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Original article

Seed dormancy and germination in *Dodonaea viscosa* (Sapindaceae) from south-western Saudi Arabia

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ABSTRACT

Dodonaea viscosa (Sapindaceae) is widespread in the mountainous highlands of the southwestern part of Kingdom of Saudi Arabia, where it is a medicinally important species for the people in Saudi Arabia. Seeds of this species were collected from Mount Atharb in Al-Baha region, at an altitude of 2100 m. The aims of this study were to determine if the seeds of *D. viscosa* have physical dormancy (i.e. a water-impermeable seed coat) and, if so, what treatments would break dormancy, and what conditions promote germination after dormancy has been broken. The dormancy-breaking treatments included: soaking of seeds in concentrated sulfuric acid (H_2SO_4) for 10 min, immersion in boiling water for 10 min and exposure to 50 °C for 1 min. After seeds had been pre-treated with H_2SO_4, to break dormancy, they were incubated at constant temperatures from 5 to 35 °C, under 12-h photoperiods or in continuous darkness, and germination recorded. Salinity tolerance was investigated by incubating acid-scarified seeds in different concentrations of mM NaCl in the light at 25 °C.

Untreated seeds had low final germination 30%. Seeds that had been acid-scarified, immersed in boiling water or exposed to 50 °C all achieved 91% subsequently when incubated at 25 °C. Thus, seeds of this species in Saudi Arabia have physical dormancy, which can be broken by all three treatments designed to increase the permeability of the testa. After pre-treatment, there was a broad optimum constant temperature for germination that ranged between 5 and 25 °C but germination was inhibited by higher temperatures (30 and 35 °C). Light had little effect on this germination response. Scarified seeds were also sensitive to salinity, with the highest germination in distilled water and complete inhibition in 400 mM NaCl. Seeds that failed to germinate in saline treatments were mostly able to germinate on transfer to distilled water, suggesting osmotic inhibition.

1. Introduction

Seed germination is one of the critical stages in the life cycle of the plant (Gutterman, 1993; Gutterman 2002; Song et al., 2005; Li et al., 2010). Seed dormancy may determine the timing of germination and various environmental factors, such as temperature, light and salinity may play a significant role in regulating germination behavior in the field (Al-Turki, 1992; Noe and Zedler, 2000; Gutterman, 2002; Koornneef et al., 2002; Khan and Gul, 2006; Saeed et al., 2011; Gulzar et al., 2013; Baskin and Baskin, 2014). One of the more important forms of seed dormancy is physical dormancy (PY), where the seed coat remains impermeable, as a result of a palisade layer of lignified malpighian cells (Baskin et al., 2000; Baskin, 2003). Physical dormancy has been reported in more than 15 dicotyledonous and one monocotyledonous families (Baskin et al., 2000, 2006; Baskin, 2003; Horn, 2004; Baskin and Baskin, 2014). Five genera of the family Sapindaceae, *Cardiospermum, Diplopeltis, Distichostemon, Dodonaea,* and *Koelreuteria,* have been reported to have PY (Johnston et al., 1979; Rehman and Park, 2000; Baskin et al., 2004; Harrington et al., 2005; Turner et al., 2006; Cook et al., 2008). Alternatively, some species have been found to have innate physiological dormancy of the embryo (Baskin and Baskin, 2014) and Naidu et al. (1999) have proved that seeds of *Sapindus trifoliatus* have physiological dormancy in addition to PY.

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Physical dormancy can be broken by using various scarification methods to render the testa water-permeable: hot water (Baskin et al., 2004), dry heat (Mott, 1982), warm moist incubation (Jayasuriya et al., 2007), long-term dry storage (Morrison et al., 1992), and soaking in concentrated sulfuric acid (Brahmam et al., 1996; Baskin and Baskin, 2014). For instance, Cook et al. (2008) reported that PY in the seeds of *D. hackettiana* was broken by exposing seeds to dry heat at 50 °C for 30 s. Turner et al. (2009) have reported that PY in the field was broken in summer, when heavy precipitation is followed by soil temperatures >50 °C within 24 h. Baskin et al. (2004) and Cook et al (2008) reported that the PY in seeds of six species of *Dodonaea* could be removed by immersing seeds in hot water.

Seed germination behavior of *Dodonaea viscosa* has been investigated previously (Burrows, 1995; Phartyal et al. 2005; Naser et al., 2013), but there are no previous reports on material from Saudi Arabia. The mature seeds of *D. viscosa* have been characterized as having a water impermeable seed coat (Demel, 1991; Negash, 1993; Phartyal et al., 2005; Naser et al., 2013). There is, however, geographical inconsistency in previous results. Seeds from Hawaii, New Zealand, Mexico, Australia and Brazil have shown physical dormancy (Baskin et al., 2004), whereas those from China, Pakistan (Qadir and Lodhi, 1971) and Botswana (Tietema et al., 1992) were reported to be non-dormant. Details of the anatomy and morphology of fruits, seeds and seedlings of *D. viscosa* are provided by Khan and Ismail (2019).

The genus *Dodonaea* L. comprises about 60–70 species that grow in a wide range of habitats, such as woodland, shrubland and forest communities (West 1984; Reynolds, 1985; Shepherd et al., 2007). *Dodonaea viscosa* is very widely distributed, including in Australia, Africa, Mexico, New Zealand, India, and South America. The center of origin of *D. viscosa* is believed to be Australia. The species is a dioecious or monoecious woody, perennial that grows up to 7 m tall, as a multi-stemmed or single stemmed shrub (Rani et al., 2009). In Saudi Arabia, *Dodonaea viscosa* subspecies *angustifolia* is found widely in high mountains in the south-west of the country, where it is an important component of the flora (Migahid, 1996; Chaudary, 1999). In fact, it is the only species in the genus *Dodonaea* in the Sapindaceae family in flora of Saudi Arabia (Migahid, 1996; Chaudary, 1999; Collenette 1999). *Dodonaea viscosa* has been reported as a medicinal plant used for skin disease in cattle, and in humans as a cure for sore throats (Jansen, 1981). It is also used for lowering fever and rheumatism in folk medicine (Khurram et al., 2009). In addition, different parts of this plant – stem, leaves, seeds, roots, bark – have been used as antibacterial, analgesics and antivirals (Rani et al., 2009).

The aims of the present study were: (1) to determine whether the seed coat of *D. viscosa* from Saudi Arabia has physical dormancy; (2) to test the effects of mechanical scarification (immersion in boiling water and heating in a drying oven at 50 °C) and chemical scarification (concentrated sulfuric acid); (3) to find the optimum temperature for germination after dormancy has been broken by investigating its responses to a wide range of constant temperatures (5–35 °C), in alternating photoperiods (12 h light: 12 h dark) and continuous darkness; and (4) to examine the effects of sodium chloride (NaCl) concentration on seed germination of *D. viscosa*.

2. Materials and methods

2.1. Study area

Al-Bahah region is located in the south of Saudi Arabia, at an elevation above sea level ranging between 130 and 2450 m (Al-Aklabi et al., 2016). The temperature ranges between 10 and 22 °C in winter and 22–32 °C in summer (Aref et al., 2011). The mean monthly rainfall is variable. January, April and December have the highest average monthly rainfalls of 85 mm, 75 mm and 75 mm, respectively, whereas September, June, August and July have the lowest, with 7 mm, 10 mm, 15 mm and 20 mm respectively (Aref et al., 2011).

2.2. Seed collection

Mature seeds of *Dodonaea viscosa* were collected from 20 to 30 plants, randomly selected from a natural population on 7 April 2017. The population sampled was at Jabal Athrab, at an elevation of 2130 m (latitude 19.694967–19.830936; longitude 41.596633–41. 759573). Seeds were air-dried, cleaned and examined immediately.

2.3. Experiment 1: Breaking dormancy

Seeds of *D. viscosa* were divided into four groups as follows (1) controls, without treatment, (2) treated with boiling water for 10 min, (3) treated with dry heat at 50 °C for 1 min, and (4) treated with concentrated sulfuric acid (97%H_2SO_4) for 10 min. All the seeds were then incubated at a constant temperature 25 °C (12 h: light: 12 h: dark) in growth a chamber (LEEC, Nottingham, UK, Model PL33). Five replicate 9-cm Petri dishes with 20 seeds in each were used for each treatment. Seeds were placed on two layers of filter papers (Whatman No.1) moistened with 7 ml of distilled water. Seed germination was counted day-to-day during 30 days. Petri dishes were randomly distributed in the incubators and their positions were changed daily. Germination was defined as the first emergence of the radicle. Final germination percentage was recorded.

2.4. Experiment 2: effects of temperature and light on germination

Seeds of *D. viscosa* were pretreated by soaking in concentrated H_2SO_4 for 10 min and then rinsing three times in distilled water, before being incubated at a range of constant temperatures (5, 10, 15, 20, 25, 30 and 35 °C), in 12 h light: 12 h dark or continuous darkness. Five replicates of 20 seeds were used, as previously described. Seed germination was counted daily for a month. For continuous darkness, the dishes were wrapped in aluminum foil to prevent any exposure to light and seed germination was counted only after 15 days at the end of experiment.

2.5. Experiment 3: effects of salinity on seed germination and recovery

The effects of 0, 100, 200, 300 and 400 mM concentrations of NaCl on seed germination were tested at 25 °C (12 h light: 12 h dark). Five replicate Petri dishes were used for each treatment, with 20 seeds per dish, as previously. Dishes were moistened with 7 ml of the appropriate solution and sealed with Nescofilm, to reduce evaporation, before being placed the incubator. The solutions were replaced at 7-day intervals. The number of seeds germinated was counted daily for 30 days and germinating seeds were removed from the Petri dishes. Seeds remaining ungerminated at the end of experiment were rinsed twice in distilled water and then transferred to dishes moistened with distilled water to assess whether salinity had inhibited germination; then they were incubated further for 15 days at 25 °C and germinations recorded daily. After this, seeds still ungerminated were tested for viability with a 1% aqueous solution of 2,3,5-triphenyl-tetrazolium chloride (TTC) (Mackay, 1972; Moore, 1985). Seeds were soaked in this solution in Petri dishes covered with aluminum foil to exclude light, and were incubated for 24 h at 20/10 °C. Dehydrogenase enzymes within the seed tissues reduce the colorless tetrazolium chloride solution to form insoluble red formazan, so living cells appear

red while dead cells appear colorless. Final germination was calculated as well as the time taken to reach 50% of the germination percentages, across all the replicates (TG_{50}).

2.6. Statistical analysis

Germination percentage were arcsine transformed before statistical analysis, in order to meet expectations of normality and homogeneity of variance, then and subjected to a one-way analysis of variance (ANOVA), with post hoc tests between treatment (Sokal and Rohlf, 1995).

3. Results

3.1. Experiment 1: breaking dormancy

Treatment of the seeds of *D. viscosa* with sulfuric acid, boiling water and dry heat at 50 °C all proved to be effective pretreatments, resulting in the highest germination (91%) at 25 °C, compared with only 30% in untreated seeds (Fig. 1). Significant differences *($P < 0.0001$, $F = 122$)* for germination percentages were found between untreated seeds and the other treatments. However, there were no significant differences ($P > 0.05$) in seed germination percentage observed between seed treatments using H_2SO_4, boiling water and dry heat at 50 °C.

3.2. Experiment 2: effects of temperature and light on germination.

The final germination percentage of *Dodonaea viscosa* seeds showed a broad, poorly defined optimum for constant temperature conditions with 12-hour photoperiods (Fig. 2). Between 5 °C and 25 °C germination was high (80–90%) and did not differ statistically. However, at higher temperatures (30 °C and 35 °C) germination was significantly lower *($P < 0.0001$, $F = 28.56$)* from that at the lower temperatures. In the dark, the germination response to temperature was extremely similar to that in the light (Fig. 3). Germination at and 30 °C and 35 °C was again significantly lower than in the range 5–25 °C *($P < 0.0001$, $F = 11.68$)*.

3.3. Experiment 3: effects of salinity on seed germination and recovery

In the salinity experiment, the highest germination percentage of *D. viscosa* seeds (91%) occurred in the distilled water control,

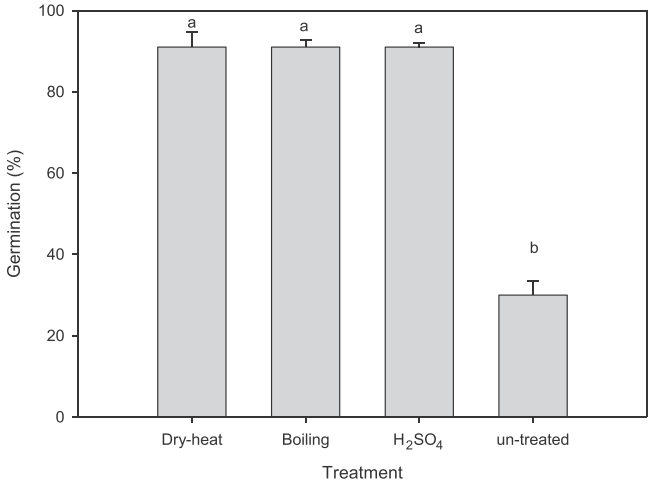

Fig. 1. The effect of sulfuric acid (H_2SO_4), boiling water and dry-heat on the final germination percentage (mean + se) of seeds of *Dodonaea viscosa* when seeds were exposed to 25 °C (12 h light: 12 h dark), (Treatments that do not share a letter are significantly different).

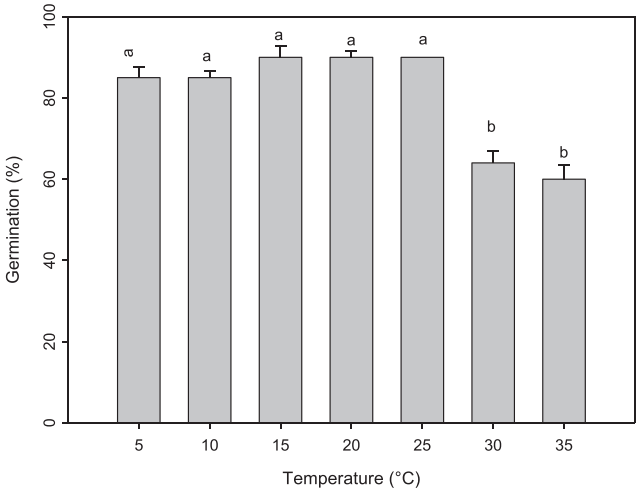

Fig. 2. The final germination percentage (mean + se) of seeds of *Dodonaea viscosa* at five constant temperatures (5, 10, 15, 20, 25, 30 and 35 °C) (12 h light: 12 h dark). (Treatments that do not share a letter are significantly different).

and germination percentage decreased linearly with increasing salinity to 46% at 300 mM (Fig. 4). No seeds germinated at 400 mM NaCl. Overall, mean germination percentage was significantly different between treatments *($P < 0.0001$, $F = 224.32$)* Furthermore, all treatments were significantly different from each other, apart from the comparison of 200 and 300 mM ($P < 0.05$) (Fig. 4). Increasing salinity also delayed the time of which 50% germination was achieved: t_{50} increased from 2 days at 0 mM to 12 days at 300 mM NaCl.

The germination of seeds after being moved to distilled water from 100, 200. 300 and 400 mM NaCl was 18%, 38%, 50%, and 93%, respectively.

4. Discussion

Dormancy is a way of creating a long-lived seed bank, which may be a mechanism for success in annual plants in unreliable habitats. The effects of innate dormancy and impermeable seed coats are significant mechanisms in delaying germination, especially among desert plant species where rainfall is irregular, until the environment is favorable for the development of the seedlings (Toole et al. 1956). Delayed or inhibited germination due to an

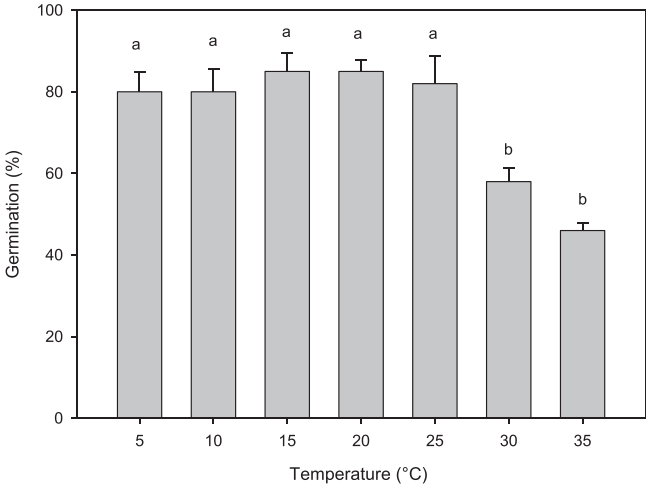

Fig. 3. The final germination percentage (mean + se) of seeds of *Dodonaea viscosa* at five constant temperatures (5, 10, 15, 20, 25, 30 and 35 °C) (continuous darkness) (Treatments that do not share a letter are significantly different).

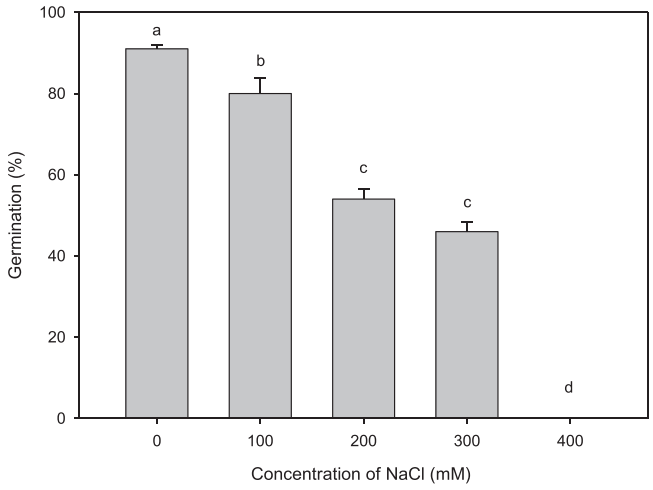

Fig. 4. The effects of salinity (NaCl) on germination percentage (mean + se) of seeds of *Dodonaea viscosa* when seeds were exposed to 25 °C, (12 h light: 12 h dark), (Treatments that do not share a letter are significantly different).

impermeable seed coat is a common phenomenon among desert plants (Koller et al., 1962; Koller 1969; Mahmoud, 1977, 1985; Mahmoud et al., 1981). It has been reported by Hudson et al. (2015) that seeds of 25% of the plants have a physical dormancy due to a hard water-impermeable testa.

The present study demonstrated clearly that the seeds of *D. viscosa* from Saudi Arabia have physical dormancy (i.e. water-impermeable seed coat) which was broken readily by three different methods: sulfuric acid, boiling water and dry heat at 50 °C. The final germination percentage of untreated seeds was much lower than that of the seeds that had been scarified in any of the three ways. These results are in good agreement with the results of most other researchers (Hussain et al., 1991; Bhagat and Singh, 1994; Baskin et al., 2000; Baskin et al., 2004; Phartyal et al., 2005; Cook et al., 2008, Naser et al., 2013), who reported that seeds of various species of *Dodonaea* can germinate rapidly under optimal conditions once physical dormancy has been removed, reaching percentages >80% over a range of temperatures. Only the germination percentage of *D. petiolaris* has been reported as being much lower, at 36%, and neither did treatment of its seeds with warm or cold stratification, or 6 months of after-ripening at 30 °C, improve germination (Turner et al. 2009).

In the case of *Dodonaea viscosa*, physical dormancy has been reported previously as present or absent in populations from different parts of the world. Our results agree with those for seeds collected from Australia, Brazil, Hawaii, Mexico and New Zealand (Baskin et al., 2004) and north-west India (Phartyal et al., 2005). However, our data for Saudi Arabia contrast with the reports that physical dormancy was absent, or nearly absent, in seeds collected from China, Pakistan (Baskin et al., 2004), Botswana (Tietema et al., 1992) and Islamabad in Pakistan (Qadir and Lodhi 1971). Its absence in that latter collections is possibly related to them having less than fully mature embryos, as suggested by Baskin et al. (2004). Where dormancy exists, it can be broken by methods similar to ours. Bhagat and Singh (1994) used sulfuric acid on seeds of *D. viscosa* collected from north-west India to obtain a final germination of 90%. Similarly, Phartyal et al. (2005) reported that manual scarification of seeds from north-west India increased germination from 24% to 84%, and boiling water increased it to 77%. Furthermore, Nasr *et al.* (2013) reported that untreated seeds of *Dodonaea viscosa* can germinate up to 40%, while the seeds treated with sulfuric acid for 45 min or boiling water can germinate up to 90.8% and 50%, respectively. In their natural habitats, species exhibiting physical dormancy will have their dormancy weakened with time by daily temperature fluctuations in the soil (Baskin et al., 2000) and also by exposure to high temperature during wildfires (Hodgkinson and Oxley, 1990; Hodgkinson, 1991). Also, some researchers (*e.g.* Gogue and Emino, 1979; Morpeth and Hall, 2000) have suggested that the impermeable seed coat of some species can be weakened by fungal activity in the soil.

This present study showed that seeds of *Dodonaea viscosa* can germinate readily over a remarkably wide range of constant temperature, in light and darkness, once physical dormancy has been removed. The indifference of its germination to light is typical of plants with relatively large seeds (Fenner and Thompson, 2005). Final germination percentage is clearly inhibited at higher temperatures (30 and 35 °C). This response could be attributed to adaptation to the environmental conditions in the habitat of this species in Saudi Arabia – cool, montane areas with predominantly winter rainfall. A similar result was reported by Al-Farraj et al., (1988), who found that the seeds of *Verbesina enceliodes* (Cav) Benth. (Asteraceae) which had been collected about 35 km to the west of Riyadh, did not tolerate temperatures in excess of 14/28 °C. Also, Al-Turki (1992) showed that seeds of *Suaeda aegyptiaca* from Al-Awashzia village (Al-Qassim region, 350 North-West of Riyadh city), attained high germination percentages (70–96%) between 15/5 and 30/25 °C temperature regimes, though only 44% at 35/25 °C. Similarly, the germination of *Suaeda monoica* was apparently inhibited by highest temperature it was tested at (35/25 °C) (Al-Turki, 1992). Recently, Hadi et al. (2018) showed that the optimum temperatures for seed germination of *Salvadora persica* were in the range 10/20–15/25 °C and its germination behavior in this respect is very similar to that of *Dodonaea viscosa*. The germination responses to temperature observed thus probably represent genetic adaptation to the habitat of this species. Also, it can be suggested from these results that germination of this species in Saudi Arabia would be more likely to occur in winter. However, seedlings have not been seen in the field in their natural habitats. Local adaptation species to the very different environmental conditions that prevail in Saudi Arabia has been considered previously by Abulfatih and Bazzaz (1985) and Al-Turki (1992). Overall, our findings agree with previous studies (*e.g.* Baskin et al., 2004) that seeds of *Dodonaea viscosa* can germinate over a wide range of constant and alternating temperatures, and under various light regimes.

The germination response of *D. viscosa* indicated clearly that it is sensitive to salinity, since the final germination percentage dropped dramatically with increasing NaCl concentration, and it was completely unable to germinate at 400 mM NaCl. Many previous workers (*e.g.* Song et al., 2005; Hadi et al., 2018) have suggested that inhibition of seed germination under saline conditions may occur due to either ionic or osmotic effects of salinity. Our study showed seeds of this species exposed to 400 mM NaCl remained viable (93%), illustrating that germination was prevented by the osmotic effect of salinity. Similar results have been reported in some desert plants. For example, seeds of *Salvadora persica* failed to germinate at 400 mM NaCl (Hadi et al., 2018), and most of ungerminated seeds of this species germinated readily when transferred to distilled water from 400 mM NaCl.

5. Conclusion

We conclude from this investigation that physical dormancy is present in the seeds of *Dodonaea viscosa* from Saudi Arabia, and that this dormancy can be broken equally by sulfuric acid (H_2SO_4), boiling water or dry heat 50 °C treatments, all of which would disrupt the impermeability of the testa. Seeds from Saudi Arabia can germinate readily with an optimum temperature ranging 5–25 °C. The germination was clearly inhibited by higher temperatures. The seeds of this species are not salt tolerant but some germination can occur up to 300 mM NaCl.

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