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Effects of storage duration and temperature of hydro-primed wheat seeds on seed and seedling quality

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ABSTRACT
Quality of seed as a propagating organ and the most valuable input to produce crops is highly important in agriculture. Thus, the objective of this study was to compare the effects of storage temperature (15, 20 and 25 °C) and storage duration (0, 2, 4, 6 and 8 days) of hydro-primed wheat seeds of Koohdasht dry land cultivar on germination and seedling characteristics. The seeds were hydro-primed then stored at each related treatment and cultivated in research farm of Lorestan University in October of 2014-2015 growing year based on randomized complete block design with three replications. Results showed that both storage temperature and duration had significant effects on seedling emergence characteristics such as speed and percentage of emergence, lateral root number, leaf number and length, seedling height, tiller number and fresh and dry weights of seedling; however, interaction of storage temperature and duration was only significant for seedling fresh and dry weights. Hydro-primed seeds stored at 15 or 20 °C had better quality in comparison to those stored at 25 °C. Extending the storage period resulted in reduced seedling quality. In overall, seeds stored at 20 °C for 2 days had better germination efficacy than those with other treatments.

Keywords: Electrical conductivity, seed storage, seed enhancement, seedling performance.

بررسی اثرات مدت و دمای نگهداری بذر هیدروپرایم شده گندم بر کیفیت بذر و گیاهچه

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چکیده
کیفیت بذر به‌عنوان اندام تکثیر و مهم‌ترین نهاده برای تولید محصولات زراعی از جایگاه ویژه‌ای در زراعت برخوردار است. بر همین اساس این تحقیق به منظور مقایسه اثر دما (۱۵، ۲۰ و ۲۵ درجه سانتی‌گراد) و مدت زمان نگهداری بذر هیدروپرایم شده (صفر، ۲، ٤، ٦ و ۸ روز) گندم دیم رقم کوهدشت بر خصوصیات جوانه‌زنی و گیاهچه، در آبان ماه سال زراعی ۹٤-۹۳ در مزرعه تحقیقاتی دانشگاه لرستان به‌صورت آزمایش فاکتوریل در قالب طرح بلوک‌های کامل تصادفی در سه تکرار اجرا شد. نتایج نشان داد که تیمار دما و مدت زمان نگهداری بذر هیدروپرایم شده به طور معنی‌داری بر خصوصیات سبز شدن گیاهچه نظیر درصد و سرعت سبز شدن، تعداد ریشه‌های جانبی، تعداد و طول برگ، ارتفاع گیاهچه، تعداد پنجه و وزن تر و خشک گیاهچه تأثیر داشت. بذور پرایم شده که به ترتیب در دمای ۲۰ و ۱۵ درجه سانتی‌گراد نگهداری شده بودند، نسبت به بذور نگهداری‌شده در دمای ۲۵ درجه سانتی‌گراد، کیفیت بهتری داشتند. همچنین با افزایش مدت زمان نگهداری بذر، کیفیت گیاهچه کاهش یافت. به‌طورکلی بذوری که در دمای ۲۰ درجه سانتی‌گراد به مدت ۲ شبانه-روز نگهداری شده بودند، از جوانه‌زنی مطلوب‌تری در مقایسه با سایر تیمارها برخوردار بودند.

واژه‌های کلیدی: افزایش کیفیت بذر، انبار کردن بذر، نمود گیاهچه، هدایت الکتریکی.

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Introduction

Wheat (*Triticum aestivum* L.) is the most vital agricultural product in the world. Although wheat consumption rate is different among countries according to their cultural and economic differences, it is one of the major food components in the world (Abotalebian *et al*., 2005). According to the estimates of the International Food Policy Research Institute, the world's wheat demand (approximately 552 million tons in 2013) will be 40 percent higher in 2020, i.e. more than 770 million tons. However, achievable resources to produce this amount of wheat demand will be much lesser at that time. Thus, it is predicted that wheat production rate will be 100 million tons lesser than the world's need in 2020 and wheat price will achieve much higher rates than the current 150 dollar per ton and purchasing from the world markets will not be possible with the current rates. According to this perspective, developing countries have made efforts to improve wheat yield which is comparable to efforts made in the past three decades and green revolution (FAO, 2013).

Seedling establishment is a critical stage in crop productions. Velocity and uniformity of seedling emergence in direct seeding method may have considerable influence on yield rate and production qualities. In recent years, efforts have been made to improve germination and seedling growth for planting in special environments; one example of these efforts is seed priming technique (Srinivasan *et al*., 1999).

Priming has positive effects, including acceleration of seedlings emergence, establishment of better and rapid seedlings, more rapid canopy closing, better competitive ability with weeds and better root development that are expected to result in better yield. Priming is more effective under stressful conditions (Eisvand *et al*., 2010).

Hydro-priming is one of the conventional priming methods, in which seeds are soaked in pure water for different time durations (Farooq *et al*., 2006) in order to improve seed germination rate (Tangh *et al*., 1999; Azarnia & Eisvand 2013).

This technique may lead to better uniformity and rapid germination of seeds, increased seedlings growth, better yield in stressful environments (Basra *et al*., 2003) and reduction in average germination time and faster seedlings establishment (Nouman *et al*., 2012).

Wheat hydro-priming and halo-priming lead to increased plumule and radicle length (Abbasdokht, 2011). Seeds priming is an effective and cheap method with low risk, resulting in better establishment of wheat seedling in various environmental conditions (Satar *et al*., 2010). In one study, wheat hydro-priming resulted in improvement of seedling characteristics and suitable establishment of seedlings (Sadat Alaei Tabatabaei *et al*., 2013).

In another study, wheat seed hydro-priming resulted in wheat yield components improvement, including grain per spike, number of spikes per area, 1000 seed weight, grain yield, biological yield and harvest index (Puran *et al*., 2014). Hydro-priming in other crops such as corn may increase germination percentage, germination characteristics and decrease the mean germination time (Ahmmad *et al*., 2014).

Seed deterioration is highly dependent on temperature and humidity. High temperature during seeds storage is one of the unsuitable factors resulting in increased respiration and rapid consumption of the storage materials, leading to seed deterioration, and affecting germination, emergence and establishment of wheat seedlings in the farm negatively (Marshall & Lewis 2004). Genetic and environmental factors (such as storage temperature and humidity) affect hydro-primed seeds quality (Rajjou *et al*., 2008). Long-term storage also reduces hydro-primed seeds life span (Hill *et al*., 2007).

Storing wheat seeds after priming for 60 days results in plumule and radicle shortening, reduction in plumule and radicle fresh as well as dry weights and subsequently decreased germination rate and speed (Shafiei Abnavi & Ghobadi, 2012).

Storage of sweet corn seeds after priming at 25 °C for 3 months resulted in decreased germination, seedling growth, and yield (Chiu *et al*., 2002).

Osmo-priming of rapeseeds for 8 hours and subsequent storage for 4 hours in comparison to hydro-priming had more positive effects on leaf area index, dry matter accumulation, crop growth rate and seed yield (Shahzad *et al*., 2003). Positive effects of priming of rice seeds are achievable when seeds are stored for only 15 days at 25 °C; higher temperatures and longer storage durations will result in lower seed yield in comparison to non-prime seeds. Thus, negative effects from priming are initially related to storage temperature (Hussain *et al*., 2015). In fact, various factors are involved for practical usage of priming in field conditions; hence, it is important to study the storage duration and temperature of wheat seeds. By achieving this goal, farmers can be informed about different storage temperature and duration conditions, for how long they have a chance to cultivate hydro-primed wheat seeds to gain maximum efficacy from priming. This research was conducted to answer these questions.

Materials and Methods

Wheat seeds (*Triticum aestivum* L. var Kouhdasht) were prepared from the Agricultural and Natural Resources Research Center, Khorram Abad, Iran. Germination and moisture content of the seeds were 100% and 8.5 %, respectively. The experiment was performed in 2014-2015 at the Research Farm of the Agricultural Faculty of Lorestan University, Khorram Abad, Iran. Experimental model was a completely randomized block design (CRBD) with 14 treatments and three replications.

This research had 14 treatments from two factors, including storage temperature (15, 20 and 25 °C) and storage period (0, 2, 4, 6 and 8 days) of hydro-primed seed of a rainfed wheat (Kouhdasht variety). In addition, non-primed seeds were planted in each block.

The seeds were hydro-primed in distilled water at 20°C for 12 hours according to Aliabadi, Farahani & Maroufi (2011). During priming process, the required oxygen for respiration was provided by aquarium pump. After priming, the seeds were dried at room temperature (25 °C) to reach the primary moisture content (8.5%). Then, they were stored at three different temperatures (15, 20, 25 °C) for different durations (0, 2, 4, 6 and 8 days). After priming and storage periods, one part of seeds was used for electrical conductivity test and the other part was cultivated in the farm. Electrical conductivity test is a rapid, inexpensive, fairly accurate, and simple method to evaluate seed vigor (Vieira *et al.*, 2002).

In order to evaluate seed membrane integrity, an electrical conductivity test was performed. Four replicates of 50 seeds from each seed treatment were weighed and immersed in 250 mL of distilled water and incubated at 20 °C for 24 hours.

Electrical conductivity of the solution was measured by (PH/mV/cond./TDP./TDS/Salt meter) instrument. Before cultivation, a complex sample of the farm soil (from 0-40 cm depth) was collected randomly in order to evaluate physical and chemical characteristics of soil and determination of fertilizer requirement (Table 1).

Workers conducted seedbed preparation, including plowing, grinding and leveling the farm. The seeds after hydro-priming and storing (according to temperature and duration mentioned above) were planted on November 8, 2014, when a rainfall had occurred four day ago. The first rain occurred 7 days after sowing. Each plot included 6 lines as P×P and R×R were 1.5 and 20 cm, respectively. Furthermore, weeds were thinned before and after tillering.

Koohdasht's rainfed wheat cultivar has high compatibility and yield potential, which can be cultivated in tropical and subtropical areas with unsuitable rainfall distribution. This cultivar has also high resistance against drought and high temperature at the end of growth period and is known as a drought resistant cultivar, being widely cultivated in the region.

The farm was daily visited and number of emerged seedlings was recorded. The speed of seedling emergence was calculated by the following formula:

$$\text{Speed of seedling emergence} = \sum_{1}^{i} \frac{ni}{Di}$$

(Agrawal, 2004)

Where n_i is number of emerged seedlings on day I, and D_i is number of days after sowing

Seedling characteristics, including lateral root number, leaf number and length, seedling height, seedling fresh and dry weights at the end of emergence stage (20 days after sowing) and tiller number at the end of tillering (45 days after sowing) were measured. Statistical analysis was performed using MSTAT-C software and Duncan's multiple range test was used to compare the means.

Results

Table 3 presents the results obtained from ANOVA. For all traits, effects of storage duration and temperature of hydro-primed wheat seeds were significant.

Table 1. Soil analysis results

Soil texture	OC (percent)	Cd (ppm)	Cu (ppm)	Zn (ppm)	Mn (ppm)	Fe (ppm)	K (ppm)	P (ppm)	pH	N (percent)
Clay-loam	1.02	0.0	1.06	0.86	8.56	4.22	285	7.8	7.6	0.101

Table 2. Precipitation and temperature during growing season (from October 2014 to June, 2015)

Month	Precipitation (mm)	Minimum temperature (°C)	Maximum temperature (°C)
October	70.30	10.19	28.68
November	27.22	4.3	18.7
December	9.6	2.6	14.4
January	9.6	-0.5	12.2
February	33.6	2.7	15.6
March	52.9	2.4	16.4
April	51.4	6.1	21.2
May	12.6	10.5	29.6
June	0.2	15.75	37.62
Average	22.17	5.77	21.85
Total rainfall during the growing season	204.24		

Percentage and Speed of Seedling Emergence

Both storage temperature and duration of hydro-primed wheat seeds had significant effects on percentage and speed of seedling emergence. Long-term storage (8 days) and high storage temperature (25 °C) had deleterious effects on the percentage and speed of emergence of hydro-primed wheat seeds. Increment of seeds storage temperature from 20 to 25°C resulted in decreased speed of emergence and percentage (Table 4). In general, seeds stored at 20 °C for 2 days had the highest emergence percentage (86.13) and rate (70.44 seedlings per day), while seeds stored at 25°C for 8 days had the lowest emergence percentage (64.68) and rate (52.90 seedlings per day). In fact, seeds stored at 15 and 20°C had significantly better emergence properties versus those stored at 25 °C. Extending the storage period from 0 to 8 days also resulted in poor emergence in the group of seeds stored at 25 °C.

Root Branches

The highest lateral root number (9.477 per seedling) was resulted from seeds stored at 20 °C for 2 days, while the lowest lateral root number (4.563 per seedling) was from those stored at 25 °C for 8 days (Table 4).

Leaf Number

The highest and lowest leaf number per seedling was resulted from seeds stored at 20 °C for 2 days (6.053) and those stored at 25 °C for 8 days (3.620), respectively (Table 4).

Leaf Length

The hydro-primed seeds stored at 20 °C for 2 days produced leaves with highest length (12.25 cm). Increase of temperature and extension of storage duration resulted in decreased leaf length (Table 4).

Seedling Height

Increase of storage temperature from 20 to 25 °C resulted in seedling shortening (Table 5). Extending storage period in seeds stored at that 25 °C also resulted in declined seedling height. Generally, seeds stored at 20 °C for up to 2 days, had the highest seedling after cultivation, whereas seeds stored at 25 °C produced the lowest seedlings regardless of their storage duration (Table 5).

Tiller Number

Seeds stored at 20 °C for 2 days produced the highest number of tillers per plant (4.637), whereas those stored at 25 °C for 8 days produced the least number of tillers per plant (2.560; Table 5).

Seedling Fresh Weight

Seedlings produced from seeds stored at 20 °C for 2 days had fresh weight of 5.163 gr, whereas seedlings resulted from seeds stored at 25 °C for 8 days had fresh weight of 2.047 gr (Table 5).

Seedling Dry Weight

Increase of storage temperature and extension of storage period resulted in declined seedling dry weight (Table 5). The greatest (2.957 gr) and lowest (1.090 gr) seedlings dry weights were attributed to seeds stored at 20 °C for 2 days and ones stored at 25 °C for 8 days, respectively.

Discussion

Seed is the most important unit in plant life cycle. Using unsuitable and low quality seeds will decrease efficiency of other inputs. In the present study, hydro-priming increased seed and seedling qualities. In addition, results indicated that hydro-primed seed could be stored for short time (2-4 days) without losses of quality. Optimal storage condition of hydro-primed wheat seeds of Kouhdasht cultivar contributes to the seed improvement and seedling quality. Shafiei Abnavi and Ghobadi (2012) reported that optimal storage condition of hydro-primed wheat seeds with regard to storage temperature and duration will result in a higher emergence percentage and speed. Other researchers also found wheat seed hydro priming as a useful technique improving seedling emergence (Sadat Alei Tabatabai *et al*., 2013; Hamidi & Pirasteh, 2013). Higher germination percentage and rate may be a consequence of decrease in germination base temperature (Abotalebian, 2005).

In some crops, usefulness of priming disappeared due to desiccation of embryo during storage (Bewley & Black 1985). Azadi and Younesi (2013) found that storage of sorghum primed seeds for extended periods will result in a significantly reduced germination owing to impaired enzyme activities of the seeds.

Table 3. ANOVA (mean of squares) for the effects of storage temperature and duration of wheat hydro-primed seeds on some evaluated seedling traits

S.O.V	df	MS								
		Seedling Em. percent	Em. speed	No. of root branches	Leaf no.	Leaf length	Seedling height	Tiller no.	Seedling FW	Seedling DW
Replication	2	878.87^{**}	591.25^{**}	61.13^{**}	11.24^{**}	55.63^{**}	118.94^{**}	8.95^{**}	8.52^{**}	2.92^{**}
Storage temp. (A)	2	448.12^{**}	299.49^{**}	20.71^{**}	3.758^{**}	26.78^{**}	13.31^{**}	2.23^{**}	8.05^{**}	0.95^{**}
Storage duration (B)	4	171.78^{**}	115.69^{**}	7.95^{**}	2.999^{**}	19.84^{**}	20.86^{**}	1.55^{**}	4.64^{**}	5.72^{**}
A * B	8	27.33^{ns}	18.25^{ns}	1.76^{ns}	0.365^{ns}	1.80^{ns}	1.46^{ns}	0.22^{ns}	0.56^{*}	0.51^{**}
error	28	15.36	10.16	1.26	0.193	0.66	2.56	0.13	0.17	0.07
CV%		5.16	5.13	16.05	9.07	8.43	8.44	10.58	12.95	15.97

ns: Not significant *, ** : Significant at 5% and 1% probability levels, respectively.
Em.: emergence

Table 4. Mean comparisons for the effects of storage temperature and duration of wheat hydro-primed seeds on some evaluated seed and seedling traits

	Storage duration (day)	Emergence Percent	Emergence Speed (seedling/day)	No. of root branches	Number of leaf	Leaf length (cm)
Non stored	0	82.09^{ab}	67.15^{ab}	8.473^{ab}	5.697^{ab}	11.89^{a}
15 °C	2	78.91^{abc}	64.54^{abc}	7.777^{abc}	5.067^{bcd}	10.57^{ab}
	4	75.38^{bcd}	61.65^{bcd}	7.150^{abc}	4.550^{cdefg}	9.507^{bcd}
	6	73.77^{bcd}	60.21^{bcd}	6.490^{bcd}	4.557^{cdefg}	8.987^{bcd}
	8	73.07^{cde}	59.75^{bcde}	5.910^{cd}	4.497^{cdefg}	8.467^{cde}
20 °C	2	86.13^{a}	70.44^{a}	9.477^{a}	6.053^{a}	12.25^{a}
	4	79.65^{abc}	65.13^{abc}	8.793^{abc}	5.303^{abc}	10.80^{ab}
	6	78.89^{abc}	64.53^{abc}	7.647^{abc}	5.043^{bcde}	9.657^{bc}
	8	78.17^{abc}	63.92^{abc}	7.370^{abc}	4.763^{cdef}	9.533^{bcd}
25 °C	2	71.18^{cde}	58.21^{cde}	5.630^{cd}	4.333^{defg}	7.957^{cdef}
	4	68.33^{de}	55.87^{de}	5.550^{cd}	4.317^{defg}	7.697^{def}
	6	64.82^{e}	53.00^{e}	4.780^{d}	3.863^{fg}	7.100^{ef}
	8	64.68^{e}	52.90^{e}	4.563^{d}	3.620^{g}	6.453^{f}
Non-primed		73.95^{bcd}	60.06^{bcde}	6.127^{bcd}	4.097^{efg}	9.813^{bc}
LSD($P \leq 0.05$)		7.744	6.404	2.083	0.8358	1.666

* Means in each column followed by similar letter(s) are not significantly different at 5% probability level, using Duncan's Multiple Rang Test.

Table 5. Mean comparisons for the effects of storage temperature and duration of wheat hydro-primed seeds on some evaluated traits

	Storage duration (day)	Seedling height (cm)	Tiller no.	Seedling fresh weight (gr)	Seedling dry weight (gr)
Non-stored	0	18.89[a]	3.917[b]	4.037[b]	2.097[b]
15 °C	2	16.22[ab]	3.640[bcd]	3.553[bc]	1.727[bc]
	4	15.86[ab]	3.250[bcdef]	2.640[de]	1.513[cde]
	6	15.79[ab]	3.207[bcdef]	2.567[de]	1.450[cde]
	8	15.22[b]	3.147[cdef]	2.470[de]	1.417[cde]
20 °C	2	18.95[a]	4.637[a]	5.163[a]	2.957[a]
	4	16.68[ab]	3.673[bc]	3.787[b]	1.763[bc]
	6	15.94[ab]	3.383[bcd]	3.783[b]	1.663[bcd]
	8	15.84[ab]	3.283[bcde]	2.850[cde]	1.617[bcde]
25 °C	2	15.16[b]	3.103[cdef]	2.463[de]	1.357[cde]
	4	14.63[b]	2.937[def]	2.310[e]	1.323[cde]
	6	14.61[b]	2.647[ef]	2.137[e]	1.173[de]
	8	13.95[b]	2.560[f]	2.047[e]	1.090[e]
Non-Primed		15.39[b]	3.333[bcde]	3.210[bcd]	1.610[bcde]
LSD(P≤0.05)		3.019	0.6189	0.7524	0.4747

* Means in each column followed by similar letter(s) are not significantly different at 5% probability level, using Duncan's Multiple Rang Test.

Satar *et al*. (2010) found rapid germination and seedling growth extremely important in suitable establishment of wheat cultivars. In fact, seed priming is an effective and cheap technique with low risk, resulting in better seedling establishment in different environmental conditions. However, temperature increment from 20 to 25 °C and extending storage period up to 8 days resulted in seed deterioration and decline in seeds quality. Seed deterioration decreases seed vigor, resulting in decreased germination and poor seedling establishment (Nash 1981). In deteriorated seeds, due to impaired function of cell organelles such as mitochondria and glyoxysomes, production rate of free oxygen radicals is increased (Bailly, 2004).

Parmoon *et al.* (2015) reported that longtime storage of milk thistle (*Silybum marianum* L.) primed seeds would result in lower seedling height. Primed wheat and barley seeds have enhanced germination and growth rate at the beginning of growing season, leading to more fertile tillers, larger spikes and extended seed development and seed filling period (Duman 2006).

Long-term storage of primed rice seeds in unsuitable temperature condition results in a decreased fresh weight of root and stem and decreased root length (Hussain *et al*., 2015). Optimal priming benefit of pansy seeds is achieved when humidity of seeds is in the range of 12 to 20% and seeds are stored for a period not longer than 4 weeks at 5°C (Carpenter & Boucher, 1991). Parmoon *et al*. (2015) reported that long-term storage of primed milk thistle seeds led to decreased catalase activity and seed storage reservoirs, leading to decreased dry seedling weight. Shahzad *et al*. (2003) found that osmo-priming of rapeseeds for 8 hours and subsequently storage of seeds for 4 hours are more effective than hydro-priming by comparing crop dry matter accumulation and growth rate indices.

Conclusion

Justification for effectiveness of priming in the farm is that it can reduce germination time and improve seed yield in various environmental conditions (Farooq *et al*., 2008).

Priming in the farm may cause increment of germination rate and percentage improvement of seedling establishment, acceleration of flowering and maturation, drought stress resistance and yield improvement. Moreover, as hydro-primed seeds had better nutritional condition, they may have greater resistance to plant pests and diseases (Harris *et al.*, 1999).

Successful priming depends on subsequent processes such as seed desiccation, optimal seed storage temperature and duration and transferring to the farm. In this study, we found that storage of hydro-primed wheat seeds for 2 days at 20 °C was effective in comparison to other storage temperatures and durations, resulting in better yield. It seems that there is no rush for the cultivation of hydro-primed wheat seeds of Koohdasht's cultivar, since they can be stored for 2 subsequent days at 20 °C before cultivation.

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