

## **Interfering of pyridoxine with NaCl on bioactive compounds and enzyme activity of germinated watermelon (*Citrullus vulgaris*)**

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**ABSTRACT:** In order to study the effect of pre treatment of watermelon seed (*Triticum aestivum* L.) by pyridoxine on germination and development characteristics of its herb as factorial in the frame of the plan was completely three times accidentally. Factors include pyridoxine with densities (witness), 0/01, 0/02 and NaCl was 0, 60 and 100 mM. Germinated seeds were counted daily for eight days. Then the percent of germination, germination speed, the average daily germination, the length of the shoot, root and the weight of dry plant and also the amount of catalase and peroxidase activity were measured. The results showed that pyridoxine affects on the percent of germination, germination speed, the average daily germination, the weight of dry plant, the length of the shoot and the root and the amount of catalase and peroxidase enzyme activity on the level of one percent ( $p>0/01$ ) meaningfully. These results showed that NaCl on the percent of germination, the weight of dry plant, the length of the shoot and the root, the amount of catalase and peroxidase enzyme activity on the level of one percent ( $p>0/01$ ) have meaningful effects but it doesn't have meaningful effect on the average daily germination and in other word the effect of pyridoxine and NaCl on the all measured characteristics on the level of one percent probability ( $p>0/01$ ). To sum up, the results showed that pre treatment seed by the pyridoxine can help the farmers in setting and producing suitable watermelon plant.

**Keywords:** pyridoxine, peroxidase, pre treatment, NaCl, catalase, watermelon

### **INTRODUCTION**

Germination is one of the most critical and sensitive stages of canola growth in facing salt stress affecting these settlement of germ and plant survival in saltiness soils. Hence, numerous attempts are made to develop policies for reducing the adverse effects of salt stress. Today, priming technique or seed preparation is one of the physiological methods to increase the germination percentage, seed efficiency, and preparation of speed and consistency of germination and deployment under saltiness conditions. Watermelon is one of the most important agricultural plants and increase in germination percent of the watermelon seeding is an important factor in improving the plant (Kafi et al 2005). Although among the plants, watermelon is one of the best adjusted agricultural categories but amount of production and performance of this plant as other agricultural plants is hardly affected by environmental factors and constantly there is a concern that whether produced watermelon can be enough for growing population in the world (Satorr and Slafar 2001). Harris et al 2001 reported that priming the seed causes the powerful development, increase the products of the watermelon. In India the effect of priming in decreasing the development duration caused that farmers can have 3 products in a year (Harris et al 2001). Karaki (1998) reported the increase of the wet weight and the length of the shoot and the root of the watermelon and barley along with priming. Determining a suitable time of priming prevents a negative effect of it. Studies by Moradshahi et al (2004) regarding the effect of salt stress on nine floral plants demonstrated that as salt stress increases, growth of the roots of all cultivars increases but aerial member of almost all cultivars decreases.

Penalosa and Eira 1993 reported that the suitable time of priming prevents a negative effect of priming on the germination seed of tomato. Seed germination has extra importance in determining the final density of the plant in the square unit so that the enough density of the plant in square unit will be achieved that cultivated seeds erupt completely with enough speed (Baalbaki et al 1990). The advantages of priming the seed is reported so that include increasing the resistance of the plant in the salty areas, Asada 1992, and under dry condition (Adams et al 1999), seed cultivation, (Benson et al 1998), increasing the performance of the seeds with low naming power, (Afzal et al 2004), and also increase the products (Dumet and Benson 2000). According to the studies, it has been specified that the role of increasing of pyridoxine among the root attract cause appearing the root that it is affected by pyridoxine and nitrogen fertilizer of the develop indicator and the amount of the leaf chlorophyll will be changed (Khan et al). Using pyridoxine cause increase the nutrients from the soil and in result increase the performance in agricultural plant (Lone et al 1999). Treating the seeds with pyridoxine was so easy. In addition increase the cropping indicator and the tank capacity (Khan et al 2001). Corn seed was prepared with pyridoxine solution. The results showed that treating the seed with pyridoxine before cultivating it, increase the characteristics of germination and final grows of the corn plant (Eradatmand Asli and Houshmand Far 2001). Ashrafi and Ramzjoo (2010) prepared three categories of Golrang in an experiment under hydro priming and asmo priming condition. Hydro priming improved germination, the amount of germination, balance of germination and amount of shoot to the root meaningfully but decreased the duration of attending to 50% germination. Ansari et al 1990 in an experiment of mash seed declared that pyridoxine application increase access to high nutrients. By studying the effect of pyridoxine on the plant, the critical role vitamin B6 (pyridoxine) was confirmed on the plants development and their resistance to the tension. And also confirmed that vitamin B6 can act as a new kind of anti oxidant in the plants (Chen and Xiong 2005). In order to study the effect of pyridoxine on canola rate an experiment was done and according to its results it was determined that its proficiency can be improved by drenching the seed in pyridoxine solution so that pyridoxine attend the seed on the suitable level of germination (Samiullah et al 1991). The end of this research is studying the effect of preparing the watermelon seeds by pyridoxine and NaCl on the germination power increase germination seed, the average of daily germination, the length of the root and the shoot, the weight of the dry plant and the amount of activity catalase and peroxidase enzyme.

## MATERIALS AND METHODS

In order to study the effect of pyridoxine and NaCl on trial watermelon germination indicators as factorial, it was done in the form of accidental plan in the laboratory of plant physiology of Enghelab institute of shahriyar city. For this purpose, we used Charleston rate of the seeds so that firstly for every pottery dish 50 healthy seeds were separated and in order to disinfect, the seeds were drenched in sodium hydro chloride 5% for 5 minutes then they were washed with water. Related seeds were put in the pyridoxine solution for 24 hours in 20<sup>0</sup> in four densities of 0, 0/01 and 0/02. After this time the seeds were transferred to the sterilized pottery dish in which bottom there was a paper filter. The diagonal of all pottery dishes was 9 cm. All petri dishes had a diameter of 9cm. then, 10ml distilled water or chlorine and sodium solutions in concentrations of 0, 60 and 100mM (depending on the type of treatment) was added to each petri dish. Then 10ml of distilled water added to each of the pottery dishes and all of them were transferred to a germinator with 25 ±1 and duration of day light 16 hours and darkness was 8 hours. Light intensity was 1500 lux. Counting the germinated seeds was done daily in a specific time. In the time of counting, the seeds were considered germinated that the length of their roots was 2mm or more. Counting will be continued till the increase in the number of germinated seed won't be observed and the number of the seed in pottery dish will be fixed. According to the data, in order to calculate the percent and speed of germination, the following equation is used.

Germination Percentage =  $S/T \times 100$

Germination Speed =  $N1/D1 + N2/D2 + \dots + Ni/Di$

Where s is the number of germinated seeds, T is the total seeds and Ni the number of germinated seed in day Di.

The average of daily germination (MDG) =  $\sum Cpsgt/T$

Where Cpsgt is the percent of the germinated seed during the period and T is the total germination period. In order to achieve the length of the shoot and the root, 1ml ruler was used then the dry plant was measured.

### **Extracting protein for assessing the enzyme activity**

One gram of the sample (seed) with 5ml of butter trace HCl 5% molar with pH=7/5 for 30minutes in ice bath was crushed in a mortar and the achieved material was transferred to the centrifuge pipe and after 10 minutes inertia in 20 minutes and 1300 rounds in 4<sup>0</sup> siliceous was done by centrifuge machine. After finishing the centrifuge

stage, the pipe was removed from the machine and a zinc solution passed through multi layer and supplied in some little vial.

**Assessing the catalase enzyme activity**

After preparing the protein extract to assess the catalase enzyme sinetic activity the following determiner was used:

Trace buffer with pH=7 and 50milimolar 2/5 ml and hydrogen peroxide 3% (volume/volume solution) 3%ml.The above cases were mixed in ice bath and immediately 60 micro liter of enzyme extract was added to it. Attraction changes curve on the wave length of 240 nanometers was read by using spectra photometer machine. Enzyme activity is calculated based on attraction unit changes per minute for every gram of sample.

**Assessing the activity of the peroxidase enzyme**

After preparing the protein extract for assessing the activity of peroxidase enzyme synthesize, the following determiner was used: Stat buffer with 2%pH=8/4 molar 2ml, distilled water (volume volume solution) 0.2 ml and gasoline 0.04 molar solution in methanol 50% and 0.2 ml

The above cases were mixed in ice bath and immediately 0.1 milliliter of enzyme extract was added to it. Attraction changes curve on the wave length of 530 nanometers was read by using spectra photometer machine. Enzyme activity is calculated based on attraction unit changes per minute for every gram of wet weight of the whole herbal material. For doing statistical action of the data we used SAS software and the average data comparison resulted from Donken test on the probable level of 5%.

**RESULTS AND DISCUSSION**

Variance analysis results (table 1) showed that pyridoxine has meaningful effect on germination percent, germination speed, the average of daily germination, dry weight of plant, the length of the shoot and the root and the amount of the activity of catalase and peroxidase enzyme on the one percent probable level (p>0/01). Also NaCl has meaningful effect on germination speed, dry weight of plant, the length of the shoot and the root and the amount of the activity of catalase and peroxidase enzyme on the one percent probable level (p>0/01). But it doesn't have meaningful effect on the germination percent and the average of daily germination.

Table 1. the results of the effect of the variance analysis of the watermelon rate and the different levels of pyridoxine on germination and biochemical indicators of watermelon

Changes resource	Free degree	Germination percentage	Germination speed	Average daily germination	Dry weight of the plant	Shoot length	Root length	Catalase	Peroxidase
Pyridoxine	3	110/528**	337/733**	4/421**	0/167**	23/956**	17/052**	151/756**	22817/034**
NaCl	3	2/306 <sup>ns</sup>	170/674**	0/92 <sup>ns</sup>	0/737**	70/997**	34/517**	822/205**	50535/737**
Pyridoxine×NaCl	9	5/269**	24/082**	0/211**	0/005**	0/345**	0/923**	3/733**	139/093**
Error	32	1/5	0/435	0/06	0/001	0/097	0/012	0/115	36/155
c.v		1/25	1/9	1/25	6/57	4/62	2/3	1/73	3/73

Interaction of pyridoxine and NaCl has meaningful effect on germination percent, germination speed, the average of daily germination, dry weight of plant, the length of the shoot and the root and the amount of the activity of catalase and peroxidase enzyme on the one percent probable level (p>0/01). The results of the average of comparison effect of different level of pyridoxine (table 2) showed that increase of the pyridoxine improve the situation of germination components related to witness and decrease the amount of peroxidase and catalase activity. The most germination indicator is related to the pyridoxine level of 0/04.

Table 2. Comparing the average effect of different level of pyridoxine on the eruptive and biochemical indicators of the watermelon

pyridoxine	Germination percent	Germination speed	Average daily germination	Dry weight of the plant	Shoot length	Root length	Catalase	Proxidase
Percent								
0/02	/578 <sup>b</sup>	27/71 <sup>d</sup>	19/7 <sup>b</sup>	0/315 <sup>c</sup>	4/93 <sup>c</sup>	3/14 <sup>d</sup>	23/71 <sup>a</sup>	216/07 <sup>a</sup>
0/01	/390 <sup>ab</sup>	35/19 <sup>b</sup>	19/866 <sup>ab</sup>	0/513 <sup>b</sup>	6/8967 <sup>b</sup>	5/06 <sup>b</sup>	20/62 <sup>b</sup>	166/57 <sup>b</sup>
0/00	95 <sup>a</sup>	40/66 <sup>a</sup>	20 <sup>a</sup>	0/593 <sup>a</sup>	8/38 <sup>a</sup>	6 <sup>a</sup>	15/17 <sup>d</sup>	110/31 <sup>d</sup>

The same letters in every column don't have meaningful statistical difference

The results of the averages of the application NaCl (table 3) showed that the increase of NaCl plunge the situation of germination components related to the witness and boost the amount of the activity of catalase and peroxidase. NaCl have much effect on the germination percent and the average of daily germination. The most germination speed, dry weight, length of the shoot and the root is related to 0 mM treatment that this treatment includes the least amount of the activity of catalase and peroxidase enzyme. The most amount of the activity of catalase and peroxidase enzyme is related to the 0.02 percent.

Table 3. Comparison the average effect of different level of pyridoxine on the eruptive and biochemical indicators of the watermelon

NaCl mM	Germination percent	Germination speed	Average daily germination	Dry weight of plant	Shoot length	Root length	Catalase	Peroxidase
100	97/17 <sup>a</sup>	29/34 <sup>d</sup>	19/43 <sup>a</sup>	0/21 <sup>d</sup>	3/98 <sup>d</sup>	2/7 <sup>d</sup>	27/8 <sup>a</sup>	236/39 <sup>a</sup>
60	98 <sup>a</sup>	34/47 <sup>c</sup>	19/6 <sup>a</sup>	0/37 <sup>c</sup>	5/58 <sup>c</sup>	4/21 <sup>c</sup>	24/87 <sup>b</sup>	188/69 <sup>b</sup>
0	97/83 <sup>a</sup>	38/15 <sup>a</sup>	19/57 <sup>a</sup>	0/78 <sup>a</sup>	9/43 <sup>a</sup>	6/65 <sup>a</sup>	9/65 <sup>d</sup>	87/6 <sup>d</sup>

The same letters in every column don't have meaningful statistical difference

Table 4. Comparing the average interaction of the pyridoxine and NaCl on the studied adjectives in this experiment

Treatment		Adjective average							
pyridoxine	NaCl	Germination percent	Germination speed	Average daily germination	Dry weight of the plant	Shoot length	Root length	Catalase	Peroxidase
0/06	100	95/6 <sup>d</sup>	25/44 <sup>k</sup>	19/13 <sup>d</sup>	0/07 <sup>a</sup>	2/53 <sup>k</sup>	1/86 <sup>l</sup>	34/45 <sup>a</sup>	289/62 <sup>a</sup>
0/06	60	98 <sup>bc</sup>	27/03 <sup>j</sup>	19/6 <sup>bc</sup>	0/17 <sup>n</sup>	4/07 <sup>i</sup>	2/4 <sup>k</sup>	27/7 <sup>b</sup>	253/42 <sup>b</sup>
0/06	0	100 <sup>a</sup>	29/8 <sup>h</sup>	20 <sup>a</sup>	0/6 <sup>e</sup>	7/15 <sup>e</sup>	4/48 <sup>h</sup>	13/38 <sup>j</sup>	136/35 <sup>h</sup>
0/02	100	97 <sup>cd</sup>	26/64 <sup>j</sup>	19/4 <sup>cd</sup>	0/2 <sup>m</sup>	3/51 <sup>i</sup>	2/46 <sup>k</sup>	27/91 <sup>b</sup>	244/65 <sup>c</sup>
0/02	60	99/6 <sup>ab</sup>	32/64 <sup>g</sup>	19/93 <sup>ab</sup>	0/37 <sup>i</sup>	5/76 <sup>g</sup>	4/7 <sup>g</sup>	25/94 <sup>c</sup>	189/87 <sup>e</sup>
0/02	0	100 <sup>a</sup>	43/16 <sup>b</sup>	20 <sup>a</sup>	0/8 <sup>b</sup>	9/99 <sup>b</sup>	7/05 <sup>b</sup>	9/59 <sup>j</sup>	90/87 <sup>j</sup>
0	100	94 <sup>e</sup>	29/05 <sup>hi</sup>	18/8 <sup>e</sup>	0/24 <sup>l</sup>	4/26 <sup>i</sup>	3/12 <sup>j</sup>	27/73 <sup>b</sup>	233/6 <sup>d</sup>
0	60	93 <sup>e</sup>	36/44 <sup>e</sup>	18/6 <sup>e</sup>	0/44 <sup>h</sup>	5/27 <sup>h</sup>	4/37 <sup>h</sup>	24/84 <sup>d</sup>	176/93 <sup>g</sup>
0	0	89/3 <sup>f</sup>	33/77 <sup>f</sup>	17/87 <sup>f</sup>	0/76 <sup>c</sup>	9/12 <sup>c</sup>	6/42 <sup>e</sup>	9/42 <sup>j</sup>	79/05 <sup>k</sup>

The average in which a column at least has one common letter with Donken test on the level of 5% is in the similar statistical group

The results of comparing the interaction effects of different levels of pyridoxine and NaCl (table 4) showed that 0.06 level of pyridoxine with 60 mM has the most germination speed, dry weight of the plant, the length of the shoot and the root and has the least amount of the activity of Catalase and the peroxidase enzyme. Also the most germination percent and the amount of daily germination related to 0.02 pyridoxine existence and 0 mM NaCl and the level of 0.02 pyridoxine with 60 mM NaCl.

### CONCLUSION

Pyridoxine can act as a new type of plant antioxidants and also are involved in a wide range of biochemical reactions, including the metabolism of glycogen and amino acid synthesis and nucleic and the synthesis and metabolism of hemoglobin, also this material in synthesis is involved, sphengomilin and other sphengo lipids neurotransmitters. 5-phosphate pyridoxine is involved in acid metabolism of gamma - aminobutyric. Vitamin B6 in the form of 5-phosphate pyridoxine plays as coenzyme of anti-enzyme, so the seeds of the proteins and carbohydrates of enzymes and reactions of hydrolysis are broken down and ready to participate in the process of germination. The reason of the increasing the germination in pyridoxine is for stimulating the respiratory inhibitor. The similar results were reported (Khan et al 1995). The probably, the reason of increasing the length of the root and the shoot is pyridoxine because of root and shoot system development by using this material that increase the nutrients attraction of in result increase the performance in agricultural plants. The similar results were reported by (Farokhi, Eradatmand, 2007), (Khan et al, 1995), (Lone et al, 1999) and (Samiullah, 1991). Also (Chen and Xiong, 2005) by studying the effect of pyridoxine on plants, confirmed the vital role of vitamin B6 (pyridoxine) on the development of the plants. Based on the research done by (Khan et al 1995) and the increasing role of pyridoxine in the amount of root drawing , cause to appear the leaf soon. It changes the ability of photosynthesis and natural attraction rate NAR. Based on the research of treating the seed with pyridoxine will have nitrogen attraction increase and phosphor in Golrang plan, vetch and lentil (Smiullah et al, 1992), watermelon (Khan et al, 1996) and

canola (Khan et al, 1995) and (Smiullah et al 1991) (Chojnowski et al 1997) reported that priming the seed of the sunflower for 3 to 5 days increase the germination speed and improve the plant development. They also declare that the reason of this reaction in respiratory activity is producing ATP, stimulating the activity of RNA and making protein in the primed seeds. Probably the reason of decreasing the activity of Catalase and peroxidase enzyme because of eliminate the free radicals directly or by antioxidant enzymes, which reduces the damage caused by reactive species, so membrane lipid peroxidation will be decreased. On one hand, applying pyridoxine reduces these enzymes. Similar results on the other materials have been reported by (Yasar et al, 2008), (Dolatabadian et al, 2009) and (Burguieres et al, 2006). The issue can be an explanation for acceleration of germination and reduction of mean germination time which the results are in accordance with the results of Bikort et al (2005), Neuman and Shalta (2001), and Dowlatabadian et al (2008). Results from the mean comparisons of different levels of ascorbic acid showed that the acid increase leads to the increase of seed resistance against salt stress and improvement in the status of germination components' growth.

Based on the results, it was demonstrated that salt stress also results in peroxidase enzyme increase that the probably stress conditions lead to the increase of H<sub>2</sub>O<sub>2</sub> in cells and since peroxidase is considered as one of the most specialized and main sweeping enzymes of H<sub>2</sub>O<sub>2</sub>, it will turn it into water and oxygen in peroxisome before any damages from H<sub>2</sub>O<sub>2</sub> homeostasis. It seems that the above enzymes all play a role through synthesis of the second cell wall. Also, salt stress probably happens with oxidative stress so that peroxidase is among the major compounds of disinfecting toxic oxygen radicals in the stresses resulting in the increase of this enzyme. However, conditions are different in ascorbic acid priming because priming with ascorbic acid at the second level leads to the enzymes reduction. It seems that it happens because if in salt stress conditions with NaCl, ascorbic acid is placed in an acidic medium, it will positively be loaded and can combine with chlorine and if in basic medium, it will negatively be loaded. So, it can combine with sodium ion and probably result in compounds like sodium ascorbic acid and the like and reduce negative effects of sodium chloride and peroxidase is no longer produced massively. As a result, the amount of them decreases at stress time. Results were in accordance with results of Botella (1994), Dehkordi (2001) and Galardo et al (2001). Generally, NaCl resulted in the reduction of seed growth process, germination indices and increase in peroxidase activity. On the other hand, ascorbic acid priming led to the reduction of salt stress negative effects.

According to the results in this experiment and the different level of treating the chemical pyridoxine of this matter we can conclude that probably, pyridoxine by increasing the root development and raising the ability of nutrient attraction by the plant represents this possibility in order to use the potential of the water and nutrient in the soil. The results of this research showed that seed treatment with pyridoxine can be as an economic simple way and also be effective on increasing the plant output.

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