Effect of salicylhydroxamic acid (SHAM) and pyridoxine on bioactive compounds and enzyme activity of germinated wheat

Bahram Behzadi¹, Davood Eradatmand Asli²* and Mohammad Sharif Moghadasi¹

1. Department of Agronomy and Plant Breeding, Islamic Azad University, Saveh Branch, Saveh, Iran
2. Department of Agriculture, Payame Noor University, Iran

Corresponding author: Davood Eradatmand Asli

ABSTRACT: This research was conducted in order to study the effect of priming on wheat seed (Triticum aestivum L.) by pyridoxine and salicylhydroxamic acid (SHAM) on germination and development characteristics as factorial design. Factors include pyridoxine with densities (witness), 0.02, 0.04 and 0.06 percent and SHAM was 0, 25 and 50 ppm. Germinated seeds were counted daily for eight days. Then the percent of germination, the length of the shoot, root and the root dry weight and also the amount of catalase and peroxidase activity were measured. The results showed that pyridoxine and SHAM affects on the percent of germination, the root dry weight, 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. However, seed priming salicylhydroxamic acid led to increase of germination indexes and the reduction of the respective enzymes’ activity such as peroxidase and catalase which is probably caused by the neutralization of free radicals. To sum up, the results showed that pre treatment seed by the pyridoxine with 0.06 percent an SHAM with 25 ppm had the best impact on germination indexes which can help the farmers in setting and producing suitable wheat plant.

Keywords: salicylhydroxamic, bioactive, enzyme, wheat

INTRODUCTION

Wheat (Triticum aestivum L.) is the most important cereal crop in the food culture of both developed and developing countries in the world. World wheat production increases by approximately 1.5% annually to meet the growing demand for food that results from population growth and economic development. A Substantial increase in grain yield potential, together with good use of water and fertilizer is required to ensure food security in the future. For improvements in photosynthetic capacity to result in additional wheat yield, extra assimilates must be partitioned to develop grains and/or potential grain weight be increased to accommodate the extra assimilates. Germination is one of the most critical and sensitive stages of wheat growth. 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. Wheat is one of the most important agricultural plants and increase in germination percent of the wheat seeding is an important factor in improving the plant (Kafi, 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, increase the products 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, 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. Studies by Moradshahi, (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, 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, 1999), seed cultivation, (Benson, 1998), increasing the performance of the seeds with low naming power, (Afzal, 2004), and also increase the products (Durnet 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, 1999). Treating the seeds with pyridoxine was so easy. In addition increase the cropping indicator and the tank capacity (Khan, 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 safflower in an experiment under hydro priming and osmotic 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, 1991). SHAM applied on wheat in 2003 by xzang et al and they were found that yield of wheat plant increased by 10 percent. Martinez Associates in 2003 breathing took place in seven species of Canola Spain and concluded with SHAM and KCN increased respiratory rate decreases, which leads to increased performance. Research on plant growth regulators is quite critical that vaccine researchers have long strings of agriculture and biology. In the end, this research was performed on effect of preparing the wheat seeds by pyridoxine and SHAM on the germination indexes such as germination seed, the length of the root and the shoot, the root dry weight and the amount of activity catalase and peroxidase enzyme.

MATERIALS AND METHODS

This study was conducted in order to study the effect of pyridoxine and SHAM on wheat germination indexes as complete factorial design in the laboratory of plant physiology of Islamic Azad University Saveh branch. For this purpose, we used back cross variety of wheat so that firstly for every pottery dish 25 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 either pyridoxine solution for 24 hours in 20° in four densities of 0, 0.02, 0.04 and 0.06 or SHAM solution in four densities 0, 25 and 50 ppm. After this time the seeds were transferred to the sterilized pottery dish in which bottom there was a paper filter. The diagonal of all Petri dishes was 9 cm. All Petri dishes had a diameter of 9 cm. Then 10ml of distilled water added to each of the Petri dishes and all of them were transferred to a germinator and duration of day light for 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 2 mm 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) = ΣCpsgt/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 30 minutes 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° 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 activity the following determiner was used: Tris buffer with pH=7 and 50 mM 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: 2ml molar Acetate buffer 2% pH 8.4, distilled water (volume volume solution) 0.2 ml and petrol 0.04 molar solution in methanol 50% . The above materials 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, root dry weight, 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 SHAM has meaningful effect on germination percent, root dry weight, 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). Interaction of pyridoxine and SHAM has meaningful effect on germination percent, root dry weight, the length of the shoot and the root and the amount of the activity of catalase and peroxidase enzyme. The results of the average of comparison effect of different levels 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 index is related to the pyridoxine level of 0.06.

<table>
<thead>
<tr>
<th>Resources</th>
<th>F</th>
<th>Germination percentage</th>
<th>Root dry weight</th>
<th>Shoot length</th>
<th>Root length</th>
<th>Catalase</th>
<th>Peroxidase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pyridoxine</td>
<td>3</td>
<td>78.681*</td>
<td>150.35**</td>
<td>45.23*</td>
<td>545.195**</td>
<td>0.219**</td>
<td>0.37**</td>
</tr>
<tr>
<td>SHAM</td>
<td>2</td>
<td>94.87*</td>
<td>197.70**</td>
<td>78.80*</td>
<td>291.75**</td>
<td>1.479**</td>
<td>0.178**</td>
</tr>
<tr>
<td>Pyridoxine×SHAM</td>
<td>6</td>
<td>85.620*</td>
<td>150.35**</td>
<td>78.80*</td>
<td>291.75**</td>
<td>1.479**</td>
<td>0.178**</td>
</tr>
<tr>
<td>Error</td>
<td>15</td>
<td>9.448</td>
<td>180.26</td>
<td>78.84</td>
<td>78.23</td>
<td>0.352</td>
<td>0.026</td>
</tr>
<tr>
<td>CV</td>
<td>9.75 %</td>
<td>6.5 %</td>
<td>11.7 %</td>
<td>7.62 %</td>
<td>11.6%</td>
<td>4.6 %</td>
<td></td>
</tr>
</tbody>
</table>

ns, * and ** respectively and non-significant and significant at the 5% level and are significant at 1%.
Table 2. Comparing average the effect of different level of SHAM on the germination and biochemical factors of wheat

<table>
<thead>
<tr>
<th>SHAM (ppm)</th>
<th>Germination percent</th>
<th>Root dry weight</th>
<th>Shoot length</th>
<th>Root length</th>
<th>Catalase</th>
<th>Peroxidase</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>61 c</td>
<td>17 b</td>
<td>32 c</td>
<td>28 c</td>
<td>1.9 a</td>
<td>1.1 a</td>
</tr>
<tr>
<td>25</td>
<td>77 a</td>
<td>21 ab</td>
<td>47 a</td>
<td>35 b</td>
<td>1.3 b</td>
<td>0.8 b</td>
</tr>
<tr>
<td>50</td>
<td>72 b</td>
<td>23 a</td>
<td>40 b</td>
<td>43 a</td>
<td>0.7 c</td>
<td>0.5 c</td>
</tr>
</tbody>
</table>

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

Table 3 Comparing average the effect of different level of pyridoxine on the germination and biochemical factors of wheat

<table>
<thead>
<tr>
<th>Pyridoxine (%)</th>
<th>Germination percent</th>
<th>Root dry weight</th>
<th>Shoot length</th>
<th>Root length</th>
<th>Catalase</th>
<th>Peroxidase</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>70d</td>
<td>13 d</td>
<td>30 c</td>
<td>26d</td>
<td>1.9 a</td>
<td>0.68 a</td>
</tr>
<tr>
<td>0.02</td>
<td>74c</td>
<td>16 c</td>
<td>33 bc</td>
<td>29c</td>
<td>1.4 b</td>
<td>0.45b</td>
</tr>
<tr>
<td>0.04</td>
<td>79b</td>
<td>22 b</td>
<td>35 b</td>
<td>35b</td>
<td>1.1 c</td>
<td>0.33c</td>
</tr>
<tr>
<td>0.06</td>
<td>83a</td>
<td>29 a</td>
<td>48 a</td>
<td>40a</td>
<td>0.95 d</td>
<td>0.14c</td>
</tr>
</tbody>
</table>

The results of comparing the interaction effects of different levels of pyridoxine and SHAM showed that 0.06 level of pyridoxine with 25 ppm has the most germination percent, root dry weight, the length of the shoot and the...
root. Also the most peroxidase enzyme activity related to 0.0 % pyridoxine with SHAM 0 ppm and the lowest point considered to $a_1b_1$ (SHAM 25 ppm + pyridoxine 0.06 %) with 0.1 OD.g$^{-1}$FW.min$^{-1}$. Moreover, it is true that about catalase enzyme activity which is 0.75 1M $H_2O_2$ min belongs to $a_2b_2$ (Sham 25 ppm + pyridoxine 0.06 %), however; reverse is true for $a_1b_1$ that is the highest amount of catalase enzyme activity with catalase enzyme activity1M $H_2O_2$ min.

CONCLUSION

Pyridoxine can act as a new type of plant antioxidants and also are involved in a wide range of biochemical reactions including: metabolism of glycogen, amino acid synthesis and metabolism of hemoglobin, also this material is involved in synthesis, sphengomilin and other sphengo lipids neurotransmitters. 5-phosphate pyridoxine is involved in acid metabolism of gamma - amino butyric. Vitamin B6 in the form of 5-phosphate pyridoxine plays as coenzyme of anti-enzyme, so increasing pyridoxine in root cause to appear the leaf soon, based on the research done by (Khan, 1995). 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 safflower plan, vetch and lentil (Smiullah, 1992), wheat (Khan, 1996) and canola (Khan, 1995) and (Smiullah, 1991) (Chojnowski, 1997) reported that seed priming of sunflower for 3 to 5 days increased the germination speed and improved the plant development. They also declared that the reason of this reaction in respiratory activity was 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 enzymes because of eliminate the free radicals directly or by antioxidant enzymes, which reduces the damage caused by reactive species, so membrane lipid peroxydation will be decreased. On one hand, applying pyridoxine reduces these enzymes. Similar results on the other materials have been reported by (Yasar, 2008), (Dolatabad, 2009) and (Burguieres, 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, (2005), Neuman and Shalta (2001), and Dowlatabadian, (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. The stored energy can be used in any component of the quantitative and qualitative performance Appears to confirm the findings of other researchers have also. (Vandorf, 1992 and Yoshiida, 1981). Some results were also distributed to form seeding method SHAM only use the additive property in other yield components indicated that it may be prepared Seeds. Salicylhydroxamic acid before planting to speed germination and seedling establish stronger According to other researchers, which can increase some of the components Their results showed that the quantitative or qualitative results of our (Cary Arthur Mitchell, 1988, and William Trevor, 1983, Kenethech, 1979, Roberts, 2002) . Therefore we must say that the use of SHAM to increase the performance of some components such as the number of grain, number of goals is useful. This applies especially for chemical preparation before planting seeds seems to be more effective for enhancing the performance of some components.

According to the results of this experiment and applying different level pyridoxine and SHAM we can conclude that pyridoxine probably due to increasing root development and raising ability of nutrient attraction of plant represents this possibility which boosts use of water potential and nutrient in the soil. The results of this research showed that seed priming by pyridoxine and SHAM can be as an economic simple way and also be effective on increasing the plant yield.

REFERENCES


Dumet D, Benson EE. 2000. The use of physical and biochemical studies to elucidate and reduce cryopreservation-induced damage in hydrated / desiccated germplasm, in Engelmann, F. and Hiroko, T. (Eds.), Cryopreservation of Tropical Plant Germplasm (Current Research Progress and Application), JIRCAS Press, Tsukuba, Japan, 2000, pp. 43-56.
Hang W. 2010. MSc Thesis. The University of Nottingham, Malaysia campus.