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Original article

Seed viability of five wild Saudi Arabian species by germination and X-ray tests

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ABSTRACT

Our objective was to evaluate the usefulness of the germination vs. the X-ray test in determining the initial viability of seeds of five wild species (*Moringa peregrina*, *Abrus precatorius*, *Arthrocnemum macrostachyum*, *Acacia ehrenbergiana* and *Acacia tortilis*) from Saudi Arabia. Usually several days were required to determine the viability of all five species via germination tests. However, X-ray test will give immediate results on filled/viable seeds. Seeds of all species, except *Acacia ehrenbergiana* and *Acacia tortilis* showed high viability in both germination (96–72% at 25/15 °C, 94–70% at 35/25 °C) and X-ray (100–80%) test. Furthermore, there was a general agreement between the germination (19%, 14% at 25/15 °C and 17% and 12% at 35/25 °C) and X-ray (8%, 4%) tests in which seed viability of *Acacia ehrenbergiana* and *Acacia tortilis* was very low due to insect damaged embryo as shown in X-ray analysis. Seeds of *Abruspreca torius* have physical dormancy, which was broken by scarification in concentrated sulfuric acid (10 min), and they exhibited high viability in both the germination (83% at 25/15 °C and 81% at 35/25 °C) and X-ray (96%) tests. Most of the nongerminated seeds of the five species except those of *Acacia ehrenbergiana* and *Acacia tortilis*, were alive as judged by the tetrazolium test (TZ). Thus, for the five species examined, the X-ray test was proved to be a good and rapid predictor of seed viability.

1. Introduction

The information about seed viability is very significant for the farmer and for the conservation of the seeds in the gene bank (Dhatchanamoorthy et al., 2016; David et al., 2016). Thus, several tests for seed viability have been carried out for testing the seed viability, such as germination, cutting, embryo excision, hydrogen peroxide, indigo carmine staining, tetrazolium staining and X-raying (Karrfalt, 2004; Antonisamy et al., 2015). All these tests except the X-ray test takes several days or weeks to complete, i.e. before the viability of the seeds is known. The X-ray method has been used in the assessment of seed viability of several species such as in *Pinus* spp. (Sahlen et al., 1995; David et al., 2016), corn, and solanaceaes and various forest species (Machado and Cicero, 2003). X-ray test has also been used to identify mechanical damages in several crops such as corn (Cicero and Banzatto-Junior, 2003) and soybean seeds. Gagliardi and Marcos-Filho (2011), stated that the X-ray radiography technique is simple, accurate, quick and that a high number of seeds can be examined in a relatively short period of time. In addition, X-ray analysis is non-destructive to the seeds (Gagliardi and Marcos-Filho, 2011). X-ray images can provide information on the internal structure and morphology of seeds, mechanical damage, and percentage of empty and filled seeds (Panchal et al., 2014).

Germination is a critical stage in the life cycle of the plants in general and particularly so in extreme environments such as desert and light and temperature are major factors that affect seed germination of desert and semi-desert plants. In contrast to X-ray analysis, seed germination tests are time consuming to determine seed viability, taking anywhere from a couple of weeks to a couple of years, depending on the seed dormancies involved. For example, seeds of Pinus species required at least three weeks to germinate (ISTA, 1985). Furthermore, a tetrazolium test (TZ) of seeds that fail to germinate takes at least another 24 h, *e.g. Francoeuria crispa* (Asteraceae), and *Aeluropus massauensis* (Poaceae), and it may be difficult if the TZ solution fails to penetrate some seeds or parts

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of seeds. Thus, although X-ray radiography provides a quick test for seed viability, it has also been used to determine maturity (viability) (Shen and Odén, 1999) and to predict early growth (germination) (Goodman et al., 2005) of tree seeds. However, no information is available on the relative benefits of using the germination vs. the X-ray test to evaluate seed viability of wild species from Saudi Arabia. Thus, the aim of this study was to compare the germination and X-ray test for determining seed viability of five species *Moringa peregrina*, *Abrus precatorius*, *Arthrocnemum macrostachyum, Acacia ehrenbergiana* and *Acacia tortilis* from Saudi Arabia.

2. Materials and methods

2.1. Study species

Five species *Moringa peregrina*, *Abruspreca torius*, *Arthrocnemum macrostachyum*, *Acacia ehrenbergiana* and *Acacia tortilis* with different uses were selected for study (Table 1).

2.2. Seed collection

Mature seeds *Moringa peregrina*, *Abruspreca torius*, *Arthrocnemum macrostachyum, Acacia ehrenbergiana* and *Acacia tortilis* were collected in Saudi Arabia at the location and on the date given in Table 2. The seeds were taken directly from at least 50 healthy plant individuals per species. Seeds were air dried, cleaned and stored in brown paper bags at room temperature (22 °C) for three weeks and then examined immediately.

2.3. Seed viability testing

Seed viability of the five species was determined using the germination test and the X-ray test.

2.4. Germination test

Seed germination tests were conducted using 9-cm Petri dishes containing two layers of filter paper (Whatman no. 1) moistened with 10 ml of distilled water, and five replicates of 20 seeds each for each species were used. Prior to the germination test, the water-impermeable seeds of *Abruspreca torius* were soaked in concentrated sulfuric acid (H_2SO_4) for 10 min to break dormancy. Petri dishes were randomly distributed in temperature-controlled incubators and their position was changed daily. Germination was defined as the first emergence of the radicle (Sanjeewani et al., 2014). Newly-germinated seeds were counted each day for 30 d and subsequently removed from the Petri dishes. Seeds were incubated in a daily photoperiod (12 h light: 12 h dark) at alternating temperature regimes of 25/15 °C and 35/25 °C that simulate possible diurnal temperature fluctuations in the habitats of the eight species. At the end of the germination tests, nongerminated seeds were tested for viability using 2,3,5-triphenyl tetrazolium chloride (TTC) solution, as described by the International Seed Testing Association (1999). The seeds were soaked in 1% TTC solution for 4 days in a glass vial in the dark at 25 °C, and a red stained embryo was used as an indication of seed viability. The final germination percentage (%) was expressed as G (%) = (A/B) × 100 (Panchal et al., 2014), where A is the total number of seeds germinated at the end of experiment (30 d) and B is the total number of seeds tested (100 seeds). Germination speed (50% = t_{50}) was calculated according to Maguire (1962) as $GSI = G_1/N_1 + G_2/N_2 + \cdots G_n/N_n$, where G_1, G_2, G_n are the number of germinated seeds and N_1, N_2, N_n the number of days.

2.5. X- ray radiography test

Two samples of 2 replications of 50 seeds of each species 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 KV/10 s. The X-ray plates were evaluated based on the presence and morphology of the embryo and endosperm. The percentage of seeds with a whole embryo, damaged embryo or no embryo was determined.

2.6. Statistical analysis

For each germination test, the results were expressed as the mean percentage ± standard error, which were subjected to the *t*-test. *T*-statistics and probabilities indicate significance differences between treatments. Data of X-ray analysis were not statistically analyzed.

3. Results

3.1. Seed viability

3.1.1. Germination test

The germination percentage of all five species was higher at 25/15 °C than at 35/25 °C (Fig. 1). Regardless of the test tempera-

Table 1
The study species and their uses.

Species	Family	Uses	Reference(s)
Moringa peregrina	Moringaceae	Nutritional and medicinal properties	Elsayed et al. (2016)
Abrus precatorius	Fabaceae	Toxic seeds, the leaves and used to treat fevers coughs and colds	Martinez et al. (2012)
Arthrocnemum macrostachyum	Amaranthaceae	Possesses high quality of edible oil having unsaturation ranging from 70–80%	Weber et al. (2007)
Acacia ehrenbergiana	Fabaceae	Medicinal and economic and Grazing plant	Javed et al. (2013)
Acacia tortilis	Fabaceae	The seeds are ingested by birds	Jaouadi et al. (2015)

Table 2
Seed collection location and date of the five study species.

No	Species	Family	Collection-date	Location
1	*Moringa peregrina*	Moringaceae	13 January 2014	Al-Ula (317 South of Tabuk Region)
2	*Abrus precatorius*	Fabaceae	17 January 2014	Jabal Shada (Al-Baha Region)
3	*Arthrocnemum macrostachyum*	Amaranthaceae	8 June 2015	Darin Island (Arabian Gulf Coast)
4	*Acacia ehrenbergiana*	Fabaceae	12 October 2013	Al-Thummamah (55 km North–East of Riyadh)
5	*Acacia tortilis*	Fabaceae	11 October 2013	Al-Thummamah (55 km North–East of Riyadh)

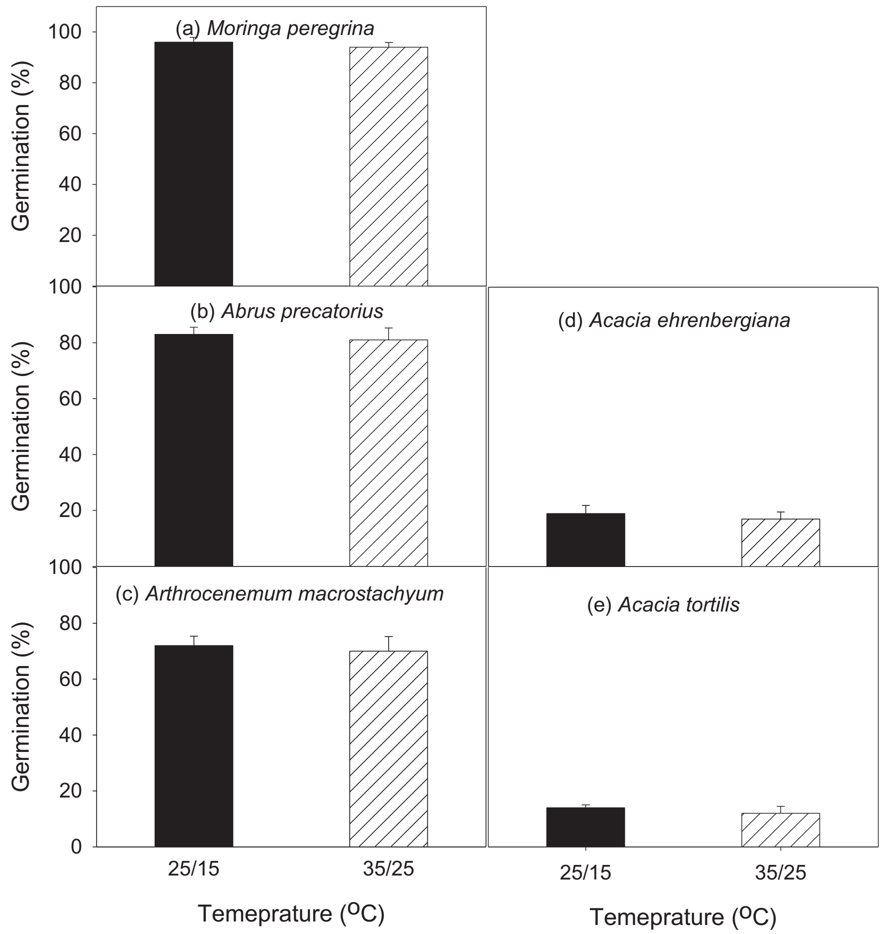

Fig. 1. The final germination percentages (mean ± Se) of (a) *Moringa peregrina*, (b) *Abrus precatorius*, (c) *Arthrocnemum macrostachyum*, (d) *Acacia ehrenbergiana* and (e) *Acacia tortilis* at two alternating temperatures.

Table 3
Time (days) taken to achieve 50% germination (t_{50}) of the five species at two alternating temperatures (12 h light/12 h dark).

Species	Alternating temperature (°C)	
	25/15	35/25
Moringa peregrina	3.5	1
Abrus precatorius	3.5	2
Arthrocnemum macrostachyum	4	2
Acacia ehrenbergiana	8	4
Acacia tortilis	8	4

Table 4
Fate of seeds that did not germinate at 25/15 and 35/25 °C (12 h light/12 h dark) Proportions (%) of original number germinating, remaining dormant and dead (as judged by the tetrazolium test) seeds of the in five species.

Species	Alternating temperature (°C)	Dormant (%)	Dead (%)
Moringa peregrina	25/15	4	0
	35/25	6	0
Abrus precatorius	25/15	10	7
	35/25	12	7
Arthrocnemum macrostachyum	25/15	20	8
	35/25	17	13
Acacia ehrenbergiana	25/15	1	80
	35/25	3	80
Acacia tortilis	25/15	0	86
	35/25	0	88

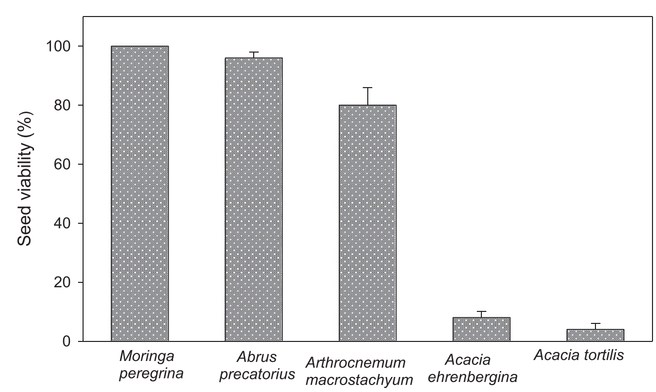

Fig. 2. Seed viability percentages (mean ±***se***) of five wild species as judged by the X-ray test. (±se = Standard error).

ture, seeds of all species, except *Acacia tortilis, Acacia ehrenbergiana* germinated to ≥70%, while those of *Acacia ehrenbergiana*, *Acacia tortilis*, germinated to only19%, 14% at 25/15 °C and 17% and 12% at 35/25 °C. Thus, temperature had no significant effect ($P > 0.05$) on germination of any of the species. Seeds of all species started to germinate after 3–4 days at both temperatures (25/15 °C and 35/25 °C). The $\mathbf{t_{50}}$ for seeds of *Acacia ehrenbergiana* and *Acacia tortilis* was quite slow (8 days at 25/15 °C) (Table 3). In contrast, seeds of *Moringa peregrina*, *Abruspreca torius* and *Arthrocnemum macrostachyum* reached 50% within 3.5, 5 and 6 days, respectively, at 25/15 °C (Table 3). The $\mathbf{t_{50}}$ of all five species decreased with increasing temperature to 1–5 days at 35/25 °C (Table 3). The tetrazolium viability test (TZ) revealed that most non-germinated seeds of *Acacia ehrenbergiana*, *Acacia tortilis* (80%, 86% at 25/15 °C and 80% and 88% at 35/25 °C) were dead (Table 4). In contrast, most of the non-germinated seeds of the other species were alive (Table 4). The seeds of *Abruspreca torius* have physical dormancy (water-impermeable seed coat) that was broken by sulfuric acid, and the germination test showed very high viability of seeds (83% at 25/15 °C and 81% at 35/25 °C) (Fig. 1).

3.2. X-ray radiography test

Exposure of seeds to 18 V radiation for 10 s enabled clear visualization of the embryo and endosperm. Based on embryo morphology as seen by X-ray radiography, 100%, 96% and 80%, of the seeds of *Moringa peregrina*, *Abrus precatorius*, and *Arthrocnemum macrostachyum,* respectively were viable(Figs. 2 and 3a–c), while only 8% and 4% of *Acacia ehrenbergiana* and *Acacia tortilis* seeds were viable (Figs. 2 and 3d and e). Endosperm was not present in seeds of *Arthrocnemum macrostachyum* (Fig. 3c).

4. Discussion

The results show that seeds of *Moringa peregrina* and *Arthrocnemum macrostachyum* do not appear to have genetically fixed (innate) mechanism of dormancy since untreated seeds samples from several plants had 96%, 72% germination at 25/15 °C, and

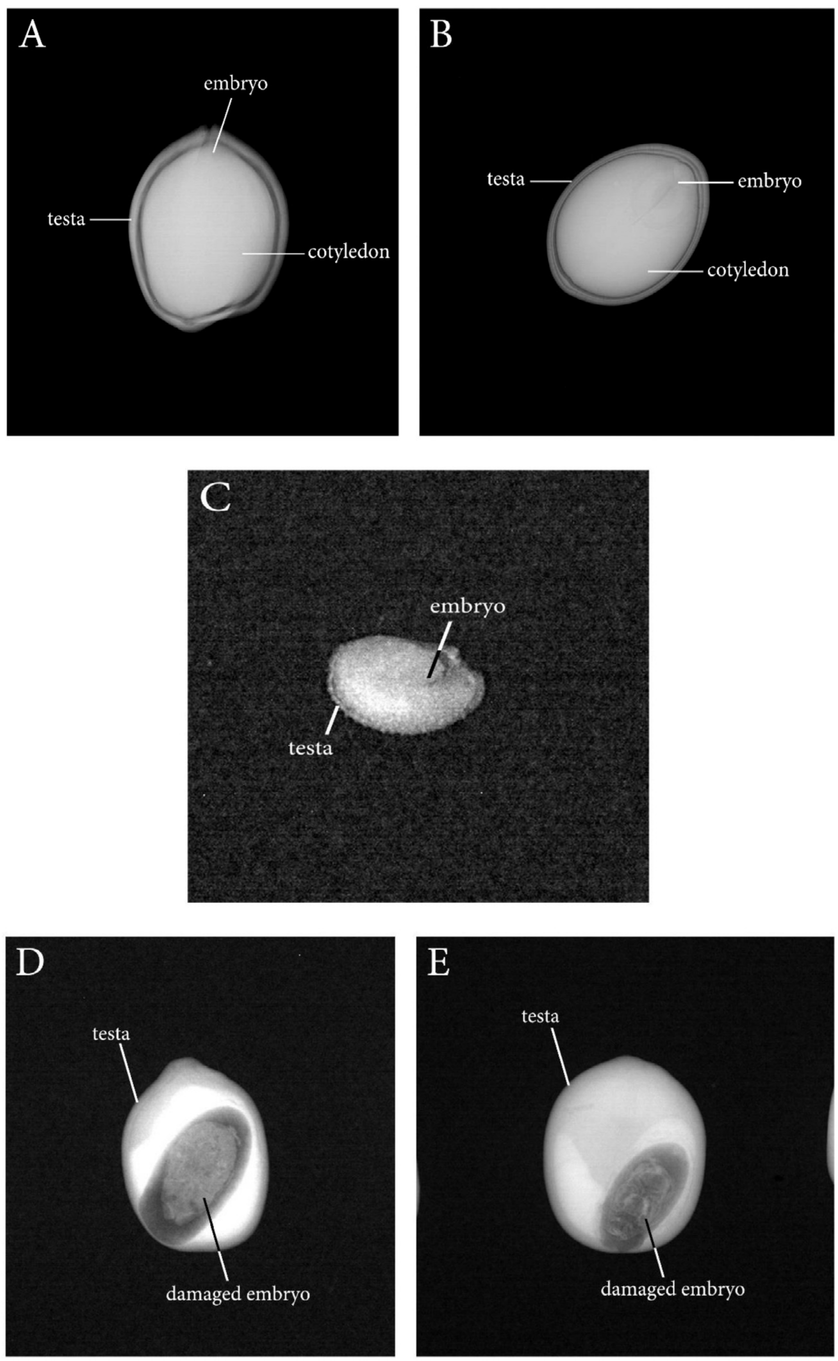

Fig. 3. X-ray photograph of *Moringa peregrina* seeds (A) and *Abrus precatorius* seeds (B) showing, testa, embryo and cotyledon (X5). X-ray photograph of *Arthrocnemum macrostachyum* seeds (C), *Acacia ehrenbergiana* seeds (D) and *Acacia tortilis* seeds (E) showing, testa, embryo, cotyledon and damaged embryo (X5).

94%, 70% germination at 35/25 °C respectively (Fig. 1). The seeds of *Abruspreca torius* have physical dormancy (water-impermeable seed coat) that was broken by sulfuric acid, and the final germination percentage (%) ranged between 83% at 25/15 °C and 81% at 35/25 °C (Fig. 1). *Acacia ehrenbergiana* and *Acacia tortilis* seeds showed very low germination (Fig. 1). Previous studies showed that seeds of different populations of *Moringa peregrina* collected from Egypt germinated easily (>94%) (Gomaa and Picó, 2011). Our study about seed viability of *Acacia ehrenbergiana* and *Acacia tortilis* confirms the earlier works of El Atta (1993). Who found that the embryo of *Acacia nilotica* from sudan was damaged by larvae of *Caryedon serratus* Olivier. Loth et al. (2005), showed that the optimum temperature of seed germination of *Acacia tortilis* from Tanzania ranged between 21 and 23 °C and the seeds of this species that had absorbed water lost their viability when kept above 35.5 °C.

In fact that both the germination and X-ray tests indicated that a high percentage of the seeds of *Moringa peregrina*, *Abrus precatorius*, and *Arthrocnemum macrostachyum* was viable (Figs. 1 and 2) while only a low percentage of the seeds of *Acacia ehrenbergiana* and *Acacia tortilis* was viable. As revealed by X-ray, the embryo in most seeds of *Acacia ehrenbergiana* and *Acacia tortilis* was damaged (Fig. 3d and e). Reliable results of both germination and X-ray test concerning seed viability for the five species agreed completely with results from previous studies. Further, using X-ray, Ferguson and Tuner (1971) found that the low germination of cotton seeds was due to damage that seeds sustained during harvest. Shen and Odén (1999) also found that X-raying seeds is an effective way to detect embryo damage and determine if seeds are filled. Although the X-ray analysis for seeds of the five species was very fast (few seconds), simple and accurate, the germination test was relatively slow but nonetheless accurate. The seeds required 2–4 days to start germination, and the speed of germination represented by $\mathbf{t_{50}}$ varied with the species and test temperature (Table 3). The time required for reaching 50% germination of *Moringa peregrina*, *Abrus precatorius*, and *Arthrocnemum macrostachyum* was 3.5 days at 25/15 °C and 1–2 days at 35/25 °C. Seeds of *Acacia ehrenbergiana* and *Acacia tortilis* had a $\mathbf{t_{50}}$ of 8 days at 25/15 °C, while the $\mathbf{t_{50}}$ for these two species was decreased to 4 days at 35/25 °C A Similar result was reported by Gomaa and Picó (2011), who found that seeds from different populations of *Moringa peregrina* required 3 days to start germination. Also, Chanyenga et al. (2012) reported that the seeds of *Widdringtonia whytei* required about 16 days to start germination at 20 °C and 15/25 °C and 21 days to start germination at 15 °C and 10/20 °C. On the other hand, Vitis et al. (2014) reported that the t_{50} of *Malcolmia littorea* (Brassicaceae) seeds decreased with increasing temperatures (9 days at 5 °C and 2 days at 25 °C). The speed of germination ($\mathbf{t_{50}}$) of different populations of several species of Amaranthaceae from Saudi Arabia (*e.g. Salicornia europaea* agg., *Suaeda aegyptiaca*, *Suaeda maritima*, *Suaeda vermiculata* and *Suaeda monoica*) decreased with an increase in temperature (Al-Turki, 1992).

5. Conclusions

From the present investigation, we concluded that the X-ray test was easier and quicker than the germination test for the five wild species from Saudi Arabia. In germination tests for these five species, at least 2–4 days were required for seeds to start germination, and the speed of germination (t_{50}) in all the species decreased with an increase in temperature from at 25/15 °C to 35/25 °C. Seed viability as determined by germination and X-ray tests was high for three species (*Moringa peregrina*, *Abrus precatorius*, and *Arthrocnemum macrostachyum*) and low for two species (*Acacia ehrenbergiana* and *Acacia tortilis).* X-ray analysis showed that the embryo in most of the *Acacia ehrenbergiana*, *Acacia tortilis* seeds was damaged. Thus, while both tests give an accurate assessment of seed viability, the X-ray test gave the fastest results as well as an explanation for low viability in the case of *Acacia ehrenbergiana* and *Acacia tortilis*.

Conflict of interest statement

None declared.

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