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ORIGINAL ARTICLE

Determination of seed viability of eight wild Saudi Arabian species by germination and X-ray tests

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X-ray test

Abstract Our purpose was to evaluate the usefulness of the germination vs. the X-ray test in determining the initial viability of seeds of eight wild species (*Salvia spinosa*, *Salvia aegyptiaca*, *Ochradenus baccatus*, *Ochradenus arabicus*, *Suaeda aegyptiaca*, *Suaeda vermiculata*, *Prosopisfarcta* and *Panicumturgidum*) from Saudi Arabia. Several days were required to determine viability of all eight species via germination tests, while immediate results on filled/viable seeds were obtained with the X-ray test. Seeds of all the species, except *Sa.aegyptiaca*, showed high viability in both the germination (98–70% at 25/15 °C, 93–66% at 35/25 °C) and X-ray (100–75%) test. Furthermore, there was general agreement between the germination (10% at 25/15 °C and 8% at 35/25 °C) and X-ray (5%) tests that seed viability of *Sa.aegyptiaca* was very low, and X-ray analysis revealed that this was due to poor embryo development. Seeds of *P.farcta* have physical dormancy, which was broken by scarification in concentrated sulfuric acid (10 min), and they exhibited high viability in both the germination (98% at 25/15 °C and 93% at 35/25 °C) and X-ray (98%) test. Most of the nongerminated seeds of the eight species except those of *Sa.aegyptiaca* were alive as judged by the tetrazolium test (TZ). Thus, for the eight species examined, the X-ray test was a good and rapid predictor of seed viability.

1. Introduction

Whether seeds are to be sown for crop production or stored in gene banks, information about their viability is very valuable. Thus, several tests for seed viability, have been developed: germination, cutting, embryo excision, hydrogen peroxide, indigo carmine staining (Kamra, 1964), tetrazolium staining and X-raying (*e.g.* Bonner, 1998, and Karrfalt, 2004). 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 (Kamra,

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1966). X-ray analysis as a way to determine that seed viability was first used in 1903 by A.N. Lundström (Kamra, 1964), but it now is widely used to determine seed quality (*e.g.* Swaminathan and Kamra, 1961; Kamra, 1964, 1966), especially for crop seeds such as *Gossypium* sp. (Ferguson and Tuner, 1971), *Solanum lycopersicum* (Van der Burg et al., 1994a), *Eugenia pleurantha* (Masetto et al., 2007), *Zea mays* (Carvalho et al., 1999), *Xylopia aromatic* (Socolowski et al., 2011) and *Capsicum annuum* (Gagliardi and Marcos-Filho, 2011) but also for trees (Simak and Gustafsson, 1953). Martin et al. (1998) and Gagliardi and Marcos-Filho, (2011) stated that the X-ray radiography technique is simple 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 (Chavagnat, 1987; Gagliardi and Marcos-Filho, 2011). Since the internal morphological structure of the seed, specifically the embryo, can be evaluated by the X-ray technique (Van der Burg et al., 1994a; Gagliardi and Marcos-Filho, 2011), the relationship between germination and seed structure has been investigated for several species (Simak, 1991; Jorgea and Ray, 2005; Gagliardi and Marcos-Filho, 2011), *e.g. C. annuum* (Dell Aquila, 2007a), *Z. mays* (Cícero et al., 1998; Carvalho et al., 1999), and *Carica papaya* (Santos et al., 2009).

In contrast to X-ray analysis, germination tests to determine seed viability may require 2-3 days, *e.g.* seeds of different populations of *Salicornia* species (Al-Turki, 1992), or several weeks, *e.g.* at least three weeks for testing seeds of several *Pinus* species (ISTA, 1985). Further, a tetrazolium test (TZ) of seeds that fail to germinate takes at least another 24 h, *e.g. Suaeda* species (Al-Turki, 1992), and it may be difficult if the TZ solution fails to penetrate some seeds or parts of seeds (Mackay, 1972). Thus, although X-ray radiography provides a quick test for seed viability, it also has been used to determine maturity (viability) (*e.g.* Belcher, 1973, 1977; Duffield, 1973; Kamra, 1976; Simak, 1980; Sahlen et al., 1995; Geo-HanDong, 1998; 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 eight species (*Salvia spinosa*, *Salvia aegyptiaca*, *Ochradenus baccatus*, *Ochradenus arabicus*, *Suaeda aegyptiaca*, *Suaeda vermiculata*, *Prosopis farcta and Panicum turgidum*) from Saudi Arabia.

2. Materials and methods

2.1. Study species

Eight species (*S. spinosa*, *Sa. aegyptiaca*, *O. baccatus*, *O. arabicus*, *Su. aegyptiaca*, *S. vermiculata*, *P. farcta* and *P. turgidum*) with different uses were selected for study (see Table 1).

2.2. Seed collection

Seeds of *S. spinosa*, *Sa. aegyptiaca*, *O. baccatus*, *O. arabicus*, *Su. aegyptiaca*, *S. vermiculata*, *P. farcta and P. turgidum* (black seeds) were collected from Saudi Arabia at the location and on the date given in Table 2. Seeds were air dried, cleaned and

Table 1 Study species and their uses.

Species	Family	Uses	Reference(s)
Salvia spinosa	Lamiaceae	Treating diarrhea and genorrhea	Lu and Foo (2002)
Salvia aegyptiaca	Lamiaceae	Treating stomachic and diarrhea	Al-Yousuf et al. (2002)
Ochradenus baccatus	Resedaceae	The seeds are ingested by birds	Al-Turki (1997)
Ochradenus arabicus	Resedaceae	The seeds are ingested by birds	Al-Turki (1997)
Suaeda aegyptiaca	Amaranthaceae	Treating burns	Razik (1986)
Suaeda vermiculata	Amaranthaceae	Soil remediation to reduce salinity and contamination by toxic metals.	Singh (2005)
Prosopis farcta	Fabaceae	Forage plant for livestock	Chaudary (1999)
Panicum turgidum	Gramineae	Grazing plant for domestic herds	Mandaville (1990)

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

No	Species	Family	Collection-date	Location
1	*Salvia spinosa*	Lamiaceae	30 March 2014	77 km north of Riyadh QassimRoad
2	*Salvia aegyptiaca*	Lamiaceae	18 April 2014	77 km north of Riyadh QassimRoad
3	*Ochradenus baccatus*	Resedaceae	29 March 2014	77 km north of Riyadh QassimRoad
4	*Ochradenus arabicus*	Resedaceae	21 May 2014	77 km north of Riyadh QassimRoad
5	*Suaeda aegyptiaca*	Amaranthaceae	11 October 2013	Al-Hufuf City
6	*Suaeda vermiculata*	Amaranthaceae	28 May 2013	Al-Zawr village
7	*Prosopis farcta*	Fabaceae	20 July 2012	Al-Awshaziyah village
8	*Panicum turgidum*	Gramineae	15 April 2014	Al-Thumamah (55 km north-east of Riyadh)

stored in brown paper bags at room temperature (22 °C) for two weeks and then examined immediately.

2.3. Seed viability testing

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

2.3.1. 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 *P. farcta* were soaked in concentrated sulfuric acid (H_2SO_4) for 10 min to break

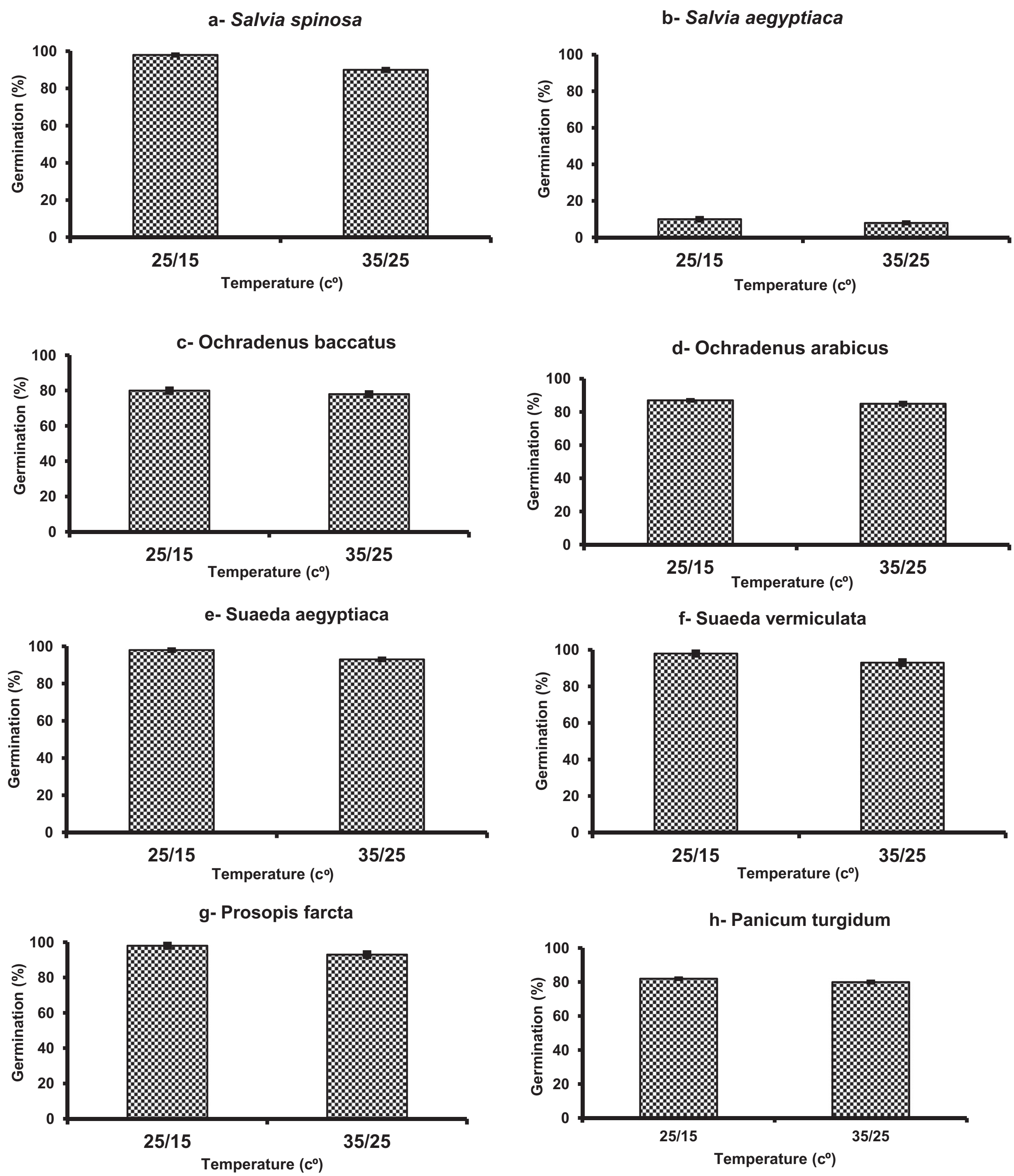

Figure 1 Final germination percentages (mean ± se) of seeds of *Salvia spinosa*, *Salvia aegyptiaca*, *Ochradenus baccatus*, *Ochradenus arabicus*, *Suaeda aegyptiaca*, *Suaeda vermiculata*, *Prosopis farcta*, and *Panicum turgidum* at two alternating temperatures (12 h upper temperature in light/12 h lower temperature in darkness).

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 (Redondo et al., 2004). 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 (%) = $(A/B) \times 100$ (Li and Shi, 2010, Wang et al., 2013), 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 = $G_1/N_1 + G_2/N_2 + \ldots G_n/N_n$, where G_1, G_2, G_n are the number of germinated seeds and N_1, N_2, N_n the number of days.

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

Species	Alternating temperature (°C)	
	25/15	35/25
Salvia spinosa	3.5	1
Salvia aegyptiaca	7	2
Ochradenus baccatus	7	2
Ochradenus arabicus	4	1
Suaeda aegyptiaca	5.5	1
Suaeda vermiculate	7	2
Prosopis farcta	4.5	1
Panicum turgidum	5.5	1

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 eight species.

Species	Alternating temperature (°C)	Dormant %	Dead %
Salvia spinosa	25/15	0	2
	35/25	0	10
Salvia aegyptiaca	25/15	0	90
	35/25	0	92
Ochradenus baccatus	25/15	20	0
	35/25	20	2
Ochradenus arabicus	25/15	10	3
	35/25	15	0
Suaeda aegyptiaca	25/15	15	8
	35/25	15	12
Suaeda vermiculate	25/15	20	10
	35/25	17	17
Prosopis farcta	25/15	2	0
	35/25	7	0
Panicum turgidum	25/15	18	0
	35/25	20	0

2.3.2. X- ray radiography test

Two replicates 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.

2.4. 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 eight species was higher at 25/15 °C than at 35/25 °C (Fig. 1). Regardless of the test

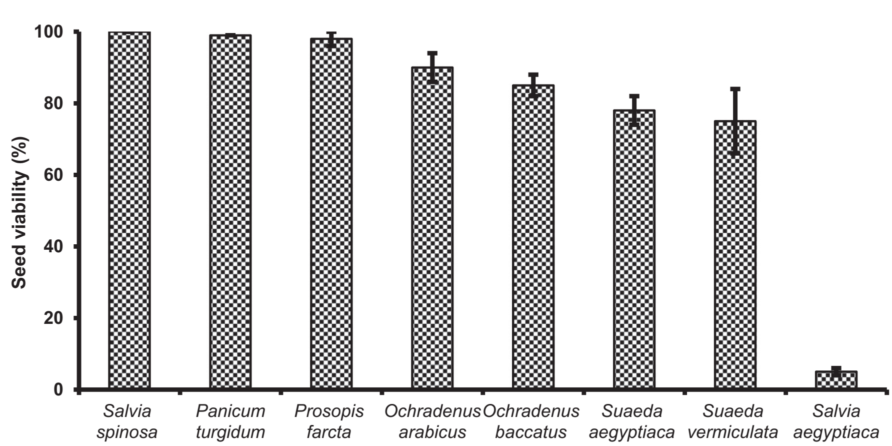

Figure 2 Seed viability percentages (mean ± se) of eight wild species as judged by the X-ray test (±se = standard error).

temperature, seeds of all species, except *Sa. aegyptiaca* germinated to ⩾80%, while those of *Sa. aegyptiaca* germinated to only 10% at 25/15 °C and 8% 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 1–2 days at both temperatures (25/15 °C and 35/25 °C) (Table 3). The t_{50} for seeds of *Sa. aegyptiaca*, *O. baccatus and S. vermiculata* was quite slow (7 days at 25/15 °C) (Table 3). In contrast, seeds of *S. spinosa*, *O. arabicus*, *P. farcta*, *Su. aegyptiaca* and *P. turgidum* reached 50% within 3.5, 4.0, 4.5, 5.5 and 5.5 days, respectively, at 25/15 °C (Table 3). The t_{50} of all eight species decreased with increasing temperature to 1–2 days at 35/25 °C (Table 3). The tetrazolium viability test (TZ) revealed that most nongerminated seeds of *Sa. aegyptiaca* (90% at 25/15 °C and 92% at 35/25 °C) were dead (Table 4). In contrast, most of the nongerminated seeds of the other species were alive (Table 4). The seeds of *P. farcta* have physical dormancy (water-impermeable seed coat) that was broken by sulfuric acid, and the germination test showed very high viability of seeds (98% at 25/15 °C and 93% at 35/25 °C) (Fig. 1).

3.1.2. 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%, 99%, 98%,

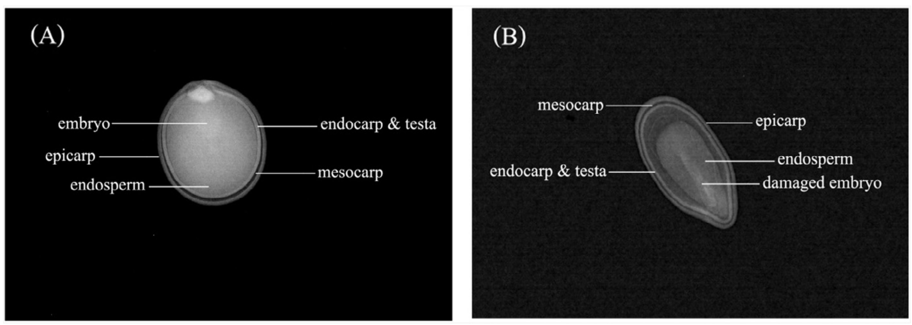

Figure 3 X-ray photograph of *Salvia spinosa* seeds (A) showing the epicarp, endocarp, testa, mesocarp, endosperm and embryo, and *Salvia aegyptiaca* seeds (B) showing the epicarp, endocarp, testa, mesocarp, endosperm and immature embryo, (X5).

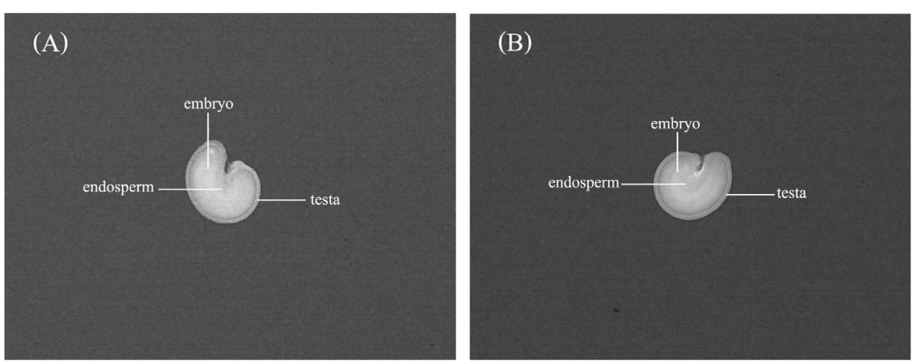

Figure 4 X–ray photograph of *Ochradenus arabicus* seeds (A) and *Ochradenus baccatus* seeds (B) showing the testa, embryo and endosperm (X5).

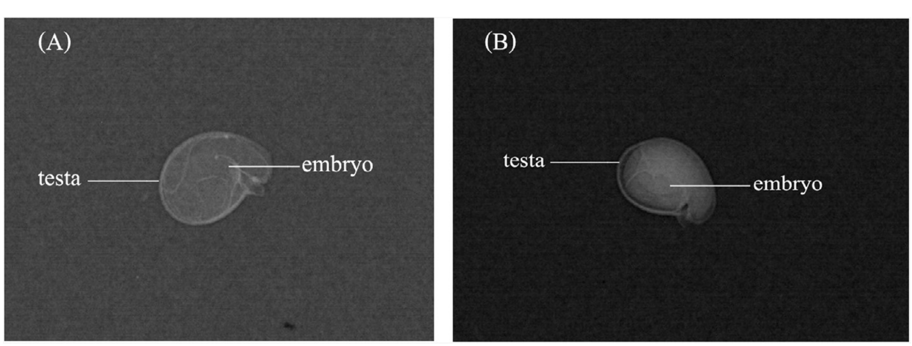

Figure 5 X-ray photograph of *Suaeda aegyptiaca* seeds (A) and *Suaeda vermiculata* seeds (B) showing the testa and embryo, (X5).

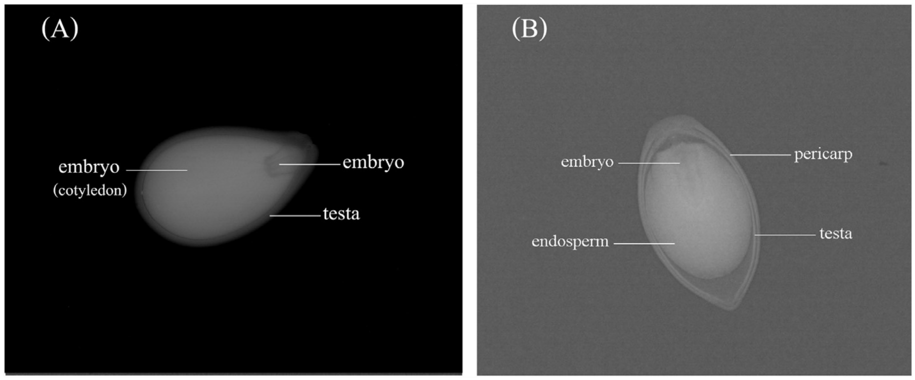

Figure 6 X-ray photographs of *Prosopis farcta* seeds (A) showing the testa and embryo, and *Panicum turgidum* seeds (black seeds) (B) showing the testa, pericarp, endosperm and embryo, (X5).

90%, 85%, 78% and 75% of the seeds of *S. spinosa*, *P. turgidum*, *P. farcta*, *O. arabicus*, *O. baccatus*, *Su. aegyptiaca* and *S. vermiculata*, respectively, were viable (Figs. 2, 3A, 4A, B, 5A,B, and 6A,B), while only 5% of *Sa.aegyptiaca*seeds were viable (Figs. 2 and 3B). Endosperm was not present in seeds of *Su. aegyptiaca* and *S. vermiculata* (Fig. 5A,B), but it was present in seeds of the other species (Figs. 3A,B, 4A,B, 6A,B).

4. Discussion

Both the germination and X-ray tests indicated that a high percentage of the seeds of *S. spinosa*, *O. baccatus*, *O. arabicus*, *Su. aegyptiaca*, *S. vermiculata*, *P. farcta and P. turgidum* was viable (Figs. 1 and 2), while only a low percentage of the seeds of *Sa. aegyptiaca* was viable. As revealed by X-ray, the embryo in most seeds of *Sa. aegyptiaca* was immature (Fig. 3B). Thus, although seeds (nutlets) of *Sa. aegyptiaca* appeared to be mature when they were collected, we suspect that plants had dried in the field before seeds had matured fully. Gorai et al. (2011) who collected seeds of *Sa. aegyptiaca* from southeastern Tunisia reported that seeds germinated over a wide range of temperatures (10–40 °C), with the highest final germination (77%) at 30 °C.

The fact that both the germination and X-ray test gave reliable results concerning seed viability for the eight species agrees completely with results from previous studies. For example, Kamra (1964, 1971) reported close agreement between the germination and X-ray tests when seeds of several forest, agricultural and horticultural species were examined. 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. Van der Burg et al., 1994b, Linington et al. (1995) and 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 eight species was very fast (few seconds), simple and accurate, the germination test was relatively slow but nonetheless accurate. The seeds required 1–2 days to start germinating, and the speed of germination represented by t_{50} varied with the species and test temperature (Table 3). The time required to reach 50% germination of *Sa. aegyptiaca*, *O. baccatus* and *S. vermiculata* was 7 days at 25/15 °C and 2 days at 35/25 °C. Seeds of *S. spinosa*, *O. arabicus*, *P. farcta*, *Su. aegyptiaca* and *P. turgidum* had a t_{50} of 3.5, 4.0, 4.5, 5.5 and 5.5 days respectively, at 25/15 °C, while the t_{50} for these species was decreased to 1 day at 35/25 °C A similar result was reported by Al-Turki (1992) who found that seeds from different populations of *Salicornia europaea* agg. (Amaranthaceae) required 2 days at 25/15 °C and only 1 day at 35/25 °C to reach 50% germination (t_{50}), 3 days at 25/15 °C and 2 days at 35/25 °C for different populations of *Su. aegyptiaca* (Amaranthaceae), nearly 5 days at 25/15 °C and 2 days at 35/25 °C for different populations of *S. vermiculata* (Amaranthaceae) and 6 days at 25/15 °C and 1 day at 35/25 °C for different populations of *Suaeda monoica* (Amaranthaceae). In these four species of Amaranthaceae, it is clear that the speed of germination (t_{50}) decreased with an increase in temperature (Al-Turki, 1992). Also, Chanyenga et al. (2012) reported that the seeds of *Widdringtonia whytei* required about 16 days to start germinating at 20 °C and 15/25 °C and 21 days to start germinating 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).

5. Conclusions

From the present study, it can be concluded that the X-ray test was faster and easier than the germination test for the eight wild species from Saudi Arabia. In germination tests for these eight species, at least 1–2 days were required for seeds to start germinating, 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 seven species (*S. spinosa*, *P. turgidum*, *P. farcta*, *O. baccatus*, *O. arabicus*, *Su. aegyptiaca* and *S. vermiculata*) and low for one species (*Sa. aegyptiaca*). X-ray analysis showed that the embryo in most of the *Sa. aegyptiaca* 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 *Sa. aegyptiaca*.

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