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Effect of Natural Seed Aging on Root and Shoot Traits in Bread Wheat (*Triticum aestivum* L.) Cultivars

Hayati Akman[1,a,*]

[1]*Department of Plant and Animal Production, Selçuk University, 42430 Konya, Turkey*
[*]*Corresponding author*

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ABSTRACT

This study targeted to elucidate the effect of seed aging on germination and emergence rates with and shoot characteristics in wheat cultivars. For this purpose, different bread wheat cultivars stored for 7 years and non-stored were compared for coleoptile length, root mass, shoot mass, root length as well as germination and seedling emergence rates. Here, the evidence suggested that seed storage over a prolonged period affected root and Shoot growth, coleoptile length, seed germination, and seedling emergence rates adversely. By linking germination and emergence rates, the data presented here indicated that a reduction in emergence rate in long-term storage was higher than that in the germination rate. It was also found that there were significant variations among the wheat cultivars about investigated traits during long-term storage. However, the emergence rates of Kate A1 and Flamura 85 were not affected substantially by long-term storage. The study suggested future studies to focus on clarification of the process controlling natural seed aging as such knowledge allows clue the eventual consequences of long-term storage.

[a] *hayatiakman@selcuk.edu.tr* *https://orcid.org/0000-0001-6878-3329*

Introduction

Wheat is a vital cereal grown over the various climatic environments worldwide. It is essential to sown the seed into deep moisture in dry-lands to induce seed germination (Schillinger et al., 1998). Deep sowing of wheat cultivars with longer coleoptile would benefit from deep moisture and emerge in hard or crusted soil (Rebetzke et al., 2004).

The high-yielding semi-dwarf wheats with *RhtB1b* and *RhtD1b* genes have a reduced response to gibberellin and decreased plant height and coleoptile length. However, Singh et al. (2001) reported that there was no significant contribution to the grain yield of the semi-dwarf isolines compared to tall genotypes under drought, but it was significant under favorable conditions. In comparison, a decline in plant height without any effect on coleoptile length was reported by other *Rht* genes (4, 5, 7, 8, 9, 12, 13, and 14) (Botwright et al., 2005). In dry-land farming, the cultivars with shorter coleoptiles may show a dramatic fall in emergence along with deep planting (Rebetzke et al., 2007). Previous reports on the effect of *Rht* genes on root traits produced conflicting results depending on various growth media and environments. It was reported that semi-dwarf wheat cultivars reduced root size, length and/or root biomass (Wojciechowski et al., 2009), while other studies showed that there were no effects on the wheat root (McCaig and Morgan, 1993). However, Miralles et al. (1997) observed that the reduced plant height increased the total root length and root mass at anthesis.

Seed longevity is important for efficient biodiversity conservation in seed banks (Fenollosa et al., 2020). The length of this time in sustaining viability of seeds is a significant trait of interest in breeding programs and food security (Dowsett et al., 2012; Finch-Savage et al., 2016). Genetic factors have a key influence on seed persistence (Bekker et al., 2003). Seed aging is known as loss of seed quality and viability during long periods (Coolbear, 1995). A chain of molecular, biochemical, physiological, and metabolic fundamentals of cell mechanism are involved on seed aging (Rajjou et al., 2012). Molecular events were observed similar to artificial and natural seed aging processes (Rajjou and Debeaujon, 2008). The seed aging is a process of practical significance as it initially leads to reduction on vigour and eventually in a drop in germination rate (Mathew, 1985). Seed aging eventually reduces emergence, seedling vigor, shoot, and dry weight and (Soltani et al., 2009).

This study aimed to elucidate the effect of seed aging on seed germination and seedling emergence traits, coleoptile length, root, and shoot traits at the early growth stage on seven years aged seeds of bread wheat cultivars.

Materials and Methods

The study was carried out at both laboratory and greenhouse conditions in 2017. Diverse 19 Turkish bread wheat cultivars were used as material. Table 1 indicates the presence of *RhtB1a*, *RhtB1b*, and *RhtD1b* genes (Yediay, 2009; Gummadov et al., 2015) in the cultivars. In the study, 7-year aged seeds stored at about 20-22°C by long-term storage and non-storage seeds were used to compare.

Germination and seedling emergence tests were made according to Soltani et al. (2009) with a few revisions. Germination test was conducted on Petri dishes on filter paper at 20°C under complete dark in the growth chamber. In the laboratory, germinated seeds were counted on the 4th d. For seedling emergence, seeds were sown to 3 cm depth into pots filled with a soil – peat mixture (70% soil and 30% peat) in the greenhouse. Emerged seeds were counted in the 7th d and 12th d. Data in germinated seeds and emerged seedlings were converted to percentage.

For coleoptile, root, and shoot length measurements, three seeds of each variety with no physical damage were line up in the middle of the paper towel, 1.5 cm apart and 8 cm from top and bottom. The paper towel and wax paper were rolled loosely, attached with a rubber band, and then designed vertically in a plastic box with holes at the base to drain excess water. The boxes were fully watered and kept for 14 days in constant darkness at 20°C under laboratory conditions. Coleoptile, root, and shoot lengths were measured with a ruler. Fresh roots were dehydrated with a towel paper and then weighted. The experiment was conducted with two repeats and three replications.

The study was arranged in a completely randomized block design, with three replicates for each treatment. Data were statistically analyzed using Analysis of Variance (ANOVA) with the software MSTAT-C (Russel, 1989). Comparisons between means were carried out using the Least Significant Difference (LSD) test at P<0.01. Percentage values were arcsine transformed before statistical analysis to correct for non-normal distribution.

Table 1. Semi-dwarfing genes in bread wheat cultivars included in the study

Cultivars	*RhtB1a*	*RhtB1b*	*RhtD1b*	Cultivars	*RhtB1a*	*RhtB1b*	*RhtD1b*
Zencirci 2002	+	-	-	Konya 2002	-	+	-
Müfitbey	ni	ni	ni	Bağcı 2002	-	+	-
Gün-91	+	-	ni	Eser	-	+	ni
Kate A1	+	-	-	Atay 85	-	+	-
Kınacı 97	-	+	-	Demir 2000	+	-	-
Mızrak	+	-	-	Bayraktar 2000	+	-	-
Yakar 99	-	+	-	Ahmetağa	-	+	ni
Harmankaya 99	+	-	-	Flamura 85	-	+	-
Sönmez 2001	+	-	-	Tosunbey	ni	ni	ni
Alparslan	+	-	-	ni shows no information			

Results and Discussion

Significant variations were found between long-term stored and control (P<0.01) seeds in terms of investigated traits. Besides, variety × storage interaction was found significant for all the traits (P<0.01).

Coleoptile length of bread wheat cultivars ranged from 2.3 to 5.3 cm for long-term stored and 3.3 to 6.6 cm for the control (Table 2). Coleoptile length at long term seed storage was reduced markedly with an average length of 4 cm compared to control cultivars with a length of 5.1 cm (Table 2). Mohan et al. (2013) reported that the coleoptile length of 662 bread wheat accessions varied from 3.4 to 11.4 cm and emergence was related to coleoptile length, however, cultivars with coleoptiles longer than 9 cm had no significant importance for emergence. A previous research was consistent with results of average coleoptile length of non-storage cultivars in this study, reporting coleoptile length of 564 European winter wheat posed that 58% of genotypes possessed 3 to 6 cm coleoptile length and only 6.4% of them had coleoptiles longer than 8 cm, and the longest coleoptile length reached up to 10 cm (Liatukas and Ruzgas, 2011). In the study, seed-aging reduced the coleoptile length of wheat cultivars. The results were in accordance with Pandey et al. (2015) who observed the effect of aging from 0 to 3 years on onion seeds, they obtained that coleoptile length and weight decreased with aging. In here, relationship between coleoptile length and seedling shoot length was weak for long-term storage (r^2=0.17; P<0.01) and strong for the control (r^2=0.70; P<0.01). Moreover, the association between coleoptile length and shoot mass was found weak. The results presented here were in accordance with Mohan et al. (2013) who indicated a positive correlation between coleoptile length and emergence.

The longest coleoptile length was found in wheat cultivars including *RhtB1a* in long-term storage and control. Müfitbey and Konya 2002 cultivars though have *RhtB1b* dwarfing gene, their coleoptile length was above 5 cm. This proposed that a longer coleoptile in decreased plant height genotypes should have selected (Mohan et al. 2013). In addition, the coleoptile length of Zencirci 2002, Mızrak and Harmankaya 99 involving *RhtB1a* gene reduced most with long-term storage. Table 2 indicates the coleoptile length of control groups mostly existed in a range of 4-5.9 cm, while in long-term storage the coleoptile length in most cultivars was found in range of 3-4.9 cm.

Table 2. Coleoptile length, shoot length, root length, and shoot mass in long-term storage (LTS) and control (non-storage seed) of bread wheat cultivars

Cultivars	Coleoptile length (cm)		Shoot length (cm)		Root length (cm)		Shoot mass (g)	
	LTS	Control	LTS	Control	LTS	Control	LTS	Control
Zencirci 2002	3.5^{j-m}	6.4^{ab}	20.5^{c-f}	25.0^{ab}	18.3^{e-k}	21.1^{a-e}	0.22^{abc}	0.24^{a}
Müfitbey	5.3^{cde}	5.5^{bcd}	20.8^{c-f}	21.5^{b-e}	16.5^{j-m}	19.6^{b-i}	0.12^{jkl}	0.17^{e-h}
Gün-91	5.3^{cde}	6.6^{a}	16.0^{g-k}	23.7^{abc}	14.7^{mno}	19.2^{d-k}	0.10^{kl}	0.21^{a-d}
Kate A1	4.9^{c-g}	5.0^{c-g}	21.6^{bcd}	22.2^{bcd}	22.5^{ab}	21.3^{a-d}	0.16^{f-i}	0.14^{hij}
Kınacı 97	3.0^{lmn}	4.8^{c-h}	9.3^{m}	18.8^{d-g}	12.7^{no}	20.2^{a-g}	0.12^{jk}	0.19^{c-f}
Mızrak	2.9^{mn}	5.5^{bcd}	17.8^{f-i}	23.6^{abc}	12.2^{o}	18.2^{e-k}	0.14^{hij}	0.17^{e-h}
Yakar 99	3.3^{klm}	3.7^{i-m}	13.6^{jkl}	16.4^{g-j}	16.5^{j-m}	18.5^{d-k}	0.13^{ijk}	0.16^{f-i}
Harmankaya 99	2.3^{n}	4.3^{f-j}	16.6^{g-j}	17.6^{f-I}	21.3^{a-d}	22.2^{abc}	0.13^{ijk}	0.18^{d-g}
Sönmez 2001	4.7^{d-h}	5.7^{abc}	17.7^{f-i}	22.1^{bcd}	17.3^{g-m}	20.5^{a-f}	0.19^{c-f}	0.23^{ab}
Alparslan	4.8^{c-h}	5.0^{c-g}	12.3^{lm}	15.4^{g-l}	15.2^{lmn}	19.1^{d-k}	0.08^{l}	0.13^{ijk}
Konya 2002	3.9^{h-l}	5.2^{c-f}	14.6^{h-l}	17.9^{e-h}	17.3^{g-m}	18.8^{d-k}	0.16^{f-i}	0.22^{abc}
Bağcı 2002	3.9^{h-l}	4.3^{f-j}	14.2^{i-l}	17.4^{f-I}	17.1^{h-m}	18.7^{d-k}	0.17^{e-h}	0.20^{b-e}
Eser	3.1^{lmn}	4.7^{d-h}	13.1^{jkl}	17.5^{f-I}	17.3^{g-m}	20.0^{a-h}	0.10^{kl}	0.14^{hij}
Atay 85	3.5^{j-m}	4.2^{g-k}	15.1^{h-l}	16.7^{g-j}	19.6^{b-i}	20.6^{a-f}	0.15^{g-j}	0.12^{jk}
Demir 2000	5.3^{cde}	6.6^{a}	16.6^{g-j}	26.0^{a}	18.3^{e-k}	22.7^{a}	0.17^{e-h}	0.19^{c-f}
Bayraktar 2000	4.5^{e-i}	5.7^{abc}	17.7^{f-i}	20.9^{c-f}	16.4^{klm}	19.4^{c-j}	0.13^{ijk}	0.18^{d-g}
Ahmetağa	3.2^{lmn}	3.3^{klm}	12.5^{klm}	14.3^{h-l}	15.1^{l-o}	18.8^{d-k}	0.14^{hij}	0.14^{hij}
Flamura 85	4.5^{e-i}	4.9^{c-g}	14.8^{h-l}	17.4^{f-I}	15.2^{lmn}	20.1^{a-g}	0.13^{ijk}	0.14^{hij}
Tosunbey	4.6^{d-i}	5.0^{c-g}	16.7^{g-j}	17.3^{f-I}	16.8^{i-m}	18.0^{f-l}	0.15^{g-j}	0.16^{f-i}
Mean	4.0^{B}	5.1^{A}	15.9^{B}	19.6^{A}	17.0^{B}	19.9^{A}	0.14^{B}	0.17^{A}
LSD $_{0.01}$ (C×T)		0.91		3.7		3.0		0.03
CV (%)		9.3		9.7		7.5		22.3

The shoot length of different wheat cultivars in long-term storage and control had a wide range of variation, varying from 9.3 to 20.8 cm and 14.3 to 26 cm, respectively (Table 2). Significant contributors to plant height variation are *RhtB1* and *RhtD1* with 15.5 and 40.9%, respectively (Würschum et al. 2015). Yediay (2009) found that *RhtB1b* was present in 37% of Turkish bread wheat, but *RhtD1b* was found in only one accession. In the study, cultivars with *RhtB1b* generally had shorter seedling height with a few exceptions. These results further revealed that the shoot length of cultivars importantly reduced at long-term storage with an average data of 15.9 cm compared to that of 19.6 cm at control (Table 2). This trend aligned with previous studies documenting a reduction in shoot length with the aged seeds (Nagel et al., 2015).

It was found in a wide range of variation among the cultivars in terms of shoot mass. The findings indicated that shoot mass varied from 0.08 to 0.22 g for long-term storage and 0.12 to 0.24 g for control (Table 2). In the present study, Alpaslan and Zencirci 2002 were ranked low and high for shoot mass in both long-term storage and control, respectively. A close look at the data in Table 2 suggested that average shoot mass was declined by long-term storage with 0.14 g compared to control with 0.17 g. The results were confirmed by results of Kandil et al. (2013) that indicated a decline in root length, shoot length, and shoot mass through long-term storage in soybean.

Significant variation in root length was found among the cultivars, ranging from 12.2 to 22.5 cm for long-term storage and 18 to 22.7 cm for control (Table 2). Inconsistent variation in root length between semi-dwarfing lines and the control lines were also reported by McCaig and Morgan (1993). The longest root length in the seedling stage was found in Kate A1, Harmankaya 99, and Atay 85 for long-term storage, while it was observed in Kate A1, Harmankaya 99, and Demir 2000 among control cultivars. Mızrak cultivar was ranked the lowest in long-term storage and control. With regard to long-term storage, root length was lowered substantially in the aged cultivars (Table 2). Root length of control groups mostly existed in a range of 18-22.7 cm, while in long-term storage most of the cultivars in terms of root length were accumulated. A similar result was also reported a decline in root length of maize with increasing aging duration (Kandil et al., 2013).

Here, there were significant variations for root mass among the cultivars, ranging from 0.04 to 0.11 g for long-term storage and 0.04 to 0.13 g for control, as previously reported by Nevo and Chen (2014) in bread wheat and Waines (2012) in spring wheat (Table 3). Effect on seedling root mass of *RhtB1b* or *RhtB1a* alleles was inconsistent for both long-term storage and control. The observed variation for root mass contributed to contrasted behavior under long-term storage. Thereby, long-term storage led to a decline in root mass with average results as opposed to control (Table 3).

Compared with other bread wheat cultivars in this study, germination rates ranged from 45 to 100% for long-term storage and 85 to 100 % for control (Table 3). The average germination rate of cultivars was 81.9% for long-term storage and 97% for control (Table 3). These results confirmed the findings of Hay et al. (2013) that indicated high germination (>70%) and variable for germinated rice seeds stored for up to 30 years. In addition, seedling emergence in 7th d varied from 15 to 90% and 53.3 to 100% for control, whereas seedling emergence at 12th d was a range from 20 to 93.3% for long-term storage and 66.7 to 100% for control. The experiments performed here clearly demonstrated that bread wheat cultivars responded distinctively to long term-storage with regard to germination and emergence rates. Alparslan, Bağcı 2002 and Yakar 99 had very low germination and emergence rates, however, several bread wheat cultivars such as Zencirci 2002, Müfitbey, Kınacı 97, Mızrak, Harmankaya 99, Konya 2002, Bayraktar 2002 and Ahmetağa possessed low emergence rates despite relatively high germination rate. Kate A1 and Flamura 85 were ranked high for germination and emergence rates that were not declined distinguishably with long-term storage.

A substantial reduction in aged seed in terms of germination and emergence rates was also reported by Badawi et al. (2007). Seedling emergence together with vigorous early growth improves stand establishment, ground cover, and reduces moisture evaporation loss (Pandey et al., 2015). Consumption of the seed starch reserve due to continual respiration deteriorated the seed quality, affecting germination and emergence (Wang et al., 2018). Besides, the seedling rate in sorghum after storage for 10 months was 78% higher than storage for12 months with 59.5% (Timotiwu et al., 2017). The results presented here showed that seed aging through the long-term storage impaired germination and seedling emergence rates significantly in the majority of cultivars with two exceptions (Table 3).

Table 3. Root mass, seed germination, and seedling emergence rates in long-term storage (LTS) and control (non-storage seed) of bread wheat cultivars

Cultivars	Root mass (g)		Germination rate (%)		Seedling rate-7 d (%)		Seedling rate -12 d (%)	
	LTS	Control	LTS	Control	LTS	Control	LTS	Control
Zencirci 2002	0.05^{hi}	0.04^{i}	90.0^{a-d}	100.0^{a}	25.0^{hij}	86.7^{abc}	28.3^{hi}	90.0^{a}
Müfitbey	0.07^{fg}	0.09^{de}	85.0^{cde}	85.0^{cde}	30.0^{ghi}	91.7^{abc}	48.3^{d-g}	91.7^{a}
Gün-91	0.08^{ef}	0.08^{ef}	97.7^{a}	100.0^{a}	53.3^{de}	90.0^{abc}	55.0^{cde}	91.7^{a}
Kate A1	0.07^{fg}	0.10^{cd}	98.3^{a}	100.0^{a}	90.0^{abc}	90.0^{abc}	93.3^{a}	95.0^{a}
Kınacı 97	0.05^{hi}	0.08^{ef}	68.3^{gh}	100.0^{a}	16.7^{ij}	93.3^{ab}	38.3^{e-h}	95.0^{a}
Mızrak	0.04^{i}	0.07^{fg}	96.7^{ab}	100.0^{a}	41.7^{efg}	86.7^{abc}	46.7^{d-g}	88.3^{a}
Yakar 99	0.06^{gh}	0.11^{bc}	61.7^{hi}	98.3^{a}	30.0^{ghi}	90.0^{abc}	35.0^{ghi}	95.0^{a}
Harmankaya 99	0.09^{de}	0.09^{de}	100.0^{a}	98.3^{a}	15.0^{j}	86.7^{abc}	36.7^{f-i}	91.7^{a}
Sönmez 2001	0.08^{ef}	0.07^{fg}	70.0^{gh}	100.0^{a}	50.0^{de}	90.0^{abc}	53.3^{c-f}	95.0^{a}
Alparslan	0.06^{gh}	0.04^{i}	45.0^{j}	100.0^{a}	15.0^{j}	91.7^{abc}	28.3^{hi}	96.7^{a}
Konya 2002	0.11^{bc}	0.09^{de}	76.7^{efg}	100.0^{a}	21.7^{hij}	90.0^{abc}	36.7^{f-i}	90.0^{a}
Bağcı 2002	0.05^{hi}	0.07^{fg}	55.0^{ij}	100.0^{a}	18.3^{hij}	78.3^{c}	20.0^{i}	86.7^{a}
Eser	0.10^{cd}	0.09^{de}	95.0^{abc}	100.0^{a}	46.7^{de}	88.3^{abc}	50.0^{c-g}	88.3^{a}
Atay 85	0.06^{gh}	0.12^{ab}	81.7^{def}	98.3^{a}	55.0^{de}	60.0^{d}	56.7^{cd}	66.7^{bc}
Demir 2000	0.08^{ef}	0.13^{a}	90.0^{a-d}	96.7^{ab}	45.0^{ef}	88.3^{abc}	55.0^{cde}	91.7^{a}
Bayraktar 2000	0.08^{ef}	0.11^{bc}	71.7^{fgh}	100.0^{a}	28.3^{g-j}	100.0^{a}	36.7^{f-i}	100.0^{a}
Ahmetağa	0.07^{fg}	0.10^{cd}	78.3^{efg}	91.7^{a-d}	31.7^{fgh}	53.3^{de}	40.0^{d-h}	66.7^{bc}
Flamura 85	0.09^{de}	0.11^{bc}	95.0^{abc}	93.3^{abc}	80.0^{bc}	83.3^{bc}	83.3^{ab}	86.7^{a}
Tosunbey	0.08^{ef}	0.08^{ef}	86.7^{b-e}	98.3^{a}	51.7^{de}	85.0^{bc}	55.0^{cde}	88.3^{a}
Mean	0.07^{B}	0.09^{A}	81.9^{B}	97.0^{A}	43.7^{B}	82.9^{A}	51.3^{B}	87.0^{A}
LSD $_{0.01}$ (C×T)		0.02		10.3		14.5		18.1
CV (%)		27.1		7.6		10.1		13.0

Here it was demonstrated that seed aging by long-term storage reduced seed germination and seedling emergence rates with shoot, coleoptile, and root length and shoot mass. Seedling emergence rate was the most affected trait when it was compared to other growth features. Furthermore, a significant variation among the cultivars in terms of investigated traits was found. Seedling emergence rates of Kate A1 and Flamura 85 cultivars were not observed a noticeable reduction during long-term storage. Compared to wheat cultivars in this study, coleoptile length and shoot length was substantially decreased in cultivars with the *RhtD1a* gene as opposed to semi-dwarf cultivars. In the study, though an important reduction in cultivars was not found in terms of germination rate with few exceptions, the decline in seedling emergence rates was significant. Based on the results of the current study, it was suggested that the seed rate in sowing should be increased for stand establishment when seeds were stored in prolonged periods under high ambient room temperature.

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