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The effect of pyridoxine and its duration application on bioactive compounds and biochemical activities of germinated wheat

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ABSTRACT

In order to study the effect of pre treatment of wheat 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), 0/02, 0/07, 0/06 % and its duration application was 8, 16 and 24 hours. 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 duration application of the pyridoxine 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 percent of germination and the average daily germination and in other word the effect of pyridoxine and its duration application on the all measured characteristics on the level of one percent probability (p>0/01). In sum the results showed that pre treatment seed by the pyridoxine can help the farmers in setting and producing suitable wheat plant.

Key words: pyridoxine, peroxidase, pre treatment, germination, catalase, wheat.

INTRODUCTION

Wheat is one of the most important agricultural plants and increase in germination percent of the wheat seed is an important factor in improving the plant (Kafi et al 2005). Although among the plants, wheat 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 wheat 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, more wheat branch fills the wheat better, increase the products and length of the branches of the wheat. 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 wheat and barley along with priming. Determining a suitable time of priming prevents a negative effect of it. 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 wheat seeds by pyridoxine and its application duration on the germination power increase germination seed, the average of daily germination, the langth 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 its application duration on trial wheat germination indicators as factorial, it was done in the form of accidental plan in the laboratory of plant physiology of agriculture college of Islamic Azad University of Saveh. For this purpose, we used Azar 2 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 8, 16, and 24 hours in 20^0 in four densities of 0, 0/02, 0/04, 0/06%. 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. 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× 100

Germination Speed = N1/D1+N2/D2+…+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) = ∑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 4^0 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

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 application duration of pyridoxine 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 wheat rate and the different levels of pyridoxine on germination and biochemical indicators of wheat.

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** **
Pyridoxine application duration	3	2/306 ^{ns}	170/674**	0/92 ^{ns}	0/737 **	70/997**	34/517**	822/205**	**50535/737****
Application duration	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**

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

pyridoxine	Germination percent	Germination speed	Average daily germination	Dry weight of the plant	Shoot length	Root length	Catalase	Proxidae
0	^{b}98/5	^{d}27/71	^{b}19/7	^{c}0/315	^{c}4/93	^{d}3/14	^{a}23/71	^{a}**216/07**
0/02	^{ab}99/3	^{b}35/19	^{ab}19/866	^{b}0/513	^{b}6/8967	^{b}5/06	^{b}20/62	^{b}**166/57**
0/04	^{a}100	^{a}40/66	^{a}20	^{a}0/593	^{a}8/38	^{a}6	^{d}15/17	^{d}**110/31**
0/06	^{c}93/3	^{c}34/55	^{d}18/66	^{b}0/505	^{b}6/955	^{c}4/83	^{c}18/98	^{c}**152/53**

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

Interaction of pyridoxine and its application duration 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.

The results of the averages of the application duration effect of pyridoxine (table 3) showed that the increase of pyridoxine application duration improve the situation of germination components related to the witness and decrease the amount of the activity of catalase and peroxidase. Pyridoxine application duration doesn't 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 24-hour 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 witness treatment.

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

Application duration	Germination percent	Germination speed	Average daily germination	Dry weight of plant	Shoot length	Root length	Catalase	Peroxidase
0	^{a}97/17	^{d}29/34	^{a}19/43	^{d}0/21	^{d}3/98	^{d}2/7	^{a}27/8	^{a}236/39
8	^{a}98	^{c}34/47	^{a}19/6	^{c}0/37	^{c}5/58	^{c}4/21	^{b}24/87	^{b}188/69
16	^{a}98/17	^{b}36/16	^{a}19/63	^{b}0/58	^{b}7/98	^{b}5/48	^{c}16/17	^{c}132/78
24	^{a}97/83	^{a}38/15	^{a}19/57	^{a}0/78	^{a}9/43	^{a}6/65	^{d}9/65	^{d}87/6

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

Table-4 Comparing the average interaction of the pyridoxine and its application duration on the studied adjectives in this experiment.

Treatment					Adjective average				
pyridoxine	Application duration	Germination percent	Germination speed	Average daily germination	Dry weight of the plant	Shoot length	Root length	Catalase	Peroxidase
0	0	95/6^{d}	25/44^{k}	19/13^{d}	0/07^{o}	2/53^{k}	1/86^{l}	34/45^{a}	289/62^{a}
0	8	98^{bc}	27/03^{j}	19/6^{bc}	0/17^{n}	4/07^{i}	2/4^{k}	27/7^{b}	253/42^{b}
0	16	99^{ab}	28/11^{i}	19/8^{ab}	0/35^{j}	6/3^{f}	3/59^{i}	21/31^{f}	184/38^{ef}
0	24	100^{a}	29/8^{h}	20^{a}	0/6^{e}	7/15^{e}	4/48^{h}	13/38^{j}	136/35^{h}
0/02	0	97^{cd}	26/64^{j}	19/4^{cd}	0/2^{m}	3/51^{j}	2/46^{k}	27/91^{b}	244/65^{c}
0/02	8	99/6^{ab}	32/64^{g}	19/93^{ab}	0/37^{i}	5/76^{g}	4/7^{g}	25/94^{c}	189/87^{e}
0/02	16	100^{a}	36/66^{e}	20^{a}	0/61^{c}	8/34^{d}	6^{d}	18/38^{h}	140/03^{h}
0/02	24	100^{a}	43/16^{b}	20^{a}	0/8^{b}	9/99^{b}	7/05^{b}	9/59^{l}	90/87^{j}
0/04	0	100^{a}	35/69^{e}	20^{a}	0/27^{k}	5/23^{h}	3/1^{j}	23/7^{e}	181/62^{fg}
0/04	8	100^{a}	39/19^{d}	20^{a}	0/46^{g}	7/15^{e}	4/92^{f}	18/84^{g}	134/88^{h}
0/04	16	100^{a}	41/47^{c}	20^{a}	0/74^{d}	9/7^{b}	7/07^{b}	11/84^{k}	86/07^{j}
0/04	24	100^{a}	44/22^{a}	20^{a}	0/89^{a}	10/96^{a}	8/38^{a}	5/68^{m}	46/7^{l}
0/06	0	94^{e}	29/05^{hi}	18/8^{e}	0/24^{l}	4/26^{i}	3/12^{j}	27/73^{b}	233/6^{d}
0/06	8	93^{e}	36/44^{e}	18/6^{e}	0/44^{h}	5/27^{h}	4/37^{h}	24/84^{d}	176/93^{g}
0/06	16	92/6^{e}	36/33^{e}	18/53^{e}	0/56^{f}	7/33^{e}	5/11^{e}	14/2^{i}	121/75^{i}
0/06	24	89/3^{f}	33/77^{f}	17/87^{f}	0/76^{c}	9/12^{c}	6/42^{e}	9/42^{l}	79/05^{k}

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 its application duration (table 4) showed that 0/04 level of pyridoxine with 24-hour application duration 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 the lack of pyridoxine existence and 24-hour application duration and the level of 0/02 pyridoxine with application duration 16 and 24 hours and the level of 0/04 pyridoxine with 0, 8, 16 and 24 hours.

DISCUSSION

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 application 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 rooot and the shoot is pyridoxine apllication 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), wheat (Khan et al, 1996) and canola (Khan et al, 1995) and (Smiullah et al 1991) (Chojnowski et al 1997) reported that primining 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).

CONCLUSION

According to the results in this experiment and the different level of treating the chemical pyridoxine and application duration 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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