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The Effects of Water Seed Pre-treatment on Soybean Vegetative and Reproductive Traits

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Abstract To study the Seed Water Pre-Treatment effects on soybeans Vegetative and Reproductive Traits, a research was carried out in Ardabil Islamic Azad University research farm, in 2010. This research was conducted in factorial based on complete block randomized design. One of the Seed Water Pre-Treatment factors was 8, 12, 16 and 20 hours which were soaked in tap water and dried to 30percent moisture. A seed sample was also considered as an observation sample (without pre-treatment). The second cultivar factor was Williams and LV (17). Results indicated that there is a significant difference at 1percent level between hydro-priming durations on Germination percentage, Seedling Weight, Main Stems Weight, Lateral Stems Weight, Number of pods per plant, Dry weight of seeds per plant and yield. In most traits 8-hour Seed Water Pre-Treatment provided the best yield. Moreover, cultivar interaction effects on Main Stems Weight trait in Seed Water Pre-Treatment were significant at 1percent. The results to the average comparison table indicated that Williams's cultivar had the most height with 8-hour Seed Water Pre-Treatment. Also, there was a significant difference among cultivars on Main Stems Weight, Lateral Stems Weight, Number of pods per plant, Dry weight of seeds per plant and yield at 1percent and in most traits LV (17) had a better yields comparing to the Williams cultivar. Considering the results, see Hydro-Priming due to the short growth period and to increase the yield and better green in farm seems to be of significance. Also, 8-hour Seed Water Pre-Treatment is suggested for soybeans.

Keywords Soybean, Seed Water Pre-Treatment, Vegetative and Reproductive Traits

1. Introduction

Soybean is the main protein and oil provider in the world. Obtaining 8necessary acid amino for human body, this plant is considered as the complete protein in a diet. The area cultivated by soybean reported in 2007 in Iran was equal to 74993hectares, among which 62038hectares were on irrigated farming and 12955hectares were on dry farming [1]. Seed germination rate and uniformity are two considerable factors in crops which go under the influence of seed quality and environmental conditions. Low germination percentage and deployment speed in seedlings are among the main problems which crops face in arid and semi-arid regions [2]. Using seed strength increase treatments, quicker germination, uniform emergence and seedlings strong deployments are accessible [3, 4 and 5]. Seeds priming is among the main treatments for increasing germination strength. Seed priming is a treatment which is conducted before seeds germination and during treatment, a controlled amount of water is absorbed by the seeds [19]. The main objective to seed priming is a partial irrigation of seeds so that the seed has passed the first (physical water absorption) and second (beginning biochemical processes and sugars hydrolysis) stages of germination but has not entered the third (using the sugar by the embryo and root germination stage [6]. One of seed pre-treatment methods is the seed water pre-treatment in which distilled water or tap water is used. Priming with water is simple and cheap and water absorption is controlled through the period which seeds are in contact with water [4, 7 and 20].

Priming by water affects DNA and RNA synthesis, alpha-amylase activities and better embryo growth. By improving the germination rate, growth consistency, seedling vigor and deployment, plant growth improves [8, 9 and 10]. It is reported that hydro-priming improves the cottonseed germination under tension and non-tension conditions [11]. Raaj and Mehra have also reported growth improvement and seedling deployment in canola under tension condition. Kaya et al [12] have reported more germination and seedling growth in hydro-primed sunflower seeds under drought and salinity tensions. Additionally, Ghassemi- Golezani et al [13] reached more seed yield in pea seeds under 16-hour hydro-priming treatment. Ghassemi- Golezani et al [13] showed that hydro-priming results in seedlings growth rate, percentage, yield and yield components. They also reported that hydro-priming has a better effect on lentil seedling growth

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rate and percentage comparing to osmo-priming. Berg et al [14] reported increase in production in subsequent to soybean seeds pre-treatment.

Since there are no comprehensive information is available on the relation between seeds water pre-treatment application and timing effects in soybean cultivars, the following research tries to study various water pre-treatment effects on soybeans vegetative and reproductive traits under field and laboratory conditions.

2. Materials and Methods

To study the water pre-treatment effects on soybeans vegetative and reproductive traits and the correlation between these traits and seeds yield, a research was conducted in Ardabil IAU research farm based on a factorial randomized complete block design. The first factor was the seed water pre-treatment including control, 8, 12, 16 and 20hours, the second factor were the cultivars of Williams and LV17. The Williams and LV17soybean seeds were provided from Moghan Agricultural Research Centre and divided into five equal parts and a sample with a most of around 10percent as the control group was kept in a plastic pack in a refrigerator at 3 to 5°C. The other four samples were watered with distilled water for 8, 12, 16 and 20hoiurs in and incubator at 17.3°C. Subsequently, pre-treated seeds were spread on a table at the laboratory environment at 20 to 22°C to reach the moist of 30oercent. To determine the seeds moist two 5gr replications from each treatment were separately pounded in porcelain containers to turn into granules. Beaten samples were weighed again and put in oven for at 130°C, for an hour. Subsequently, samples were taken out of the oven and weighed. The seeds moist percentage was calculated through the following formula:

Seeds Moist Percentage = Samples Wet Weight- Samples Dry Weight/ Samples Wet Weight × 100

Three 20-seed replications were randomly separated from each sample. Seeds for each replication were put on a wet filter paper and another wet filter paper was put on them. Papers where folded 2 to 3cm to the paper end and tubed. The tubed papers for each sample was kept in a plastic pack and put in a beaker at 45degree angle to be kept in an incubator for at 10°C. After 24hours, seeds for all treatments were separately taken out of germinator. After counting germinated seeds on the same day, they were folded in filter papers and kept in the incubator. Emergences of roots with 2mm were counted as a criterion for seeds germination. Germinated seeds were counted daily, for 10days in a row. At the end of the research, the normal and abnormal buds number was determined. After germination test, seeds normal seedling was separated. Roots and shoots were cut from the seeds junctions and dried in an oven at 70°C for 24 hours. Seedlings were weighed and the seedlings dry weight average was recorded.

The field research was conducted at Ardabil Research Filed in 05.29.2008. Seeds density was considered to be 20seeds in square meters. During crop ripening, to determine the main stem weight and number of pods per plant, 10plants from each treatment were randomly harvested. The main stem weight and number of pods per plant were measured. The final harvests of each research unit were finished when the moist reached to 17 percent. In this stage, the plants available in 1square meter in each plot were harvested. Seeds were separated from the pod. And the seeds yield was recorded. All statistical analyses and means comparison were done by SPSS software. Diagrams were drawn by Excel software.

3. Results and Discussion

3.1. Germination Percentage

A result to the pre-treatment effect on germination percentage variance analysis was significant at 1percent. (Table 1) Results to the mean comparison suggested that controlled and 8, 12 and 16hour pre-treatments had the highest germination percentage in the same level and the 20hour pre-treatment had the lowest germination percentage. (Table 3) In 20hour pre-treatment, most seeds cotyledon exhaustion happened and as a result, germination percentage was decreased. Basra et al [15] reported that water pre-treatment increase low-quality rice seeds germination, so that, water pre-treatment lead to an increase from 38percent of controlled group germination to 77percent in pre-treated seeds. Gary et al [16] claimed that the germination improvement influenced by seeds pre-treatment is due to the activation of repair mechanisms and metabolic process which occur during water absorption. Basra et al [8] on a study on wheat seeds came to this conclusion that seeds water pre-treatment for 24hours had a high effect in accelerating germination. Also, Casiro et al [17] came to this conclusion that water pre-treatment is the most effective method for improving germination in onions. In wheat, water and metric pre-treatments on germination rate and percentage were higher than NaCl Osmotic pre-treatment [8].

3.2. Seedling Weight

Considering the variance analysis table for studied factors, except seed pre-treatment, all factors and their interactions showed no significant differences on seedling weight. (Table 1) Considering mean comparison table (Table 3) controlled and 8 and 12hour water pre-treatments had the highest seedling weight in the same level. In controlled and 8hour water pre-treatments, germination time, healthy seed and its high strength could lead into the increase in seedling weight. Alvord and Bradford [18] came to this conclusion that tomato seeds pre-treatment increase the seedling growth and this supremacy is preserved during growth period for a long time. This increase in plants strength in pre-treated seeds is related to their germination and uniformity. Basra et al [15] believe that the increase in

seedlings weight in pre-treated wheat seeds is due to the high germination rate and uniformity which lead to the quick deployment of seedlings. The main seed pre-treatment effect on seedling's growth is due to the quicker germination which leads the seedling to have longer growth period. Farooq et al [5] reported that rice pre-treated seeds seedlings dry weight is significantly higher than the control group seedlings. They came to this conclusion that the highest seedling weight was due to the 48hour water pre-treatment. Other findings on have been reported on the relation between seedlings weight increase due to the water pre-treatment. (8, 19) Water treatment effect in sunflower seedlings wet weight is more considerable, comparing to the osmotic pre-treatment in osmotic conditions [20].

3.3. Grain Yield

According to the variance analysis table (Table 2) there is a significant difference between economic yield in replications, cultivars and treatments. The interaction between cultivar and treatment is not significant in this trait. Average comparison results (Table 4) show that among the cultivars, LV (17) has the highest economic yield and among the treatments, 8-hour aqueous pre-treatment has the highest yield. It should be mentioned that there was no significant difference found between 8-hour and 12-hour treatments. 20-hour aqueous treatment had the lowest economic yield. Pod number, plant height and grain dry weight is among the factors which could affect the yield. In aforementioned traits also, 8-hour aqueous pre-treatment had the highest yield. Rashed et al [23] reported that barley seed pre-treatment could increase the yield up to 53percent. Increase in seed yield has been observed in corn and rice seeds due to the seed pre-treatment. Hussain et al [22] and Harris et al [10] believe that the rice seed yield due to the pre-treatment is a result of growth percentage improvement and yield execution such as seed weight. Ghassemi-Golezani et al [13] showed that hydro-priming could lead into seedling growth rate and percentage and also yield and yield components. Kahlon et al [20] Hussain et al [22] reported higher seed yield in hydro-primed seeds of sunflower and wheat, respectively. Moreover, Ghassemi-Golezani et al [13] obtained a higher pea seed yield in 16-hour hydro-priming. Harris et al [10] reported that hydro-priming results in corn, pea and Upland rice better seedling deployment and vigor which increases the growth, flowering maturity and yield.

3.4. Main and Lateral Stems Weight

Considering the variance analysis table (Table 2) and comparing the replications, cultivar, treatment and cultivar interaction with treatment effects means on main stem (Table 4). Considering the plant height trait, since 8hour water pre-treatment and Williams cultivar had the most height, has the highest main stem height. Among the interaction between treatment and cultivar, Williams's cultivar with 8hour pre-treatment and LV17with a 20hour pre-treatment had the most and the least main stem weights, respectively. Replications and interaction between treatment and cultivar effects were not significant on lateral stem weight, but considering the variance analysis table (Table 2), cultivar and treatment effects on this trait were significant. Comparison between data means suggests that LV17 had the highest lateral stems and among treatments, 16 and 20hour water pre-treatments had the highest weight. Hydro priming affects DNA and RNA synthesis, ATP availability, alpha amylase activity and better embryos growth. Hence, improvement in germination rate, growth uniformity, seedling vigor and deployment lead to improvement in plant growth. [8, 9 and 10].

Table 1. Analysis of variance for the evaluated traits at different Seed Water Pre-Treatment levels in Williams and Lv_{17} cultivars in Laboratory in 2008

Source of Variations	df	Mean Square	
		Germination percentage	Seedling Weight
Cultivar	1	414.408 ^{ns}	0.003 ^{ns}
Seed Water Pre-Treatment	4	673.346 *	0.217 *
C*SWPT	4	160.129 ^{ns}	0.016 ^{ns}
Error	20	129.908	0.016
CV (%)		13.07	11.24
* and ** Significantly at p < 0.05 and < 0.01, respectively			

Table 2. Analysis of variance for the evaluated traits at different Seed Water Pre-Treatment levels in Williams and Lv_{17} cultivars in Field in 2008

Source of Variations	df	Mean Square				
		Main Stems Weight	Lateral Stems Weight	Number of pods per plant	Dry weight of seeds per plant	Yield
Rep	2	2.867 **	0.245 ^{ns}	11.700 ^{ns}	6.395 ^{ns}	49409.085 **
Cultivar	1	5.994 **	7.762 **	929.633 **	107.390 **	17079.941 **
Seed Water Pre-Treatment	4	3.843 **	2.917 **	915.967 **	85.675 **	19956.800 **
C*SWPT	4	0.490 **	0.161 ^{ns}	3.633 ^{ns}	1.443 ^{ns}	1456.316 ^{ns}
Error	18	0.089	0.429	51.959	9.087	2975.028
CV (%)		4.37	10.11	9.14	10.53	16.66
* and ** Significantly at p < 0.05 and < 0.01, respectively						

3.5. Number of Pods per Plant

According to the variance analysis table (Table 2), replication and cultivar interaction in treatment were not significant on number of pods per plant. However, there was a significant difference found between treatments and various cultivars at 1 percent. Data means comparison results (Table 4) indicate that, among cultivars, LV17and Williams had the most and the least number of pods per plant, respectively. This trait shows that the more LV17 height is increased, yield increases and among the treatments, control and 8hour water pre-treatments had the most number of pods per plant, while 12, 16 and 20 hour water treatments had the least number of pods per plant. Results to this trait show that the quicker the germination, germination growth and seed growth are done, pods positions are increased.

3.6. Dry Weight of Seeds per Plant

Dry weight of seeds is a trait which is dependent on the number of seeds and seeds weight. According to the variance analysis table (Table 2), there was no significant difference found between replication and cultivar interaction and treatment. However, there was a significant difference found between treatments and various cultivars at 1percent. Considering the mean comparison table (Table 4), LV17 had the highest dry weight of seeds and 8hour water pre-treatment had the dry weight of seeds. Considering the number of pods per plant, other treatments had the highest number of pods per plant. This shows that, in addition to the number of pods per plant, seeds weight had the highest effect on plant yield. Considering the environmental conditions and growth period length in this research, it could be concluded that seed formation time and the seeds weight have the highest effect on yield.

Considering Iran's location which is situated in arid and semi-arid region and two crises of moisture and temperature which are considered to be of significant factors in seed germination and seedling deployment stage, especially rain fed conditions, rapid deployment could be of a great help in better water resources use. In such situation, using seed priming technique to reach a scientific result from laboratory to farm is of importance. One of the main existing concerns is conducting laboratory research in this field without evaluating their results in greenhouse and farm conditions. Hence, if there is a possibility to use this technique well, we could benefit from each condition of water cultivation in more rapid deployment with lower irrigation and success in delayed plants in rain fed condition with temperature and moisture fluctuations. According to the results in this research, due to the growth short period and using seed aqueous pre-treatment in increasing the yield and improvement in growth, the activities before seed aqueous pre-treatment seem to be of essence and the 8-hour aqueous pre-treatment is recommended for soybeans.

Table 3. Comparison of Means of traits at different Seed Water Pre-Treatment levels in Williams and Lv_{17} cultivars in Laboratory

Seed Water Pre-Treatment **levels**	**Characters**	
	Germination percentage	Seedling Weight **(gr)**
Without pretreatment	87.37 ab	0.6202 ab
8 hours	97.82 a	0.7902 a
12 hours	93.02 a	0.7030 ab
16 hours	88.05 ab	0.5773 b
20 hours	69.83 b	0.2882 c
Differences between averages of each column which have common characters are not significant at probability level of 5%.		

Table 4. Comparison of Means of traits at different Seed Water Pre-Treatment levels in Williams and Lv_{17} cultivars in Field

Seed Water Pre-Treatment **levels**	**Characters**			
	Yield**(gr/m^2)**	Lateral Stems Weight**(gr)**	Number of pods per plant	Dry weight of seeds per plant**(gr)**
Without pretreatment	326.7 abc	5.88 c	89.67 a	31.49 ab
8 hours	409.6 a	5.88 c	94.33 a	33.72 a
12 hours	348.3 ab	6.24 bc	74.33 b	27.17 bc
16 hours	297.3 bc	7.10 ab	69.00 b	25.57 c
20 hours	255.3 c	7.33 a	67.17 b	25.23 c
Differences between averages of each column which have common characters are not significant at probability level of 5%.				

Table 5. Comparison of Means of cultivar interaction effects with trait

Seed Water Pre-Treatment **levels* cultivars**	**Characters**
	Main Stems Weight**(gr)**
Williams* Without pretreatment	7.66 b
LV17* Without pretreatment	6.50 cde
**Williams ***8 hours	8.85 a
**LV17 ***8 hours	7.20 bc
**Williams ***12hours	7.08 bcd
LV17*12 hours	6.40 de
**Williams ***16 hours	6.51 cde
**LV17 ***16 hours	6.40 de
**Williams ***20 hours	6.30 e
**LV17 ***20 hours	5.44 f
Differences between averages of each column which have common characters are not significant at probability level of 5%.	

Table 6. Comparison of Means of traits at cultivars

cultivars	**Characters**			
	Yield**(gr/m^2)**	Lateral Stems Weight **(gr)**	Number of pods per plant	Dry weight of seeds per plant(gr)
Williams	303.56 b	5.96 b	73.33 b	26.74 b
LV17	351.28 a	6.98 a	84.46 a	30.52 a
Differences between averages of each column which have common characters are not significant at probability level of 5%.				

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