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Wild Barley (*Hordeum spontaneum* Koch) Seed Germination as Affected by Dry Storage Periods, Temperature Regimes, and Glumellae Characteristics

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ABSTRACT

After dry storage, germination of newly harvested intact and naked seeds of wild barley (*Hordeum spontaneum* Koch) were determined at 20° C. Intact seeds did not germinate after 8 weeks whereas, naked seeds germinated and no significant differences between dry storage periods were observed for germination of these seeds. Cold stratification periods had no effect on germination percentages of non-dormant seeds of wild barley. The minimum, optimum, and maximum temperatures for germination of wild barley seeds were 5, 20, and 30 °C, respectively. Results showed that wild barley glumellae had either physical or chemical effects on seeds germination because, all naked seeds germinated but when intact seeds were rinsed in ethanol 70% and distilled water, the germination percentage were 0 and 54%, respectively, which was lower than that of naked and intact seeds.

Keywords: Cold stratification; *Hordeum spontaneum*; Wheat; Cardinal temperature; glumellae.

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INTRODUCTION

Seed dormancy is a major adaptive trait in weeds which facilitates the survival of them and provides for resistance to sprouting of preharvest in members Poaceae family (Benech-Arnold et al., 2000). In addition, the seeds of many weed species can remain viable in the soil seed bank because they possess some types of inherent dormancy (Fenner & Thompson, 2005). The dormancy can further reassure that the germination of seeds to occur only at an appropriate time and space (Benech-Arnold et al., 2000), and that is an important reason why most weeds are successful, though each individual will differential experience а success (Gutterman & Nevo, 1993).

There are several factors that have been found to trigger germination or break the seed dormancy including cold stratification, dry storage and/or exposure of dry seeds to elevated temperature, leaching, scarification and treatments with chemicals may be involved in breaking (Bradbeer, 1994). dormancy The conditions required to break dormancy often vary among species, but also vary within species or populations. (Alen & Meyer, 1998).

Wild barley (*H. spontaneum* Koch) such as wild wheat is one of the principal grain plants on which Neolithic food production in the Near East was founded. Wild barley is the progenitor of cultivated barley and this is indicated by its cross-compatibility, full fertility and sporadic spontaneous

hybridization with other cultivars. The high genetic diversity of wild barley makes it as the best resources for improving the narrowing genetic base of the cultivated barleys (Nevo, 1997). Wild barley is an annual, brittle, two-row diploid (2n=14), and is predominantly self-pollinating (Brown et al., 1978). Wild barley is widespread in the Near East Fertile Crescent and its population involves abundant genetic variation against drought and salinity while its highest resistant genotype is significantly correlated with the high stress environment and with the highest genetic polymorphism (Nevo, 1992).

It has been founded that freshly harvested seeds of wild barley do not germinate in a range of temperatures, in light or darkness (Gutterman & Nevo, 1993; Gutterman et al., 1996). An important survival strategy of wild barley under unpredictable small amounts and infrequent winter rains in dry regions is after-ripening (Evenari, 1965; Gutterman, 1998). The need for afterripening prevents germination of mature seeds after a late rain at the beginning of the long, dry summer (Evenari, 1965). The primary dormancy in wild barley seeds is a result of glumellae, the seed covering tissue. Such covering structures of the seed may limit the oxygen supply to the embryo (Gutterman, 1996) and thus inhibit germination. Gutterman et al., (1996) reported that the dormancy breaking of wild barley seeds occur during storage in dry conditions at 35 °C or in the natural habitat during summer. Gozlan & Gutterman, (1999) found that seeds of wild

barley ecotypes that originating from different regions, show different germination responses to dry storage temperatures and duration.

The aims of this investigation were to study (1) the effect of constant temperatures on seed germination, (2) after-ripening patterns of wild barley seeds, (3) effect of cold stratification on seed germination, and (4) physical and chemical effects of glumellae on seed germination of wild barley.

MATERIALS AND METHODS

All experiments were conducted in laboratory and germination tests were done in dark condition. Caryopses of wild barley were harvested on 15 May 2004 from the Experimental Station Farm of Shiraz University at Kushkak located 1650 meters above the average sea level with a longitude of 52° 34' E and latitude of 30° 7' N.

Experiment 1

Effect of Constant Temperatures on Wild Barley Non-Dormant Seed Germination

To determine the cardinal temperatures for the germination of wild barley seeds compared to wheat (*Triticum aestivum* cv. Pishtaz), surfaced-sterilized wild barley and wheat seeds were germinated in sterilized 9-cm Petri dishes containing two sheets of Whatman # 2 filter papers moistened with 5 ml of distilled water. Petri dishes were then placed at 8 constant temperature regimes of 5, 10, 15, 20, 25, 30, 35, and 40 °C under dark condition. Germination counts were made after 3 and 7 days. Germination was considered to occur when radicle length was 1 mm or longer.

Experiments were conducted in a completely randomized design (CRD) with four replications. Twenty five seeds in each Petri dish were considered as a replicate. Data were subjected to analysis of variance procedure. Statistical analysis of data was conducted using MSTAT-C software.

Experiment 2

Effect of Dry Storage Periods on Germination of Wild Barley Seeds

Immediately after harvesting, the seeds were stored in fine-mesh bags at room temperature $(25\pm3 \ ^{\circ}C)$ with relative humidity ranging from 15-20%. At 7-day intervals (for 14 weeks), two samples of 100 intact and hand dehulled (naked) seeds (four replicates of 25 seeds) were selected and germinated in a germinator set to constant temperature of 20 ± 1 °C in the darkness (based on results of experiment 1). For each experiment, seeds were placed in 9-cm sterilized Petri dishes on two sheets of Whatman #2 filter paper and moistened with 5 ml of distilled water. Germination was considered to occur when radicle length was 1 mm or longer. Experimental design was split factorial in which the storage time was considered as main plot and seed type was considered as sub-plot.

Experiment 3

Effect of Cold Stratification on Dormant and Non-Dormant Seed Germination of Wild Barley Wild barley dormant (freshly harvested) and non-dormant (from the previous year) seeds were placed in 9-cm sterilized Petri dishes on 2 sheets of Whatman # 2 filter paper and moistened with 5 ml distilled water and stored in an incubator at constant temperature of 2 ± 1 °C for 4 weeks. At 7day intervals, dishes were randomly selected and transferred to germinators and were kept at constant temperatures of 10, 20 and 40 °C in the dark condition for 7 days. After 7 days the germinated seeds were counted. During cold stratification, the dishes were checked to make sure the seeds remain moist. Experiments were conducted using a split-plot design with four replicates. Twenty five seeds in each Petri dish were considered as a replicate. The main factor constituted three temperature levels (10, 20 and 40 °C) and the sub-factor constituted five stratification periods of 0, 1, 2, 3 and 4 weeks at 2 ± 1 °C. No germination was occurred when the dishes were incubated at 2 ± 1 °C.

Homogeneity were calculated for all of the experiments (as variance) and those data not linearly distributed were \log_{10} transformed and detrains formed data are present in the results. Data were subjected to analysis of variance, and mean separation was made using Duncan's new multiple range test at the 0.05 level of significance.

Experiment 4

Effects of Glumellae Characteristics on Wild Barley and Wheat Seeds Germination Germination of intact and dehulled wild barley and wheat seeds were examined under 13 conditions as described below:

1) Wild barley intact seeds (with glumellae) in order to reveal the physical effects of glumellae on germination;

2) Wild barley naked seeds along with the separated glumellae in the Petri dish to reveal the chemical effects of glumellae;

3) Wild barley intact seeds were rinsed in ethanol 70% for 5 min to remove any possible ethanol-soluble compound(s) from the glumellae (Chen *et al.*, 2004);

4) Wild barley intact seeds were rinsed in distilled water for 5 min to remove any possible water-soluble compound(s) from the glumellae;

5) Wild barley naked seeds were rinsed in ethanol 70% for 5 min;

6) Wild barley naked seeds were rinsed in distilled water for 5 min;

7) Wild barley naked seeds alone;

 Wild barley naked seeds and their separated glumellae were rinsed in ethanol 70% for 5 min and were then placed in the same Petri dishes;

9) Wheat seeds with wild barley glumellae(25:25 in each Petri dish);

10) Wheat seeds with wild barley naked seeds (25:25 in each Petri dish);

11) Wheat seeds with wild barley intact seeds (25:25 in each Petri dish);

12) Wheat seeds with wild barley glumellae that rinsed in 70% ethanol for 5 min (25:25 in each Petri dish);

13) Wheat seeds alone.

Following 7-days incubation at 25 ± 1 ^OC, seed germination, shoot and root length, and number of seminal roots for both plants were measured. Germination was considered to occur when radicle length was 1 mm or longer.

The experiment was conducted in a completely randomized design (CRD) with four replications. Homogeneity were calculated for all of the experiments (as variance) and those data not linearly distributed were \log_{10} transformed and detransformed data are present in the results.

RESULTS AND DISCUSSION

Experiment 1

Effects of Constant Temperatures on Wild Barley Seeds Germination

Wild barley seeds required temperatures lower than 30 °C for germination. Maximum germination occurred at 20 °C (Figure 2). This optimum temperature for wild barley seeds germination was similar to that of reported by (Gutterman *et al.*, 1996).

Wheat seeds germinated nearly 100% after 7 days at 25 °C or lower temperature, but declined to 24 and 0% at temperatures of 35 and 40 °C, respectively (Figure 3). The rate of germination for wild barley seeds was lower than that of wheat in both counting dates (Figures 2 & 3). The wheat also maintained a high germination percentage at high temperature. Compared to wheat, final germination values were lower for wild barley at all temperatures. The results of this study indicated that lower temperatures (i.e. 5-10 °C) were more favorable for germination of wild barley seeds than higher ones (30-40 °C). These results agree with the results of (Egley & Duke, 1984) who reported that high temperatures may denature the enzymes and change lipid phase. However, the lack of germination of wild barley seeds at temperatures higher than 30 °C can be due to lower temperature requirements of this species for germination. However, (Gozlan & Gutterman, 1999) reported that after 20 months of storage of H. spontaneum seeds at 10-30 °C, a negligible percentage of seeds germinated at 30 °C.

Experiment 2

Effects of Dry Storage Periods on Wild Barley Seed Germination

No germination of intact seeds occurred until 8 weeks after storage (Figure 1). All these seeds were found to be dormant, as shown for *Aegilops cylindrica* (Fandrich & Mallory-Smith, 2005), *Tripsacum* dactyloides (Gibson et al., 2005) and Phalaris sp. (Matus-Cadiz & Hucl, 2005). The germination of intact seeds increased with dry storage durations. After 12 weeks, the germination of intact seeds reached its maximum rate (65%). This delay in germination, usually called after-ripening, is an important survival strategy for many Poacea family members such as Lolium rigidum (Steadman et al., 2003), weedy rice (Oryza sativa) (Gu et al., 2005), and Alopecurus myosuroides (Colbach & Durr, 2003) which may confront unpredictable rainfall in the summer (Evenari, 1965). On the contrary, no significant differences

between dry storage periods were observed for naked seeds germination (Figure 1). The dormancy of wild barley freshly harvested seeds results mainly from inhibitory action of glumellae which is reduced during dry storage (Figure 1).

It was suggested by (Lenior et al., 1986) that in wild and cultivated barley (Hordeum vulgare L.) glumellae prevents germination by placing the seed in fix hypoxia, because they oxygen. However, a good oxygen supply under the glumellae would be necessary only during the first hours of germination. In fact, after 30 hour, oxygen uptake by the glumellae is the same for dormant and non-dormant seeds. This oxygen absorption would be sufficient to prevent germination of freshly harvested seeds. But, after a long period of dry storage, seeds would not be subjected to this inhibition action because germination would have started at the beginning of imbibition, when the glumellae absorb very little oxygen. However, primary dormancy of *H*. spontaneum is much deeper than that of seeds of cultivated barley. According to (Baker, 1974), the success of a weed may depend in part on a delay in germination until conditions are suitable for plant

growth therefore, high wild barley population density in wheat fields can be attributed to this phenomenon.

Experiment 3

Effects of Stratification Periods on Wild Barley Dormant and Non-Dormant Seed Germination

No significant differences were observed stratification among periods all at temperature regimes, whereas, the mean germination percentages varied significantly between temperature regimes. Overall. the highest germination percentage was occurred at 20 °C (87%) followed by 10 °C (65%) with no germination at 40 °C (Table 1). None of the dormant seeds germinated after 4 weeks (data not shown), indicating those four weeks of cold stratification could not alleviate the dormancy of wild barley seeds. Baskin & Baskin, (1998) reported that cold stratification is not required for seed germination of all species, but is required for embryo growth in seeds with non-deep dormancy. Since, the dormancy of wild barley seeds is more likely to be related to glumellae characteristics (Figure 1) and not to embryo, this may explain why cold stratification is not effective for dormancy breaking.

Constant	Weeks of stratification at $2 1 \pm ^{\circ}C$					
temperature	0	1	2	3	4	Means
(°C)			%			
10	66.00 a	66.00 a	65.00 a	64.00 a	66.00 a	65.40 B
20	87.00 a	86.00 a	88.00 a	87.00 a	87.00 a	87.00 A
40	0.00 a	0.00 a	0.00 a	0.00 a	0.00 a	0.00 C
Means	51.00 a	50.66 a	51.00 a	50.33 a	51.00 a	

Table 1. Effects of cold stratification periods on germination of wild barley non-dormant caryopses	Table 1. Effects of co	ld stratification periods or	germination of wild barle	v non-dormant carvopses ^{ab}
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^aMeans within each row with the same letters (small letters) are not significantly different at

the 5% level according to Duncan's new multiple range test.

^bMeans within each column with the same letters (capital letters) are not significantly different at the 5% level according to Duncan's new multiple range test.

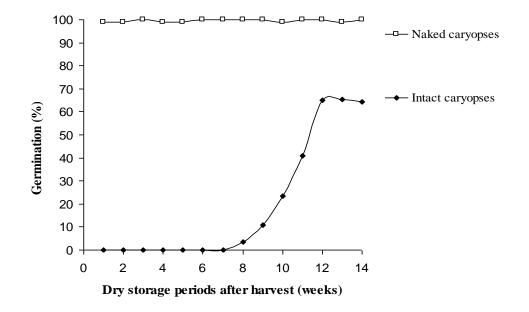


Figure 1. Effects of dry storage periods on germination of wild barley intact and naked seeds at 20 \degree C

Experiment 4

Effect of Glumellae Characteristics on Wild Barley and Wheat Seed Germination

The effects of different treatments on wild barley intact and naked seeds germination and the seedling growth are shown in Table 2 Only 59% of intact seeds were able to germinate within 7 days of incubation (T1). When the glumellae was removed, the naked seeds germinated up to 100% (T7). Naked seeds placed adjacent to glumellae germinated 95% (T2). Very similar results were obtained with naked seeds and detached glumellae that were inserted in ethanol 70% for 5 min (T8). Intact seeds treated with ethanol (T3) did not germinate but, those inserted in distilled water (T4) had germinated as great as 54%. The germination percentages of naked seeds that were treated with ethanol (T5) or distilled water (T6) were 90 and 95%, respectively (Table 2).

Shoot length of intact seeds that were treated with distilled water (T4) were 54 mm and less than that showed no differences (T1), whereas, root length, and number of seminal roots were not affected by all treatments (Table 2). Wheat seed germination, root length, and number of seminal roots were not influenced by any of the treatments (Table 2), but the shoot length was increased as affected by wild barley glumellae.

The results presented above clearly show that glumellae had either physical and chemical effects on wild barley seed germination. When intact seeds were rinsed in ethanol 70% (T3) and distilled water (T4), their germination percentages were 0 and 54%, respectively. This was lower than that of naked (T7) and intact seeds (T1). This may be the main reason

chemical for stimulating effects of glumellae on germination. The presence of some phenolic compounds in seeds of family Graminae has been reported 1961: (Belderock, Glennie, 1981: Jayachandran-Neir & Sridhar, 1975). In nature and under high interference pressure, there are many known and unknown mechanisms that may be involved in enhancing the seed seedling germination and plant establishment. Results from this study show that ethanol- and/or water-soluble compound exist in glumellae and are desirable and suitable to enhance seed germination and seedling growth of its own plant. In this relation, absence of the glumellae chemical compound (T3 and T4) decreased germination of seeds (Table 2). Hamidi et al., (2006) reported that some extract concentrations made from the intact seeds of wild barley stimulated germination, shoot and root lengths, shoot and root dry weights of wheat and its own plant and this phenomenon could be considered as an ecological adaptation.

Growth parameters						
Treatments	Germination	Shoot length	Root length	Seminal roots		
	(%)	(mm)	(mm)	(no.)		
		Wild	barley			
T ₁	59 b	94.43 ab	116.00 a	3.16 a		
T_2	95 a	99.43 a	104.40 a	3.42 a		
T ₃	0 c	0 c	0 b	0 b		
T ₄	54 b	54.17 b	93.17 a	3.16 a		
T ₅	90 a	106.30 a	119.30 a	3.32 a		
T ₆	95 a	82.65 ab	113.60 a	3.10 a		
T_7	100 a	85.87 ab	109.40 a	3.66 a		
T ₈	99 a	87.33 ab	111.00 a	3.33 a		
		Wheat				
T ₉	100 a	129.50 a	123.70 a	5.81 a		
T ₁₀	99 a	108.20 b	114.00 a	5.71 a		
T ₁₁	99 a	106.50 b	123.40 a	5.63 a		
T ₁₂	100 a	121.70 a	129.40 a	5.22 a		
T ₁₃	100 a	112.70 b	126.30 a	5.07 a		

Table 2. Effects of physical and chemical characteristics of wild barley glumellae on wheat seed and its own seed germination, shoot and root length, and number of seminal roots ^{a,b}.

^aFor each species, means within each column with the same letters are not significantly different at the 5% level according to Duncan's new multiple range test. ^bT1: intact seeds; T2: naked seeds with the glumellae; T3: intact seeds treated with 70% ethanol; T4: intact seeds treated with distilled water; T5: naked seeds treated with 70% ethanol; T6: naked caryopse treated with distilled water; T7: naked seeds; T8: naked seeds with the glumellae treated with 70% ethanol; T9: wheat seeds with wild barley gelumellae; T10: wheat seeds with naked seeds; T11: wheat seeds with intact seeds; T12: wheat seeds with glumellae that treated with 70% ethanol; and T13: wheat seeds.

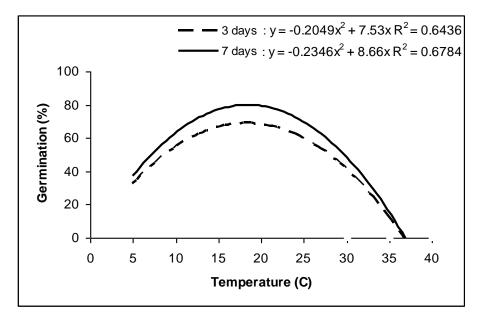


Figure 2. Effects of constant temperatures on wild barley seeds germination after 3 and 7 days.

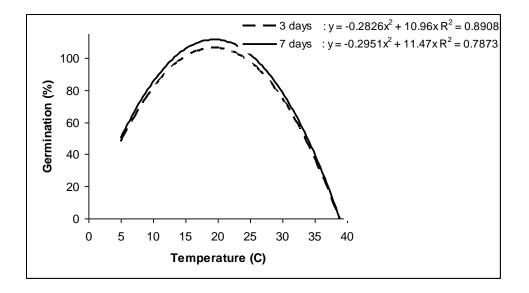


Figure 3. Effects of constant temperatures on wheat seed germination after 3 and 7 days.

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جوانه زنی گندمههای تازه رسیده جو وحشی در دمای ۲۰ درجه سانتی گراد تعیین گردید. گندمه های دست نخورده تا هشت هفته جوانه نزد ند ولی جوانه زنی آنها پس از ۱۲ هفته بیشترین مقدار رسید . جوانه زنی گندمه های پوست کنده از هفته اول آغاز و در جوانه زنی آنها تفاوت معنی داری وجود نداشت. نتایج نشان دادند که خواب گندمه های دست نخورده از نوع پس رسی بوده و پوشینه ها مانع فیزیکی جوانه زنی میباشند.گندمه های تازه سواشت شده و آنهایی که دوره پس رسی را گذرانیده بودند در دمای ۱ \pm ۲ درجه سانتی گراد برای چها هفته، مته گیداری شدند. مها*نگی درصد* جوانه زنی میباشند.گندمه های تازه در دماهای ۱۰، ۲۰ و ۴۰ درجه سانتیگراد برای گندمه های بدون خواب به ترتیب ۶۶، ۸۶ و صفر درصد بود. پس از ۲، ۳ و ۴ هفته، ه یچ گونه تفاوت معنی داری بهی در دماهای ۱۰، ۲۰ و ۴۰ درجه سانتیگراد برای گندمه های بدون خواب به ترتیب ۶۶، ۸۶ و صفر درصد بود. پس از ۲، ۳ و ۴ هفته، ه یچ گونه تفاوت معنی داری بهی درصد جوانه زنی گندمه های که ۱۰، ۲۰ و ۴۰ درجه سانتیگراد برای گندمه های بدون خواب به ترتیب ۶۶، ۶۸ و صفر درصد بود. پس از ۲، ۳ و ۴ هفته، ه یچ گونه تفاوت معنی داری بهی درصد جوانه زنی گندمه های بدون خواب به ترتیب ۶۶، ۶۸ و صفر درصد بود. پس از ۲، ۳ و ۴ هفته، ه یچ گونه تفاوت پس از ۴ هفته سرمادهی، جوانه زنی گندمه های که ۲۰، ۳، ۳، ۳ و ۴ هوشی به و حشی و گندم در دماهای ثابت ۵، ۱۰، ۲۰، ۱۰، ۲۵، و ۴۰ در جانه زنی گندمه های جو وحشی و گندم در دماهای ثابت ۵، ۱۰، ۲۵، ۲۰، ۲۵، ۳۵، و ۴۰ درجه سانتی گراد اندازه گوی شد. پس از ۴ موند یکی شد، پس از ۴ روز، بهتری درصد جوانه زنی گندمه های جو وحشی در دمای ثابت ۲۰، ۲۰، ۲۵، و ۴۰ درجه سانتی گراد درد داد در حالی که پس از ۴ هفته سرامادهی جوانه زنی کمتری برخوردار بودند. به منظور تعین اثر فغریکی و شریع به یه در دامنه ۵ تا ۳۰ در دمای کراد در داد در مای که در حمای ۲ به ۲۰، ۲۰، ۲۰، ۲۰، ۲۵، ۲۰، ۲۵، ۲۰ در دای مقای مقایحه با گندم از سرعت جوانه زنی کمتری برخوردار بودند. به منظور تعین اثر فغریکی و شریع بهی بو درمانه ۵ تا ۳۰ در دم سانتی گراد دران ی خود و گندم، مقایحه با گندم از سرعت موانه زنی کمتری برخوردار بودند. به منظور تعین اثر فغریکی و شریع بهی پوشینه های جو و مشی روی مینه های بزری موند روی می مور و ساز کی بور وخون یک بر ری جوانه زنی بند جو وحشی درم، بندی ری میه هرا مورد

کلمات کلیدی: فراشدهی سرمائی، جودره، گندم، دمای پایه، پوشینه