p<0.05$) (growth traits), where the seed socked with pyridoxine (1750) mg. $L^{-1}$ gave the highest means of dry root weight, dry leaves' weight, biomass, percentage of leaves/plant and total chlorophyll content (1.33gm, 2.56gm, 3.89gm, 65.81%, 3.33 Minolta SPAD index) respectively, while the seeds that's soaked with pyridoxine (0) mg. $L^{-1}$ were given the lowest values in all those traits, (0.65gm, 0.86gm, 1.51gm, 56.95%, 2.34 Minolta SPAD index) respectively.

Increasing the concentration of pyridoxine lead to the increasing in the average of the growth traits at the certain limit (1750 mg. $L^{-1}$), and then the rates gradually decreased after that concentration. The correlation analysis shows that there is a significant positive correlation ($p<0.01$) between the pyrodoxine and average of dry root weight, dry leaves' weight, biomass, percentage of leaves/plant and total chlorophyll content ($r= 0.95^{**}$, $r= 0.99^{**}$; $r= 0.96^{**}$, $r= 0.96^{**}$; $r= 0.98^{**}$) respectively, this result indicating that the wheat seeds that's treated with an aqueous solution of production at a certain limit (1750 mg. $L^{-1}$) lead to enhance the ability of the wheat plant to prevent the adverse effects of salt making it give the highest levels of the growth parameter.

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Our results are consistent with the results of Desouky (1995) and Hamad and Khulaef (2000) which they reported that the soaking seeds with pyridoxine (vitamin B6) stimulated photosynthetic pigment and net photosynthetic rate. Likewise, Khan et al. (2001) Noticed that the application wheat plants at certain limit gave the maximum values for growth parameters, this is consistent with our findings.

Dalatabadian and Modarressanavy (2008) stated that, the increasing pyridoxine concentration till 400 ppm increased significant plant dry weight in sunflower plants. Asli and Houshmandfar (2011) reported that socking seed with pyridoxine solution could enhance the seedling growth characteristics of the corn plant, it seems that pyridoxine play a key role in cell division, This is identical to what we found in this experiment.

Enzyes activities (DHAR, GPX and POD) also showed that these enzymes were influenced by the of pyridoxine (vitamin B6) concentrations, the results of this experiment showed significant differences ($p$<0.05), the result showed that when the pyridoxine is not used at the level of (0 mg. $L^{-1}$) all of these enzymes gave the heigh activities (0.42, 1.83, 15.13) $U.mg^{-1}$ protein for (DHAR, GPX and POD), respectively, and then these activities were gradually decreased till to level (1750 mg. $L^{-1}$), then begin to rise gradually. Correlation analysis shows that there is a significant negative correlation ($p$<0.01) between the pyrodoxine and antioxidant enzymes studied (DHAR, GPX and POD) ($r$=-0.75*, $r$= -0.73*; $r$= -0.71*) respectively. This result indicates that the use of an aqueous solution of pyrodoxin at this level 1750 maintained plant growth excellent by protecting plant cells from the harmful effects of salts and the evidence is that antioxidant enzyme activity is not elevated at this level of aqueous solution of pyrodoxine compared to the first level (0 mg. $L^{-1}$).

Many studies reported that the salt induced increases in the activity of antioxidant enzymes such as GPX in plants (Hampson and Simpson, 1990; Begum et al. 1992). In the present study significant low changes were observed in DHAR, GPX and POD activities using an increase concentration in pyridoxine, maximum response was observed at (1750 mg. $L^{-1}$) while the activity increased significantly to after this level.

The results showed that salinity induced a marked effect on the antioxidant activities at level pryrpdoxine (0 mg. $L^{-1}$), an increase in the activity of antioxidant enzymes (DHAR, GPX and POD) in this present study under salt stress at this level could be indicative of ROS production thus increasing the protection to reduce the oxidative damage that caused by the salt stress (Gosset et al., 1994; Chaparzadeh et al., 2004).

The (DHAR, GPX and POD) enzymes withdraw polyphenols and ascorbate Which is considered a growth inhibitor of growth at higher salinity condition, it is possible that these antioxidant enzymes succeed to capture ROS (O2 and H2 O2 ) at this condition, our results are consistent with the results of (sairam et al. 2005; Mullineaux and Rausch, 2005; Ksouriet al. 2007).

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| Traits | 0 | 250 | 1000 | 1750 | 2500 | 3250 | 4000 |
|---|---|---|---|---|---|---|---|
| | | | | | | | |
| Roots. gm | 0.65$^{c}$ ±0.21 | 0.76$^{c}$ ±0.20 | 0.98$^{a}$ ±0.22 | 1.33$^{a}$ ±0.32 | 1.11$^{a}$ ±0.22 | 0.87$^{b}$ ±0.11 | 0.87$^{b}$± 0.12 |
| Leaves. gm | 0.86$^{d}$ ±0.06 | 1.09$^{c}$ ±0.11 | 1.65$^{b}$ ±0.17 | 2.56$^{a}$ ±0.32 | 1.44$^{b}$ ±0.24 | 1.08$^{c}$ ±0.05 | 1.22$^{c}$± 0.11 |
| Biomass. gm | 1.51$^{d}$ ±0.2 | 1.85$^{c}$ ±0.1 | 2.63$^{ab}$ ±2.08 | 3.89$^{a}$ ±1.22 | 2.55$^{b}$ ±0.11 | 1.95$^{c}$ ±0.16 | 2.09$^{b}$± 0.24 |
| % leaves/plant | 56.95$^{ab}$ ±9.21 | 58.92$^{ab}$ ±7.21 | 62.74$^{a}$ ±6.06 | 65.81$^{a}$ ±5.48 | 56.47$^{ab}$ ±3.25 | 55.38$^{b}$±4.17 | 58.37$^{ab}$± 2.66 |
| SPAD index | 2.34$^{c}$±1.33 | 2.83$^{b}$±0.32 | 2.90$^{b}$±0.36 | 3.33$^{a}$±0.25 | 2.81$^{b}$±0.20 | 2.72$^{bc}$±0.3 | 2.91$^{a}$±0.31 |
| DHAR.(U.mg$^{-1}$ protein) | 0.42$^{a}$± 0.11 | 0.28$^{ab}$± 0.54 | 0.20$^{b}$± 0.02 | 0.15$^{c}$± 0.01 | 0.25$^{b}$± 0.03 | 0.33$^{ab}$± 0.05 | 0.35$^{a}$± 0.03 |
| GPX.) .(U.mg$^{-1}$ protein) | 5.83$^{a}$± 0.17 | 5.21$^{b}$± 0.04 | 5.31$^{b}$± 0.13 | 4.95$^{c}$± 0.14 | 5.43$^{b}$± 0.05 | 5.52$^{b}$± 0.03 | 5.61$^{ab}$± 0.05 |
| POD.(U.mg$^{-1}$ protein) | 15.13$^{a}$± 1.45 | 16.23$^{a}$± 2.73 | 9.5b$^{c}$± 1.73 | 7.13$^{c}$± 0.27 | 11.6$^{b}$± 1.99 | 12.78$^{b}$± 0.02 | 14.50$^{ab}$± 1.88 |
| | | | | | | | |

**Table (2):** Effect of pyridoxine (mg. L$^{-1)}$ on the growth and antioxidant enzyme activities of the wheat plant

*Note:* Means within the column followed by the same letter are not significantly different to each other at $p > 0.05$.

## CONCLUSION

The present study revealed a significant negative effects promoted by the salinity stress on the reduction of plant growth rates and, and the pyridoxine has a significant role in reducing the harmful effects of salts, so that the increasing concentrations of pyridoxine and to a certain extent can protect the plant cells and increases its resistance to the environmental stresses as salinity

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