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Conservation of genetic resources for five traditional crops from Jazan, SW Saudi Arabia, at the KACST Gene-Bank

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ABSTRACT

The conservation of five traditional crops is an important aspect of achieving national food security. In the present study, we aimed to collect and conserve germplasm of five local crops from the Jazan region of southwestern Saudi Arabia: Sorghum: *Sorghum bicolor* (L.) Moench); Barley (*Hordeum vulgare* L.) Millet (*Pennisetum glaucum* (L.) R. Br.); Sesame (*Sesamum indicum* L.) and Guar (*Cyamopsis tetragonoloba* (L.) Taub). Forty-one seed accessions of these five crops were collected and tested to determine seed moisture content (MC%) and quality as indicators of their potential to survive during long-term dry storage at −18 °C (i.e. *ex-situ conservation* of genetic resources). Seed viability was assessed using germination tests, the tetrazolium chloride (TZ) test and X-ray imaging. Seeds of the five crops had very low MC% and high viability (fully developed embryos and germination >91%), indicating that they were of good quality and had high potential for long-term survival in gene banks. The genetic resources of these crops (seeds) have now been preserved at the gene-bank of King Abdulaziz City for Science and Technology (KACST-BGB), Riyadh, Saudi Arabia.

1. Introduction

Sorghum (*Sorghum bicolor*), Barley (*Hordeum vulgare*), Millet (*Pennisetum glaucum*), Sesame (*Sesamum indicum*) and Guar (*Cyamopsis tetragonoloba*) are economically very important as a food (for humans and livestock) and for fibres in many parts of the world, particularly semiarid regions of Africa, Middle East, Russia, the Himalayas and the Andean countries of South America, (FAO, 1995; Schulte et al., 2009; Dahlberg et al., 2011; Upadhyaya et al., 2014). These five crop species are originally from Africa, but have been spread globally including to the Arabian Peninsula (*e.g.* Saudi Arabia, Yemen) (De Wet, 1967, 1971, 1972, 1978; Assar et al., 2005; Upadhyaya et al., 2014). They play a significant role in food security, especially in tropical and northeastern Africa.

As far back as the 1920s and 1930s scientists (*e.g.* N.I. Vavilov and J. Harlan) realised that traditional crop varieties were being lost from cultivated fields around the world. Consequently, scientific efforts to conserve plant genetic diversity started focusing on collecting material and placing it into *ex-situ* storage. Thus, the establishment of gene-banks (*ex-situ conservation*) began in the 20th century through the work of Harrington in the United States and Ellis and Roberts in the United Kingdom (Hong et al., 1996). The viability of seeds became a very important issue for crop plants, and it was shown that seeds survive for several decades when stored in dry and low-temperature conditions (e.g. Agacka et al., 2014). It has been reported by several authors (*e.g.* Linington and Pritchard, 2001) that seed longevity can be predicted by seed moisture content. Furthermore, there are genetic influences on the relationship between seed longevity and moisture content. Many authors (*e.g.* Harrington, 1970, 1972; Roberts, 1973; Ellis and Roberts, 1980a,b; Ellis et al., 1986, 1988; Dickie et al., 1990; Smith, 1992; Steiner and Ruckenbauer, 1995; Obunyali, 2007) have conducted detailed studies of the effects of seed moisture content and storage conditions on seed longevity. In addition, Probert and Hay (2000) have shown that the longevity of seeds is influenced by seed quality at the time seeds are placed

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into storage. Smith et al. (2011), Godefroid et al. (2011) and Merritt et al. (2014) have shown that *ex-situ* conservation is one of the most effective strategies for preserving plant biodiversity. Gene-banks started storing seeds in cold rooms (at -18 °C or lower) in the second half of the 1970s when it was appreciated that reduced seed moisture content and storage temperature promoted seed longevity (Ellis and Roberts, 1980b). For example, seeds of wheat and barley stored dry at 0 °C for 34 years germinated to 87% and 95%, respectively (Nagel et al., 2009, 2010).

The Jazan region is located in the southwestern corner of Saudi Arabia, between latitudes 16° 22′ and 17° 46′ N, and longitudes 41° 33′ and 43° 09′ E (including the Farasan Archipelago). The many geomorphological characters of the region include islands and the coastal belt of the southeastern Red Sea, a relatively wide coastal plain, a transitional rocky habitat and a chain of mountains up to 2621 m a.s.l. (Masrahi, 2012). The climate varies from hot and humid near the sea in the coastal plain with mean annual rainfall of only 150 mm to relatively cool and humid in the mountains with rainfall of >600 mm year^{-1} (Masrahi, 2012). The main agricultural area of Jazan lies in the coastal plain where alluvial silts and fine sands from the highlands are deposited in the valleys, thus enhancing the fertility of the soil. Although annual rainfall is low in the coastal plain, it falls typically as thunderstorms, filling the main channels and tributaries of wadis in many cases. This erratic rainfall represents the main source of irrigation for seasonal agriculture. Other agricultural areas are located on mountain terraces.

The Jazan region has many crops, of which 5 represent the most important in the area over a long time, including Sorghum, Millet, Barley, Sesame and Guar. Sorghum (*Sorghum bicolor* (L.) Moench) is the most cultivated crop, mainly in the coastal plain (red and white grain varieties). Millet (*Pennisetum glaucum* (L.) R. Br.) is restricted to the sand-dune belt in the coastal plain. Both sorghum and millet are C_4 plants, meaning that these species are well adapted to a hot climate and drought. Barley (*Hordeum vulgare* L.) is restricted to some mountains, such as Al-Hashr and Fyfa mountains (in the east of Jizan region). Sesame (*Sesamum indicum* L.) and Guar (*Cyamopsis tetragonoloba* (L.) Taub) are cultivated in the coastal plain and rocky habitats to the east of the coastal plain.

Our objective in the present study was to collect and conserve the germplasm of these five different crops: *Sorghum bicolor (S. bicolor* red variety, *S. bicolor* white variety *and Sorghum bicolor* Shahla variety), *Pennisetum glaucum, Hordeum vulgare, Sesamum indicum* and *Cyamopsis tetragonoloba*). We sought to assess the suitability for storage of multiple seed accessions from the Jazan region, southwestern Saudi Arabia, before conserving their germplasm outside their natural habitats (*ex-situ*), in long-term storage at about −18 °C in the King Abdul-Aziz City gene-bank.

2. Materials and methods

2.1. Germplasm collections

The method of collection involved visits to farming areas (Figs. 1–3) and interviews with farmers. Seeds of 41 accessions representing five crops: *Sorghum bicolor (*Red variety, White variety and Shahla variety*), Pennisetum glaucum, Hordeum vulgare, Sesamum indicum and Cyamopsis tetragonoloba*, were collected from different locations in the Jizan region during 2017 (Table 1). Some collections/samples were 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, blown with air to remove debris, and placed in the cold room (5 °C) of the KACST gene-bank. For each accession moisture content was determined and viability was assessed.

2.2. Determination of moisture content (MC)

Seed MC (fresh weight basis) was determined using three replicates (200 seeds each) from each accession using the high constant – temperature oven method (ISTA, 2013). The samples were weighed, dried at 130 °C for 12 h, allowed to cool for 1 h over silica gel and then weighed again. Moisture content percentage = (weight of fresh seeds –weight of dry seeds)/weight of fresh seeds × 100 (Witte, 1995; Touchell et al., 1998).

2.3. Seed germination tests

Five replicates of 20 seeds each were sown on two layers of Whatman No. 1 paper moistened with 7 mL distilled water in 90-mm diameter Petri dishes. Seeds were incubated at alternating

Fig. 1. Agricultural field of *Sorghum bicolor* red variety in Jazan region.

Fig. 2. Agricultural field of *Pennisetum glaucum* in Jazan region.

Fig. 3. *Sesamum indicum* plant in Jazan region.

temperatures of 30/20 °C and 35/25 °C (12 h light and 12 h dark cycle). Seed germination was counted daily for 30 days, and a seed was scored as germinated when the radicle had emerged by at least 2 mm. The final germination percentage (%) was expressed as G (%) = (*A*/*B*) X 100 (AL-Turki, 1992; Li and Shi Fukuda, 2010; Wang et al., 2013; Baskin and Baskin, 2014), where *A* is the total number of seeds germinated at the end of experiment (30d) and *B* is the total number of seeds tested (100 seeds).

2.4. Tetrazolium chloride tests

Seeds were soaked in a 1% solution of 2,3,5-tripheneyl tetrazolium chloride (TTC), as described by the International Seed Testing Association (ISTA, 1999) 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.

2.5. X-ray radiography

Three replicates of 100 seeds of each accession were radiographed with the aid of digital equipment (Faxitron X-ray brand, model MX-20 DC12) connected to a computer. The seeds were exposed to 18 HV/10 s. The X-ray plates were evaluated based on the presence and morphology of the embryo and endosperm. The percentage of seeds with an intact embryo, damaged embryo or no embryo was determined. X-rays showed the internal structure

Table 1
Forty-one germplasm accessions of five crops collected from different sites in Jazan region.

NO	Accession- no.	Scientific name	Local- name	Family	Location
1	70	*Sorghum bicolor* red var.	hamra	Poaceae	Al-Hashr mount.
2	409	*Sorghum bicolor* red var.	hamra	Poaceae	Ad'dayer
3	418	*Sorghum bicolor* red var.	hamra	Poaceae	Baish
4	419	*Sorghum bicolor* red var.	hamra	Poaceae	Sabya
5	429	*Sorghum bicolor* red var.	hamra	Poaceae	Abu Arish
6	446	*Sorghum bicolor* red var.	hamra	Poaceae	Wa'alan
7	467	*Sorghum bicolor* red var.	hamra	Poaceae	Al Aredha
8	469	*Sorghum bicolor* red var.	hamra	Poaceae	Farasan island
9	480	*Sorghum bicolor* red var.	hamra	Poaceae	Farasan island
10	66	*Sorghum bicolor* white var.	baidha	Poaceae	Al-Hashr mount.
11	69	*Sorghum bicolor* white var.	baidha	Poaceae	Al-Hashr mount.
12	416	*Sorghum bicolor* white var.	baidha	Poaceae	Baish
13	428	*Sorghum bicolor* white var.	baidha	Poaceae	Abu Arish
14	445	*Sorghum bicolor* white var.	baidha	Poaceae	Mogor
15	458	*Sorghum bicolor* white var.	baidha	Poaceae	Jazan
16	465	*Sorghum bicolor* white var.	baidha	Poaceae	Al Aredha
17	484	*Sorghum bicolor* white var.	baidha	Poaceae	Farasan island
18	426	*Sorghum bicolor*	shahla	Poaceae	Abu Arish
19	447	*Sorghum bicolor*	shahla	Poaceae	Badawi village
20	469	*Sorghum bicolor*	shahla	Poaceae	Al Aredha
21	67	*Pennisetum glaucum*	dukhn	Poaceae	Al-Hashr mount.
22	71	*Pennisetum glaucum*	dukhn	Poaceae	Sala mount.
23	116	*Pennisetum glaucum*	dukhn	Poaceae	Sabya
24	164	*Pennisetum glaucum*	dukhn	Poaceae	Jazan
25	300	*Pennisetum glaucum*	dukhn	Poaceae	Faifa mount.
26	411	*Pennisetum glaucum*	dukhn	Poaceae	Faifa mount.
27	425	*Pennisetum glaucum*	dukhn	Poaceae	Abu Arish
28	448	*Pennisetum glaucum*	dukhn	Poaceae	Sawarya village
29	461	*Pennisetum glaucum*	dukhn	Poaceae	Jazan
30	470	*Pennisetum glaucum*	dukhn	Poaceae	Al Aredha
31	471	*Pennisetum glaucum*	dukhn	Poaceae	Al Aredha
32	478	*Pennisetum glaucum*	dukhn	Poaceae	Farasan island
33	65	*Hordeum vulgare*	shaeer	Poaceae	Al-Hashr mount.
34	126	*Hordeum vulgare*	shaeer	Poaceae	Tallan mount.
35	414	*Hordeum vulgare*	shaeer	Poaceae	Al-Hashr mount.
36	473	*Hordeum vulgare*	shaeer	Poaceae	Al Aredha
37	472	*Sesamum indicum*	semsem	Pedaliaceae	Al Aredha
38	443	*Sesamum indicum*	semsem	Pedaliaceae	Al Mowassam
39	432	*Sesamum indicum*	semsem	Pedaliaceae	Abu Arish
40	125	*Sesamum indicum*	semsem	Pedaliaceae	Sabya
41	422	*Cyamopsis tetrgonoloba*	gawar	Fabaceae	Al Aredha

of the seed clearly, including the embryo; when the embryo is alive then this area has high homogeneity, whereas dark, heterogenous areas indicate damage to the embryo or one of its parts (i.e. the embryo is not alive) (Al-Turki and Baskin, 2017; Al-Hammad and Al-Ammari, 2017).

2.6. Statistical analysis

Results from the seed moisture content and the seed viability tests were expressed as the mean percentage ± standard error (mean ± se). SPSS 11.5 for windows was used for data analyzed. The data for this work were analyzed using one-Way ANOVA followed by Duncan Post Hoc to compare the means with 5% probability level.

3. Results

3.1. Seed moisture content (MC)

The seed moisture content percentages of *Sorghum bicolor* red variety was 1.13 ± 0.19% (Table 2). Statistical analysis shows that the mean seed moisture content percentage of *Sorghum bicolor* red variety was significantly different ($P < 0.05$) from other crops (Table 2). On the other hand, the mean seed moisture content percentages of *Sorghum bicolor white* variety, *Sorghum bicolor* shahla variety, *Pennisetum glaucum, Hordeum vulgare* and *Sesamum indicum* were very similar (Table 2). ANOVA shows that overall the mean seed moisture content percentage for these crops was not significant ($P > 0.05$) (Table 2). Although the mean seed moisture content of *Cyamopsis tetragonoloba* was highest at 0.43 ± 0.24% (Table 2), no significant differences ($P > 0.05$) were evident between *Cyamopsis tetragonoloba* and other crops (Table 2).

Table 2
Seed moisture content (MC %) (mean ± se) of five crops.

Scientific-name	Seed moisture content (MC%)
Sorghum bicolor red variety	$1.13 \pm 0.19^{a*}$
Sorghum bicolor white variety	0.32 ± 0.02^{b}
Sorghum bicolor shahla variety	0.31 ± 0.01^{b}
Pennisetum glaucum	0.15 ± 0.01^{b}
Hordeum vulgare	0.34 ± 0.08^{b}
Sesamum indicum	0.18 ± 0.03^{b}
Cyamopsis tetragonoloba	0.43 ± 0.24^{ab}

Means ± se = (standard error) followed by the same letter are not significantly different ($P > 0.05$).
[*] Significantly higher.

3.2. Seed viability percentages

The seed viability for all crops examined was very high and ranged between 91.75 ± 1.23% –100 ± 0.00% (Table 3). Seed viability of

Table 3
Seed viability percentage **(mean ± se)** for the five crops (as judged by germination, Tetrazolium testing (TZ) and X-ray imaging).

Species	Seed Viability %			
	Germination % at		TZ %	X – Ray %
	30/20 °C	35/25 °C		
Sorghum bicolor - red variety	91.77 ± 1.54[a]	92.22 ± 1.54[a]	93 ± 1.44[a]	97.44 ± 0.58[a]
Sorghum bicolor – white variety	91.75 ± 1.23[a]	92.87 ± 1.36[a]	94.62 ± 1.3[a]	98 ± 0.46[a]
Sorghum bicolor – shahla variety	99.33 ± 0.66[a]	99.66 ± 0.33[a]	100 ± 0.00[a]	99 ± 0.10[a]
Pennisetum glaucum	93.66 ± 1.60[a]	94.42 ± 1.3[a]	95 ± 0.98[a]	96.25 ± 0.87[a]
Hordeum vulgare	99.25 ± 0.47[a]	100 ± 0.00[a]	100 ± 0.00[a]	99.75 ± 0.25[a]
Sesamum indicum	100 ± 0.00[a]	100 ± 0.00[a]	100 ± 0.00[a]	100 ± 0.00[a]
Cyamopsis tetragonoloba	100 ± 0.00[a]	100 ± 0.00[a]	100 ± 0.00[a]	100 ± 0.00[a]

Means ± se = (standard error) in the same row or column followed by the same letter are not significantly different ($P > 0.05$).

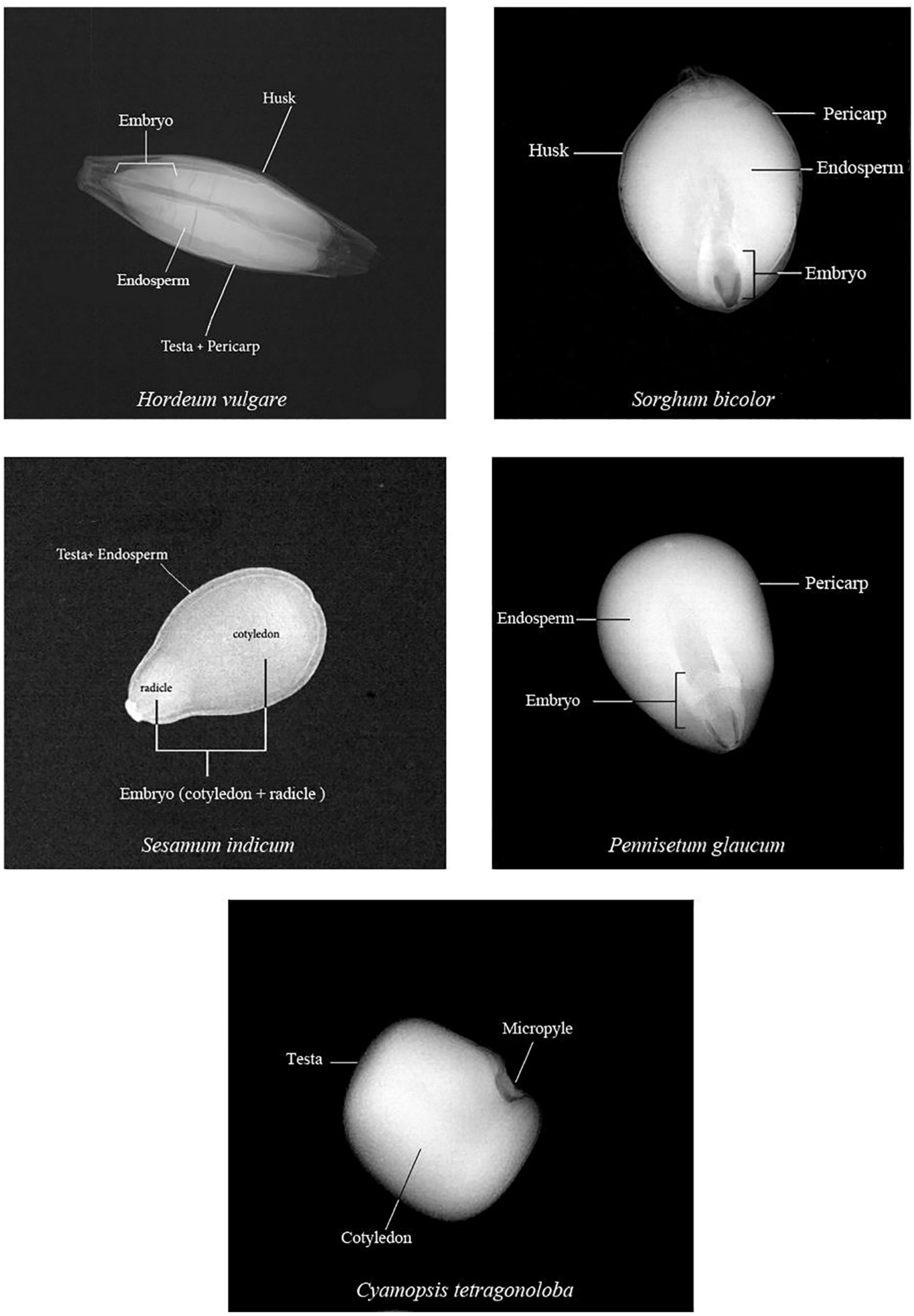

Fig. 4. X-ray images of viable seeds of five crops showing internal characteristics of embryo and endosperm.

Sorghum bicolor red variety was between 91 ± 1.54 and 97 ± 0.58%. Statistical analysis shows no significant differences ($P > 0.05$) between seed viability tests for *Sorghum bicolor* red variety and those for the other crops, which did not differ significantly from each other ($P > 0.05$). X-ray imaging of seeds of the five crops from the Jazan region revealed intact/non-damaged embryos and endosperm (Fig. 4), which appeared as clear visualization. Clear visualization of both embryo and endosperm indicate an absence of any signs of insect or microbial invasion and reflect low moisture content and good conditions for preservation in the gene bank.

4. Discussion

The conservation of genetic resources of agricultural crops developed through selection by generations of farmers in any country is of paramount importance to food security. This conservation of crop genetic resources is especially important in dry and warm environments like those of Saudi Arabia, where scarce water resources and climatic conditions are suitable only for highly adapted crops. In the present era, the genetic origins of agricultural crops are an invaluable treasure, but they are under the constant threat of negative human activities. Thus, conserving the genetic resources has great importance (FAO, 2010). The five crops collected from Jazan region represent varieties of food and fodder crops of high value for the population. In addition, hundreds of years of selection have resulted in crops that are highly adapted to the region. Thus, it is important to preserve the genetic diversity of these crop species in seed banks (*ex-situ Conservation*), as well as to continue cultivating them in Jazan region (*in-situ Conservation*).

The moisture content of seeds is one of the most important factors affecting seed deterioration, microbial damage and loss of vitality during storage (Lazzari, 1988). It has been found that the longevity of the seeds increases with a decrease in the amount of moisture to a range between 4 and 6%. The natural seed MC of the five crops studied was low, ranging from 0.15 ± 0.01% and 0.18 ± 0.03% (for some varieties of *Pennisetum glaucum* and *Sesamum indicum*) to 1.13 ± 0.19% (for some varieties of *Sorghum bicolor*). This contrast with other crops with others where the natural MC often exceeds 15% (Silberstein et al., 2010). The average MC of the seeds placed in seed banks should not exceed 12% (FAO, 1999). The reduction in the MC of the five varieties may be enhanced by the good storage of these crops by the farmers in addition to the genetic influence and genetic assets. The MC of the seeds is significantly affected by relative humidity in the surrounding environment (El-Daw, 1998; Filho, 2005). In most Jazan region, humidity is >60% in most months of the year. The low MC of the seeds of these five crops may explain the success and continuity of planting these crops over long periods of time despite the conditions of the hot climate with high humidity. On the other hand, it is desirable to help preserve these crops in gene banks. The seed viability of all five crop varieties was high, ranging from 91% to 100% in all viability tests. This confirms the above-mentioned idea of the success of planting these five crops and maintaining their viability for long periods despite difficult climatic conditions in the Jazan region.

The Jazan region is considered as one of the richest regions for genetic resources in Saudi Arabia. Climatic factors like temperature, rainfall patterns and topography make Jazan a major region of genetic diversity for many crop plants like Sorghum, Barely, Sesame, Coffee, Musa (Al-Turki, 2002). Several other regions in Saudi Arabia, such as Al-Hufuf and Al Qaseem contain very important genetic resources (Al-Turki et al., 2010), and all these important genetic resources led to the establishment of the Botanical Gene-Bank in 1994 at King Abdulaziz City for Science and Technology (KACST-BGB) in Riyadh, along with the Gene-Bank of the Ministry of Agriculture, which was established to collect and save these genetic resources from extinction.

Vavilov (1964), believed that several crops like Sorghum (*Sorghum bicolor*), Barley (*Hordeum vulgare*), Sesame (*Sesamum indicum*) have their center of diversity in Africa (Ethiopian region). In addition, some scientists (e.g. De wet and Harlan, 1971; Upadhyaya et al., 2014) regard Africa as the primary center of Sorghum diversity. The great similarity between the Jazan region and East Africa, especially in the topography and climate, has led to the similarity of large agricultural crops and they were imported from Africa to the southwestern part of the Arabian-Peninsula long time ago, specifically to the Jazan region. Consequently, the establishment of the Botanical Gene-Bank in King Abdul Aziz City for Science and Technology (KACST-BGB) represents a major step towards achieving food security and self-sufficiency in the germplasm of local crops important in feeding humans and their livestock.

5. Conclusion and recommendations

This study has revealed that the seeds of the combined varieties of the five crops (Sorghum, Barley, Pennisetum, Sesame and Guar) from the Jazan region had low MC and high viability and thus were suitable for storage in the plant gene-banks for long periods of time. Based on the results of this study and high competitiveness of these five crops relative to other crops (according to the results of moisture content and their ability to survive), we recommend the following points:

1- Supporting farmers in each region of the Kingdom of Saudi Arabia through the provision of agricultural facilities.
2- The establishment of several local plant gene-banks to conserve the genetic resources of all crops in each main agricultural region of the Kingdom of Saudi Arabia.
3- The preparations of programs and workshops by specialists at King Abdulaziz City for Science and Technology Gene-Bank (KACST-Gene-Bank) targeting farmers and workers in branches of agricultural activities in various regions to raise awareness of the importance of preserving the genetic assets of local agricultural crops and ways of conservation.

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