



Original article

Ex-situ conservation of wheat genetic resources from Saudi ArabiaT.A. Al-Turki^a, A.A. Al-Namazi^a, B.S. Al-Ammari^b, M.S. Al-Mosallam^a, M.A. Basahi^c^aThe herbarium and gene-bank of the king Abdelaziz City for Science and Technology (KACST), Box 6086, Riyadh 11442, Saudi Arabia^bAl-Imam Mohammad ibn Saud Islamic university, College of Science, Biology Department, Saudi Arabia^cCollege of Science and Arts Sajir, Shaqra University, P.O. Box 33, Shaqra 11961, Saudi Arabia

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ABSTRACT

Wheat (*Triticum* L.) is one of the major food crops of the world, and an important component of food security. The aim of this study was to collect and preserve seeds of wheat growing in eight regions of the Kingdom of Saudi Arabia (Al-Qassim, Asir, Al-Taif, Najran, AL-Baha, Jazan, Al-Madinah and Wadi Al-Dawasir) where wheat has been cultivated since ancient times. Sixty-one accessions/samples of wheat (*Triticum aestivum*) were collected and placed in dry storage (*ex-situ* conservation) at $-18\text{ }^{\circ}\text{C}$ (i.e. permanent storage). The accessions of local wheat have the ability to grow under harsh environmental conditions such as (high temperature, drought and salinity). Most of these samples were collected directly from farms, but a few were collected from markets. The most important criteria for *ex-situ* conservation is that seeds need to have a low moisture content (MC) and a high percentage viability. Seed MC was measured for all 61 accessions by the oven-drying method and seed viability was tested in three ways: percentage of germination, tetrazolium chloride testing, and X-ray radiography.

The seed MC of the 61 accessions was uniformly very low (0.10–0.12%), and 97 to 100% of the seeds were viable. Thus, all 61 wheat accessions collected in this study have the initial requirements to remain viable for long periods of time in *ex-situ* conservation in the gene seed bank.

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1. Introduction

Wheat (*Triticum* L., Poaceae) is one of the major food crops in the world and is important for global food security. As a result of the increasing world population, there is an urgent need to protect this strategic food crop (FAO, 1998; 2010). The earliest cultivated forms of wheat were diploid ($2n = 2x = 14$) and tetraploid ($2n = 4x = 28$), and genetic evidence indicates that they originated from the south-eastern part of Turkey some 10,000 years ago (Heun et al., 1997; Nesbitt and Samuel, 1996; Dubcovsky and Dvorak, 2007). Subsequently, hybridization gave rise to hexaploid wheats and cultivation of several wheat species spread widely into the near East, Asia and Europe (Feldman and Kislev, 2007). Currently, *Triticum aestivum* (bread wheat), accounts for about 95% of the world production (Ortiz et al. 2008a).

Much of the land in the Kingdom of Saudi Arabia is not usable for agriculture, due to the topography, insufficient rainfall and high salinity. However, in suitable areas, farmers in the Kingdom have traditionally planted locally selected landraces of *Triticum aestivum*, notably in Alhaba, Al-Samma, Al-Qaimi, Hanttah Al-Qassim, Hamra, Samira and Madinah (Sayed, 1979). These varieties have been inherited by many generations of farmers in the Kingdom and their seeds represent genetic assets that need to be preserved before a decline in farming, as a result of human and other activities, results in them becoming extinct. The importance of these local wheat varieties lies in their capacity for adaptation to the extreme climatic conditions of heat and drought in the Kingdom. All of these factors contribute to the importance of collecting and conserving agricultural genetic resources in plant gene banks as pure genetic resources for future generations. Therefore, the wheat genetic resources of the Kingdom need to be preserved by *ex-situ* conservation in gene-banks so that they will be available in the future (Al-Turki, 2002; Al-Turki et al., 2010; Al-Turki et al., 2019).

De Carvalho, et al. (2013) reported that 859,472 accessions of wheat germplasm are being maintained in Gene-Banks in 90 countries in Europe, America, Asia, Africa and Oceania. The top six

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wheat germplasm collections are CIMMYT, NSGC, ICGR-NBPGR, ICARDA and NIAS (de Carvalho, et al. 2013). >850,000 wheat samples are maintained in a living condition in the world, and these samples are stored in 229 collections. Russia, United States, China, India, Italy and Japan have the most significant national wheat collections, as well as CIMMYT in Mexico and ICARDA in Syria (FAO, 1998). In Saudi Arabia, 61 and 126 accessions of wheat germplasm are conserved in the Gene-Banks of King Abdulaziz City for Science and Technology (KACST-BGB) and the Ministry of Environment, Water and Agriculture, respectively. However, despite these germplasm collections, the collection and preservation of the wheat germplasm of Saudi Arabia is far from complete.

The objective of the present work was to collect and conserve the germplasm (as seeds) of wheat (*Triticum aestivum* L.) from eight geographical regions of Saudi Arabia. Also, we sought to assess the suitability for storage of these seed accessions, before conserving them *ex-situ*, in long-term storage at about -18°C in the King Abdulaziz City for Science and Technology Gene-Bank (KACST-BGB).

2. Materials and methods

2.1. Germplasm collection

We visited numerous wheat farms (Fig. 1) scattered across the Kingdom of Saudi Arabia and interviewed many wheat farmers about their agricultural experience. Seeds of 61 accessions of *Triticum aestivum* L. were collected from eight different geographical areas (Al-Qassim, Asir, Al-Taif, Najran, Al-Baha, Jazan, Al-Madinah and Wadi Al-Dawasir) in Saudi Arabia, during 2017–2019 (Table 1). The minimum sample size 1 kg. Some samples were from freshly harvested seeds but others were from seeds that had been stored by farmers. All samples were transferred to the laboratory of the KACST Gene-bank in Riyadh, winnowed by blowing with air to

remove debris, and placed in the cold room (5°C short term storage). For each accession seed moisture content and viability were determined.

2.2. Determination of seed moisture content

We used four replicates (100 seed each) of each accession. These were weighed, dried at 130°C for 2 h, allowed to cool for 1 h over silica gel and then reweighed (Witte, 1995; Touchell et al. 1998; ISTA, 2013). Moisture content percentage = (weight of fresh seeds – weight of dry seeds)/weight of fresh seeds \times 100.

2.3. Seed germination tests

Five replicates of 20 seeds each for each accession were placed in 90-mm diameter Petri dishes on two layers of Whatman No. 1 paper moistened with 7 ml distilled water. Dishes were incubated at under two alternating temperature regimes $15/5^{\circ}\text{C}$ and $20/10^{\circ}\text{C}$, with a 12 h light and 12 h dark, cycle. Germination was monitored daily for 30 days, and a seed was scored as germinated when the radicle had emerged to a length of ≥ 2 mm. The final germination percentage (%) was expressed as $G (\%) = (A/B) \times 100$ (Al-Turki, 1992, Baskin and Baskin, 2014; Basahi, 2018), where A is the total number of seeds germinated at the end of experiment and B is the total number of seeds tested.

2.4. Tetrazolium chloride tests for viability

According to the International Seed Testing Association protocol (ISTA, 1999), seeds were soaked in a 1% solution of 2,3,5-triphenyl tetrazolium chloride (TTC) for 4 days in a glass vial in the dark at 25°C . A red-stained embryo was counted as viable and a non-stained embryo as nonviable.



Fig. 1. A wheat field at the flowering stage, surrounded by palm trees, located in the village of Al-Awashzia (15 km east of Unayzah city), in the Al-Qassim region (photo taken by Prof. T. A. Al-Turki on 20 March 2019).

Table 1

Sixty-one germplasm accessions of Wheat (*Triticum aestivum*) collected from different sites in Saudi Arabia.

No.	Accession no.	Location
1	35	AL-Qassim
2	36	AL-Qassim
3	73	AL-Qassim
4	78	AL-Qassim
5	79	AL-Qassim
6	96	AL-Qassim
7	83	AL-Qassim
8	143	AL-Qassim
9	144	AL-Qassim
10	145	AL-Qassim
11	225	AL-Qassim
12	243	AL-Qassim
13	235	AL-Qassim
14	236	AL-Qassim
15	264	AL-Qassim
16	268	AL-Qassim
17	290	AL-Qassim
18	292	AL-Qassim
19	301	AL-Qassim
20	323	AL-Qassim
21	355	AL-Qassim
22	431	AL-Qassim
23	433	AL-Qassim
24	444	AL-Qassim
25	456	AL-Qassim
26	459	AL-Qassim
27	476	AL-Qassim
28	497	AL-Qassim
29	102	Asir
30	103	Asir
31	104	Asir
32	105	Asir
33	106	Asir
34	107	Asir
35	108	Asir
36	109	Asir
37	130	Asir
38	131	Asir
39	133	Asir
40	137	Asir
41	138	Asir
42	237	Asir
43	500	Asir
44	510	Asir
45	511	Asir
46	517	Asir
47	157	Najran
48	330	Najran
49	415	Najran
50	477	Najran
51	495	Najran
52	156	AL-Taif
53	159	AL-Taif
54	165	AL-Taif
55	166	AL-Taif
56	457	AL-Medinah
57	460	AL-Medinah
58	68	Jazan
59	408	Jazan
60	60	AL-Baha
61	331	Wadi – AL-Dawasir

2.5. X-ray radiography

Four replicates of 100 seeds of each accession were X-rayed with a Faxitron X-ray machine (model MX-20 Dc12) connected to a computer. Seeds were exposed to 18 HV/10 s, and the X-ray plates were evaluated based on presence of the embryo and endosperm. The percentage of seeds with an intact embryo, damaged embryo or no embryo was determined. X-ray images clearly showed the internal structure of the seed, including the embryo. A viable embryo has high homogeneity, but a dead one has dark

heterogenous areas, indicating damage to the embryo (Al-Turki and Baskin, 2017; Al-Hammad and Al-Ammari, 2017; Al-Turki et al., 2019).

2.6. Statistical analysis

Results from the seed moisture content determinations and viability tests were expressed as the percentages (mean \pm standard error). Data were analyzed using one-way ANOVA followed by Duncan post Hoc to compare the means with 5% probability level (Sokal and Rohlf, 1995). SPSS 11.5 for Windows was used for data analysis.

3. Results

3.1. Germplasm collections

Sixty-one accessions of wheat (*Triticum aestivum* L.) were collected from eight different regions of Saudi Arabia (AL-Qassim, Asir, Najran, AL-Taif, Jazan, AL-Madinah, AL-Baha, Wadi Al-Dawasir) (Table 1). Twenty-eight accessions were collected from AL-Qassim region, 18 from Asir region, five from Najran, four from AL-Taif, two from AL-Madina, two from Jazan, and one each from AL-Baha and Wadi Al-Dawasir (Table 1). This preliminary study also shows that the geographical distribution of wheat crops is clearly concentrated in the AL-Qassim, AL-Taif and Asir regions, which have relatively low temperatures and high rainfall.

3.2. Seed moisture content

The moisture content of the 61 accessions of wheat (*Triticum aestivum* L.) was uniformly low, ranging from 0.10 ± 0.00 to $0.12 \pm 0.00\%$ (Table 2), with no significant differences between accessions ($P > 0.05$).

3.3. Seed viability

Seed viability as assessed by tetrazolium testing for the 61 accessions was uniformly high, ranging from 97 ± 0.10 to $100 \pm 0.00\%$ (Table 3) with no significant differences between them ($P > 0.05$). X-ray imaging of seeds of the 61 accession of seeds revealed that almost all had intact, undamaged embryos and endosperms (e.g. Fig. 2) indicating absence of any sign of insect or microbial invasion. Seeds in all the wheat samples studied were long and oval in shape, with a clear embryo at the base of the seed.

3.4. Spike morphology

Spike morphology is considered a crucial trait in identifying landraces and for taxonomic studies of them. Morphological

Table 2

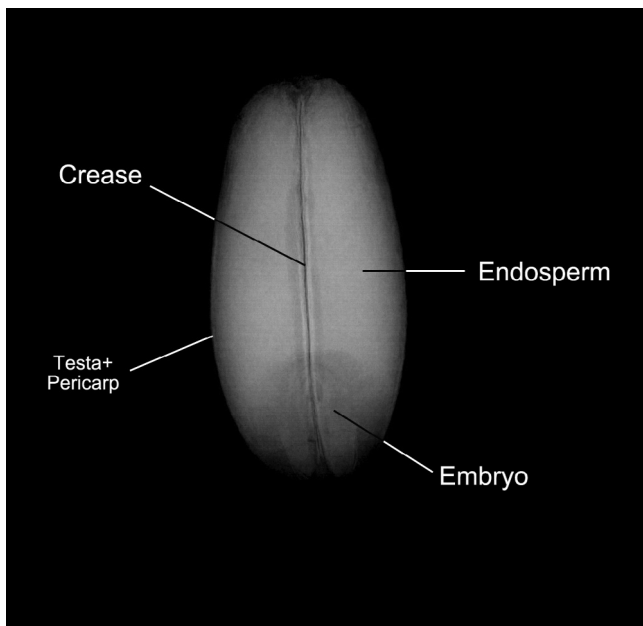
Seed moisture content (mean \pm se) of 61 samples of *Triticum aestivum*.

No	Seed moisture content (%)	Location
1	0.12 ± 0.00^a	AL-Qassim
2	0.10 ± 0.11^a	Asir
3	0.10 ± 0.12^a	Najran
4	0.11 ± 0.13^a	AL-Taif
5	0.10 ± 0.00^a	AL-Medinah
6	0.11 ± 0.00^a	Jazan
7	0.10 ± 0.00^a	AL-Baha
8	0.12 ± 0.00^a	Wadi –AL-Dawasir

Means \pm standard error (se) followed by the same letter are not significantly different ($P > 0.05$).

Table 3Seed viability percentage (mean \pm se) of 61 samples of *Triticum aestivum* (as judged by tetrazolium testing (TZ) and X- Ray imaging).

Seed viability %				Location
Germination % at		TZ %	X- Ray %	
15/ 5 °C	20/10 °C			
97 \pm 0.10 ^a	98 \pm 0.10 ^a	97 \pm 0.21 ^a	100 \pm 0.00 ^a	Al- Qassim
98 \pm 0.12 ^a	100 \pm 0.00 ^a	97 \pm 0.11 ^a	97 \pm 0.21 ^a	Asir
98 \pm 0.10 ^a	100 \pm 0.00 ^a	98 \pm 0.10 ^a	99 \pm 0.10 ^a	Najran
99 \pm 0.10 ^a	100 \pm 0.00 ^a	100 \pm 0.00 ^a	100 \pm 0.00 ^a	Al- Taif
100 \pm 0.00 ^a	100 \pm 0.00 ^a	100 \pm 0.00 ^a	100 \pm 0.00 ^a	Al- Medinah
98 \pm 0.10 ^a	100 \pm 0.00 ^a	100 \pm 0.00 ^a	100 \pm 0.00 ^a	Jazan
98 \pm 0.11 ^a	99 \pm 0.10 ^a	100 \pm 0.00 ^a	100 \pm 0.00 ^a	Al-Baha
97 \pm 0.20 ^a	100 \pm 0.00 ^a	98 \pm 0.10 ^a	100 \pm 0.00 ^a	Wadi –Al- Dawasir

**Fig. 2.** X-ray photograph of a viable seed (caryopsis) of wheat (*Triticum aestivum*) showing the fused testa and pericarp, crease (ventral furrow), endosperm and embryo (x3).

observations were made on flowering spikes of typical examples of the more important landraces.

3.5. Morphological characterization of wheat landraces

Al-Qassim region is famous for its agriculture with five landraces of wheat: *T. aestivum* var. Al-Juraba (Fig. 3a), *T. aestivum* var. Al-Ma'a-, Halba (Fig. 3b), *T. aestivum* var. Al-Ma'a- Samma (Fig. 3c), *T. aestivum* var. Hanttah-Najed (= *T. aestivum* var. Hanttah-Al-Qassim) (Fig. 3d), and *T. aestivum* var. Al-Qaimi. The Asir area is also famous for the presence of some varieties of local wheat, including: *T. aestivum* var. Al-Mabia, *T. aestivum* var. Al-Qayyad, *T. aestivum* var. Smyran. In Al-Taif region, three different varieties of wheat were distinguished: *T. aestivum* var. Al-Nakhla, *T. aestivum* var. Al-Naqra and *T. aestivum* var. Hamis. In Al-Madinah region, the wheat farmers refer to the existence of another variety of Wheat (*T. aestivum* var. Sindhi).

4. Discussion

The main aim of the study was to collect some of the genetic resources of local wheat (*Triticum aestivum* L.) in Saudi Arabia and preserve them in the KACST Gene-bank. Long-term seed storage is a basic and essential step to prevent the ancient wheat

**Fig. 3a.** Spike of *T. aestivum* var. Al-Juraba, distinguished by absence of awns from spikelet (or very short awns) (photo taken by Prof. T. A. Al-Turki on 20 March 2019).



Fig. 3b. Spike of *T. aestivum*. var. Al-Ma'a -Halba, characterized by the presence of tall awns on spike and the length of the spike is between 10.5 and 12 cm. (photo taken by Prof. T. A. Al-Turki on 20 March 2019).



Fig. 3c. Spike of *T. aestivum*. var. Al-Ma'a-Samma characterized by the presence of a medium-length awns and the length of the spike between 7 and 10 cm. (photo taken by Prof. T. A. Al-Turki on 20 March 2019).

varieties of the Arabian Peninsula from going extinct. Our 61 accessions of the hexaploid *Triticum aestivum*, belonging to different landraces (varieties) from eight regions of the kingdom, were concentrated in areas with relatively cold climates in winter and high rainfall, particularly Al-Qassim, Asir and Al-Taif regions.

Many researchers (e.g. Lazzari, 1988) have pointed out that seed moisture content (MC) is one of the most critical factors that must be controlled to maintain viability of seeds during storage. If seed MC exceeds 12%, seeds are more prone to damage by fungal infection. On the other hand, seed longevity is increased when MC is reduced to 4–6% (Ellis et al. 1991; Witte, 1995; Joshi and Singh, 2004, Ellis and Hong, 2006). However, a decrease to as low as < 1% in our work did not have any negative effect on the seed

viability. Storing seeds with a high MC (e.g. 15%) at a low temperature (−18 °C) would result in seed death. Cell damage would occur in the frozen seeds due to formation of ice crystals that cause damage to the membranes and embryonic axis, resulting in loss of viability. However, values for the moisture content vary among the seeds of different species, and sometimes, even between varieties within the same species and this consequently can affect the longevity of the seeds i.e. their retention of viability (e.g. Joshi and Singh, 2004).

The low seed MC in our 61 accessions of wheat may be due to the preservation of these seeds by farmers who have extensive agricultural experience and are aware of the benefit of dry storage in maintaining seed viability. This low MC is a highly desirable and



Fig. 3d. Spike of *T. aestivum*. var. Hanttah -Najed, characterized by the presence of a short awn not exceeding 3 cm and the length of the spike is between 10 and 11 cm. (photo taken by Prof. T. A. Al-Turki on 20 March 2019).

important trait that ensures that seeds in the *ex-situ* seed bank can remain viable for long periods of time. Germination is one of the most important events in the life cycle of plants (Al-Turki 1992; Gutterman, 1993, 2002; Basahi, 2018). Stress-inducing factors such as high temperatures and salinity may inhibit germination in the field. In fact, high or low temperatures can play a major role in the inhibition of germination of wheat seeds (Lobell and Ortiz-Monasterio, 2007). Several studies indicate that the optimal temperature for the germination of a wheat crop ranges from 12 °C – 25 °C (e.g. Acevedo et al., 2002). Buriro et al. (2011) have found that seeds of some wheat varieties from Pakistan have an ability to germinate with high percentages at 10, 20, and 30 °C. In our study, final germination percentages were extremely high at both 15/5 and 20/10 °C, which is consistent with the results of Buriro, et al. (2011) for wheat varieties from Pakistan.

Our study also shows that seeds of *Triticum aestivum* from Saudi Arabia do not appear to have genetically fixed (innate) mechanisms of dormancy since untreated seed samples from all 61 accessions of wheat had 97 and 100% germination at 15/5 and 20/10 °C, respectively. Freshly harvested seeds of all 61 accessions showed a very high germination percentage (94–100% at 20/10 °C). This result shows clearly that neither stored nor fresh seeds of wheat have innate dormancy in Saudi Arabia.

In Saudi Arabia, the King Abdulaziz City for Science and Technology (KACST) has established the botanical Gene-bank (KACST-BGB) along with the KACST-MUZ-Herbarium, and these are important accomplishments in the Kingdom in terms of securing food security and preservation of wheat genetic diversity. We suggest that these achievements must be followed by large national research projects that contribute to the collection and preservation of plant genetic resources for all local and developing agricultural crops in all the Arabian Gulf countries (Al-Turki et al. 2010; Al-Turki et al. 2019).

5. Conclusion

Seeds of all 61 accessions of wheat (*Triticum aestivum*) had good and desirable qualities (low MC and high viability) that contribute directly to their preservation in the Gene-Bank of King Abdulaziz City for science and technology (KACST-BGB). We estimate that

these seeds will remain viable in the seed bank for ≥ 25 years. It is clear from visiting different farms that >126 varieties of wheat can be found in Saudi Arabia. For example, Al-Qassim region alone is famous for its cultivation of five landraces of wheat. Thus, more collecting and preservation of wheat genetic diversity is urgently needed before these varieties disappear. This study also concludes that the geographical distribution of wheat crop in the Kingdom is largely concentrated in three different regions (Al-Qassim, Asir and Al-Taif) due to relatively low temperatures in winter in these areas. Also, some varieties of wheat are concentrated in high mountains in Jazan region (south-western of Saudi Arabia), such as in the Jabal- Fayfa and Jabal Al-Hashr mountains, which are also characterized by low temperatures and high rainfall. This study is the first to show the importance of collecting and preserving plant genetic resources (seeds in *ex-situ* seed banks) of the local wheat varieties in the Kingdom of Saudi Arabia.

6. Recommendations

Plant genetic resources for food and agriculture are considered one of the most important resources for world food security. Thus, it is urgently important that the genetic diversity of wheat, a staple food source for people, is preserved. To promote this preservation effort, the following recommendations are made:

- 1- The establishment of several national centres for plant gene banks in various important environmental regions of Saudi Arabia.
- 2- The establishment of a regional centre for plant genetic banks to preserve plant genetic resources with the aim of collecting plant germplasm in the Arabian Peninsula and the Arabian Gulf region.
- 3- The establishment of botanical gardens in a strategic environmental region would contribute positively to the work of gene banks by identifying plant biological properties, by cultivating and maintaining many local plants and tracking their life cycle and stages of development. Botanical gardens, herbaria and gene banks have the potential to greatly enhance plant science research in the Kingdom of Saudi Arabia and the Arabian Peninsula.

- 4- Herbaria, botanical gene banks and botanical gardens will also contribute to the definition and classification of plant species and therefore indirectly the preservation of genetic resources.

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