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Germination of *Cassia italica* from Saudi Arabia

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ABSTRACT *Cassia italica*, an evergreen under-shrub, is widely distributed in Saudi Arabia, and is used extensively as a medicinal plant. It is associated with depressions and water runnels. Germination was found to be dependent on scarification of the seed-coat, a process which could be achieved in the laboratory by acid treatment. The seeds germinated rapidly to high percentages over a wide range of alternating temperatures. Germination was also tested with various concentrations of seawater, under two fluctuating temperature regimes. The seeds germinated in salinities higher than those encountered by and tolerated by adult plants in their natural habitat.

Cassia italica (Mill.) Lam. ex Steud. (= *C. obovata* Collad.) is an evergreen under-shrub of the family Leguminosae. It is not grazed, being purgative; and, because of this, it ranks high as a medicinal plant. The species is widely distributed in Saudi Arabia, and is almost present in almost all of the phytogeographical regions of Saudi Arabia recognized by Migahid (1978). It is associated with areas in which runoff water collects.

The geographical distribution of any plant species depends on its adapted responses to temperature, day length and rainfall. Thompson (1970) showed that the distribution of different species of the family Caryophyllaceae in Europe was reflected in their germination temperature responses. The mechanisms which synchronize the event of germination with the season of optimal environmental conditions for subsequent growth and establishment of the seedlings are of great importance among the adaptations of plants to the desert (Koller 1969, Mahmoud 1977; Mahmoud *et al.* 1983a, 1984). Of the mechanisms responsible for the coordi-

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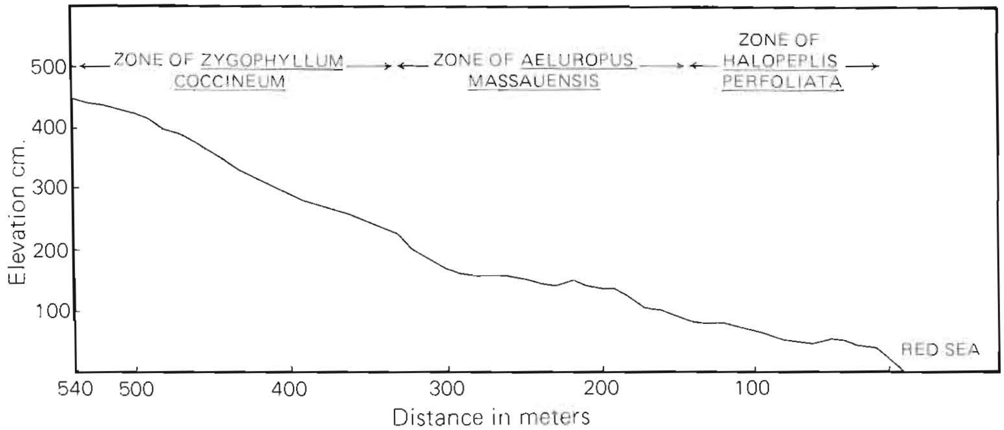

Fig. 1. A transect across the salt marsh at Rabigh showing the different zones of vegetation in relation to ground level (Mahmoud *et al.* 1982).

nation of germination with physical parameters of the environment, two of the most important relate to temperature responses and the proportion of the seeds with dormancy restrictions (Thompson 1973). The work described below reports the germination temperature responses of the seeds of *C. italica*, and their germination regulatory mechanism.

Saline habitats are very common in Saudi Arabia. At Rabigh salt marsh on the Red Sea coast, Saudi Arabia, *C. italica* is associated with the *Zygophyllum coccineum* community type. This represents the landward edge of the march (Fig. 1). The salinity is lower here than in the zones of *Aeluropus massauensis* and *Halopeplis perfoliata* (Mahmoud *et al.* 1982). The inhibition of germination by high salinities constitutes a major factor in the zonation of plant growth (Toole *et al.* 1956, Kassas and Zahran 1967). Seed germination was therefore investigated in various concentrations of seawater at two fluctuating temperature regimes.

Experiment 1

Methods

Fully developed undamaged seeds were liberated from freshly collected pods. There was no discrimination between large and small seeds. The seeds of *C. italica* have impermeable seed-coats. In order to increase the permeability of the testa, batches of 100 seeds each were soaked separately in concentrated sulphuric acid for 5, 15, 30 and 60 min. The seeds were stirred vigorously every two minutes with a glass rod to prevent the deposition of carbon on their surfaces which might have interfered with the action of the acid. After the appropriate time in the acid, seeds were washed thoroughly with tap water and then soaked in distilled water for 10 min.

Table 1. Final germination percentages attained by the seeds of *C. italica* subjected to different acid treatments prior to incubation at 36/24°C and also the length of incubation (days) for 50% of the maximum germination to be reached. Ninety-five percent confidence limits are included.

Treatment		Percentage germination	Time for 50% germination (days)
Concentrated H_2SO_4 for	5 minutes	95 ± 6.09	0.35 ± 0.09
	15 minutes	98 ± 3.67	0.33 ± 0.08
	30 minutes	96 ± 5.19	0.33 ± 0.08
	60 minutes	89 ± 6.09	0.45 ± 0.09
Distilled water for	60 minutes	No germination	

One hundred seeds, soaked in distilled water for one hour prior to incubation, provided the control treatment. The seeds were placed in germination flasks in a dark incubator maintained at 36/24°C. Four replicates of 25 seeds were used. A seed was considered to have germinated when the radicle emerged. Germinated seeds were discarded immediately and counts were made daily until no more germination over seven successive days. Counting involved the removal of the lid of the flask which allowed a change of air and brief exposure to light.

Results

In the control treatment none of the seeds germinated during two weeks of incubation; in fact, they did not imbibe water. The acid treated seeds germinated rapidly to high percentages (Table 1).

Experiment 2

Methods

Seeds, which had been scarified by soaking them in concentrated sulphuric acid for 30 min, were germinated over a range of alternating temperature regimes: 18/8, 21/10, 23/13, 26/15, 29/18, 33/22, 36/24 and 40/26°C, selected in accordance with meteorological data from two stations within the habitat range of the species, Al-Seyl Al Kabier in South Hijaz and Khurais, 150 km from Riyadh in Najd.

Results

The seeds of *C. italica* germinated rapidly to high percentages (> 90%) over the whole temperature range, though at 18/8°C germination was comparatively slower than in the other temperature regimes (Table 2).

Table 2. Final germination percentages attained by the seeds of *C. italica* at different fluctuating temperatures; the time (days) to reach 50% of the maximum germination percentages as well as the 95% confidence limits is also included.

Temperature (C°)	% germination	Time for 50% germination
18/8	97 ± 6.09	0.98 ± 0.15
21/10	99 ± 3.18	0.33 ± 0.08
23/13	97 ± 6.09	0.33 ± 0.08
26/15	96 ± 5.20	0.38 ± 0.08
29/18	97 ± 6.09	0.33 ± 0.08
33/22	96 ± 5.20	0.35 ± 0.09
36/24	96 ± 5.20	0.33 ± 0.08
40/26	97 ± 6.09	0.33 ± 0.08

Experiment 3

Methods

Seeds were germinated over the following range of salt concentrations obtained by diluting Red Sea water: 2305.5 ppm (5 percent), 4611 (10 percent), 9222.5 (20 percent), 18445 (40 percent), 36 890 (80 percent) and 46112 (100 percent). Germination took place in germination flasks kept in dark incubators maintained at 21/10°C, and 40/26°C. The selection of these temperature regimes was based on the germination temperature responses of the seeds in distilled water (Experiment 2). The two temperature regimes represent upper and lower limits of the optimal range. After treatment with concentrated sulphuric acid, the seeds of *C. italica* were washed in the appropriate saline solution (instead of tap water) for subsequent germination studies.

Results

High percentages (> 90%) of the seeds germinated rapidly at both temperature regimes in 5, 10 and 20 percent seawater. In 40% seawater germination percentages attained at 21/10°C and 40/26°C were somewhat lower. However, in these four concentrations, the rate of germination was slower at 21/10°C than at 40/26°C. At both temperature regimes no seed germinated in the 80% and 100% seawater concentrations (Table 3).

Table 3. The final germination percentages attained by the seeds of *C. italica* at two fluctuating temperature regimes in different salinities; the length of incubation (days) to reach 50% of the maximum germination percentage as well as the 95% confidence limits is also included.

Treatment	21/10 °C		40/26 °C	
	% germination	Time for 50% germination	% germination	Time for 50% germination
Distilled water	99 ± 3.18	0.33 ± 0.08	97 ± 6.09	0.33 ± 0.08
2305.5 ppm (5% seawater)	98 ± 3.67	0.55 ± 0.16	99 ± 3.18	0.30 ± 0.00
4611 ppm (10%)	98 ± 3.67	0.85 ± 0.09	99 ± 3.18	0.30 ± 0.00
9222.5 ppm (20%)	97 ± 3.18	1.23 ± 0.24	98 ± 3.67	0.30 ± 0.00
18445 ppm (40%)	77 ± 8.01	2.40 ± 0.33	81 ± 3.18	1.50 ± 0.35
36890 ppm (80%)	No germination	–	No germination	–
46112 ppm (100%)	No germination	–	No germination	–

Experiment 4

Methods

The seeds of *C. italica* which did not germinate in high salinities (80 and 100 percent seawater) at 21/10°C and 40/26°C for 15 days were thoroughly washed with distilled water and then set to germinate at the same temperatures in distilled water. The procedure then adopted was similar to that in Experiment 1.

Results

There was no germination at all of seeds previously in 80 and 100 percent seawater at both temperature regimes.

Discussion

In deserts, where rainfall is low and unpredictable, it is important that seeds should not germinate unless the soil contains enough water to enable the resulting seedlings to complete their life cycles. Germination controlled by a regulated response to the environment would clearly enhance biological success. One mechanism that regulates germination and contributes to the survival of desert plants is the presence, in their seeds, of water-soluble, water-leachable germination inhibitors. These need minimal levels of precipitation before they are leached out (El-Naggar 1965, Koller 1969, 1972). Another regulatory mechanism involves the germination temperature responses which synchronize germination with the season of optimal environmental conditions (Mahmoud *et al.* 1983a,b).

The mechanism in *C. italica* is obviously different from these. Inhibition is here attributed to the impermeability of its seed-coat. This may be overcome experimentally by scarification with sulphuric acid (Experiment 1, Table 1). Untreated seeds soaked for one hour in distilled water prior to incubation did not show signs of imbibing water for up to 15 days, while the treated seeds germinated without delay. Delayed germination, as resulting from an impermeable seed-coat, is very common among desert plants (Koller 1969, Mahmoud 1977, Mahmoud and El-Sheikh 1978, Mahmoud *et al.* 1984). It is an adaptation to irregular rainfall because the break-down of the seed-coat in the natural habitat may take several years. Consequently, seeds from a single generation become ready to germinate at widely separated intervals and it is likely that a few will germinate and establish successfully. This ensures that some seeds remain dormant for long periods and provide a stock for subsequent occasions when seedling establishment may occur which increases the changes of survival of the species (Koller 1969).

Since the seed-coat becomes permeable to water, as a result of prolonged wetting, and most probably by the increased activity of soil microorganisms during the rainy season, the thick seed-coats of *C. italica* restrict germination to the time when abundant water is available. It also restricts the distribution of the species to habitats which normally receive abundant water. Mahmoud (1977) showed that the impermeability of the seeds of three species of *Acacia* (*A. nilotica* > *A. raddiana* > *A. tortilis*) is related to the water demand of the three species and the amount of water likely to be available within their habitats. The same is true of other *Acacia* spp. (Mahmoud *et al.* 1984). *C. italica* is associated with water runnels ranging from narrow channels to *wadis* and depressions. In the Sudan, the species also inhabits similar habitats, in addition to drainage ditches and river banks (Obeid and Mahmoud 1971) which are inundated for 1-2 months annually. In the water runnels in the desert, seeds are scarified by running water in mixtures of sand, gravel, rock and water.

The acid-treated seeds of *C. italica* germinated rapidly to high percentages (> 90%) over the range of alternating temperatures tested (Table 2). These simulate temperature cycles that prevail both during the rainy and the dry season within the regions where the species occurs (Fig. 2). This indicates that with adequate moisture the seeds can germinate over a wide temperature range. *C. senna*, which is always associated with *C. italica*, when tested over a similar temperature range (Mahmoud 1984) showed similar temperature responses. Species with common distribution tend to possess germination responses that are broadly similar (Thompson 1973).

The wide amplitude of temperatures at which seeds of *C. italica* can germinate (Table 2), the synchronization of germination with the optimal conditions for subsequent seedling establishment, the massive production of seeds throughout the year, and the unpalatability of the plant (vegetative and reproductive parts), all contribute to the success and wide distribution of *C. italica* in Saudi Arabia.

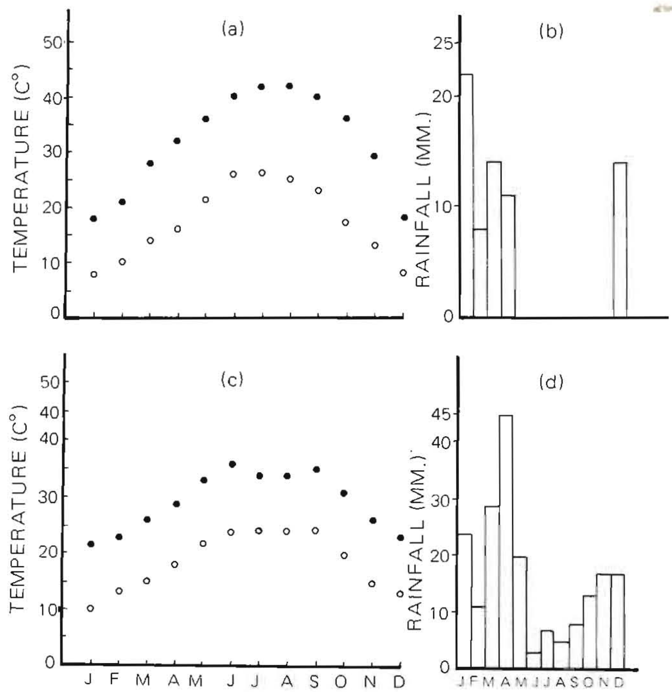

Fig. 2. Meteorological data for Khurais station (a) and (b) 150 Km from Riyadh and Al-Seyl Al-Kabier (c) and (d) in South Hijaz: (a) and (c) represent the mean daily temperature maxima and minima; (b) and (d) represent monthly rainfall (Data are averages of the records 1969-79).

When moisture and temperature are favourable, the seeds can germinate in salinities higher than those encountered by or tolerated by adult plants in their natural habitat (Tables 3 and 4). Successfull germination may thus occur with the advent of winter rains even without earlier leaching of the soil. Seeds of *C. italica* have a higher salt tolerance than those of *C. senna* (Mahmoud 1984).

The absence of *C. italica* from the coastal salt marsh at Rabigh on the Red Sea coast (Zones of *Aeluropus massauensis* and *Halopeplis perfoliata*) (Fig. 1, Mahmoud *et al.* 1982) might be explained by the failure of its seeds to germinate on account of excessive salinities in the surface of the soil (Tables 3 and 5). Leaching of salts during the brief rainy season is insufficient to reduce the salt content to levels that are tolerated by the seeds. *C. italica* is associated with the *Zygophyllum coccineum* community type at Rabigh salt marsh. This represents the landward edge of the march (Fig. 1). The salinity is lower here than in the rest of the marsh (Tables 4 and 5).

Table 4. Total water soluble salts in soil samples collected from the habitat of *C. italica* within Makkah Jiddah region.

Habitat	Profile	Depth (cm)	Total water soluble Salts (ppm)
Wadi Fatima	1	0-5	3,200
Makkah Jiddah road		5-25	1,500
Water runnel between Makkah and Jiddah	2	0-5 5-25	1,400 1,024
Depression along Jiddah–Jamoum Road	3	0-5 5-25	2,400 1,312
Depression along Jamoum–Makkah road	4	0-5 5-25	1,600 1,120
In the habitat of *Zygophyllum coccineum* community type – Landward edge of Rabigh salt marsh (Mahmoud *et al.* 1982.)	5	0-5 5-25	201 102
Same as (5)	6	0-5 5-25	243 115

Table 5. Total water soluble salts in soil samples (0-5 cm depth) collected within the habitats of *Halopeplis perfoliata* and *Aeluropus massauensis* at Rabigh salt marsh (Mahmoud *et al.* 1982).

Species	Habitat	Soil Sample No.	Total water soluble salts ppm
Halopeplis perfoliata	Shore line	1 2 3	112,000 128,000 134,000
	Shallow creek	4	102,400 89,000
Aeluropus massauensis	Mud-flat	1 2 3 4 5	22,400 35,200 24,000 52,160 28,800

A salty habitat can inhibit germination in two ways: (a) by poisoning the embryo due to toxic effects of certain ions (Uhvits 1946) or (b) by preventing uptake of water due to high osmotic potential of the medium (Ayers and Hayward 1948, Ayers 1952, Ungar 1962, Boorman 1968, Macke and Ungar 1971, Mahmoud *et al.* 1983c). Evidence from Experiment 3 indicates that inhibition is due to toxic ionic effects. However, irreversible damage may also be caused in seeds even by pure osmotic stress of the medium. 'This is supported by the fact that transfer of the caryopses of *Aeluropus litoralis* into distilled water, which did not germinate in a mannitol solution, raised germination percentage only slightly, if at all' (Waisel 1972 p. 395).

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إنباب بذور نبات *Cassia italica* فى المملكة العربية السعودية

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الرياض ص. ب ٢٤٥٨ المملكة العربية السعودية

نبات *Cassia italica* من النباتات العشبية المعمرة واسعة الانتشار في المملكة العربية السعودية والتى ترتبط بأماكن تجمُّع المياه (في المنخفضات والمجارى المائية بأنواعها المختلفة). يُستعمل النبات فى العلاج.

يعتمد إنبات البذور على خدش القصرة حتى تصبح منفذة للماء، الشيء الذى يمكن تحقيقيه فى المختبر بمعالجة البذور بحمض الكبريتيك المركز. تم إنبات البذور إلى نسبة عالية وبسرعة فى مدى واسع من درجات الحرارة المتبادلة.

تمت دراسة تأثير تركيزات مختلفة من ماء البحر على الإنبات تحت نظامين حراريين. تحملت البذور تركيزات عالية من الملوحة تفوق كثيرا ما تتحمله وتواجهه النباتات البالغة فى بيئاتها الطبيعية.

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