Pak.J.Bot., 32(1): 211-226, 2000.

FECUNDITY, SEED CHARACTERISTICS AND FACTORS REGULATING GERMINATION OF *RHYNCHOSIA MINIMA* (L.) D.C.

S. SHAHID SHAUKAT AND NAZIA BURHAN

Department of Botany, University of Karachi, Karachi-75270, Pakistan.

Abstract

Fecundity of three *Rhynchosia minima* populations was compared. The old-field population exhibited highest fecundity as well as average reproductive output weight, followed by the population existing in a vacant lot while the population in a natural undisturbed shrub-dominated community showed lowest fecundity and average output weight.

Variation in seed weight within and between populations was demonstrated. The implications of seed size variation are discussed. Seeds have water impermeable seed coats and thus they have physical dormancy. Physical scarification with sand paper was more effective in breaking seed dormancy than chemical scarification with hydrochloric acid. Dry heat treatment (50° and 70°C) markedly increased the germination percentage. Under field conditions dry heat during summer apparently breaks seed dormancy thereby allowing the seeds to germinate in considerable numbers after rain showers.

Greater germination percentage was observed in light compared to dark. Final germination percentage varied with temperature (25°- 40°C) with the optimum at 35°. Significant inhibition of germination occurred at 6, 7, and 8 bar NaCl salinity, while in polyethylene glycol (PEG 4000) germination was reduced at 7 and 8 bar. At iso-osmotic concentrations PEG 4000 caused lesser inhibitory effect compared to NaCl salinity.

Introduction

The potential of a species to colonize, establish and perpetuate at a site depends upon its fecundity, seed and germination characteristics. *Rhynchosia minima* (L.) DC., is a twining annual widespread in the tropics. In Karachi, Pakistan and its suburbs, populations of *Rhynchosia minima* prevail in relatively undisturbed desert shrub communities as well as in disturbed habitats such as vacant lots and abandoned fields. It is known to exhibit considerable phenotypic plasticity (Quraish & Farooqui, 1970) and possesses the ability to colonize disturbed areas as well as gaps in natural communities.

Within a plant population, reproductive output is highly correlated with plant size structure (Samson & Werk, 1986), and size variation is considered to be a reflection of variation in Darwinian fitness (Weiner, 1988). In annuals, plasticity coupled with a high reproductive output allows a population to persist, even if there is much herbivory (Crawley & Brown, 1988) and seed predation (Crawley, 1983; Islam & Crawley, 1983; Thomson, 1985).

The germination pattern of a species in nature is determined by an interaction of intrinsic properties of the seeds and a multitude of external factors. Seed size, dispersal and dormancy are regarded as ecologically important life history traits (Harper *et al.*, 1970; Baker, 1972; Gross & Kromer, 1986; Venable & Brown, 1988; Stamp, 1990).

Seed size variation may have several important ecological implications including germination (Weis, 1982; Zhang & Maun, 1990; Stamp, 1990), seedling establishment (Schaal, 1980; Gross & Werner, 1982; Winn, 1985; Zhang & Maun, 1990), competitive ability (Harper *et al.*, 1970; Gross & Werner, 1982; Gross, 1984) and the reproductive ability of adult plants (Stanton, 1984; Wulff, 1986a,b).

Seeds of many taxa of Leguminosae exhibit physical dormancy due to the presence of a water impermeable seed coat (Barton, 1965; Rolston, 1978; Tran & Cavanagh, 1984) which can be broken by various treatments (Martin *et al.*, 1975; Mayer & Poljakoff-Mayber, 1989). In addition to mechanical and chemical scarification (Clemens *et al.*, 1977; Cavanagh, 1980; Khan *et al.*, 1984) dry or moist heat can break physical dormancy of legume seeds (Martin *et al.*, 1975; Cavanagh, 1987).

The objectives of this study were to: (1) estimate the fecundity of *R. minima* in different habitats, (2) examine seed size (weight) variation, (3) determine the effectiveness of various breaking-dormancy treatments, and (4) ascertain the influence of certain environmental variables, including light, temperature, salinity and water stress on germination of non dormant seeds.

Materials and Methods

Fecundity: Three sites were selected to study reproductive output: (1) a natural community dominated by shrubs such as *Euphorbia caducifolia, Acacia senegal, Grewia makranensis* and *Prosopis juliflora*, (2) an old-field where *Rhynchosia minima* was dominant along with *Digera muricata* and *Corchorus* spp., as codominants, and (3) a vacant lot (waste ground) where the community was dominated by undershrubs like *Sida pakistanica, Trichodesma amplexicaule* and *Heliotropium subulatum* with *R. minima* being the most abundant annual. Site 1 is located near the Super Highway while sites 2 and 3 are located on the Karachi University Campus. At each site number of fruits (pods) in fifteen randomly-chosen plants was counted in October 1994. Frequency of one- and two-seeded pods was determined for 100 randomly-picked fruits at each site. Fruits were air-dried, and 200 seeds from each site were individually weighed. The frequency distribution of seed weight of each population was constructed and departure of the distribution from normality was tested (Zar, 1994). Average output weight was calculated as 'average seed output x average seed weight' (Salisbury, 1942).

Dormancy: Three month old seeds collected from Karachi University Campus were used in all the experiments. To break physical dormancy of the seeds, the following treatments were applied.

1. **Physical and chemical scarification**: The seeds were scarified by (i) immersing them in 1N HCl for 1, 2 and 5 minutes, or by (ii) mechanically rubbing them on sand paper No. 1.5.

2. **Hot water treatment**: Seeds were immersed in hot water (80°C) for 2 and 5 minutes.

3. **Dry heat treatment**: Seed contained in a wire basket were exposed to temperatures of 50° and 70°C for 4 minutes in an oven.

After each treatment, seeds were tested for germination in light. Seeds were germinated in sterilized glass Petri plates. Twenty seeds were placed on two discs of Whatman No. 1 filter paper moistened with distilled water. Treatments and controls were replicated four times. Germination was carried out in a growth chamber maintained at 35±1°C. Light was provided by fluorescent tubes (36 μmol m^{-2} s^{-1}). Seeds were inspected at 2-day interval and germination counts made upto 18 days.

Water uptake: To determine the degree of net seed hydration, 20 unscarified seeds (control) and mechanically scarified seeds were placed on two layers of moist Whatman No. 1 filter paper in sterile Petri plates. The seeds were weighed hourly for 12h and then at 2h- intervals upto 24h, and the percentage increase in weight determined.

Effect of environmental factors:

1. **Light and dark**: In addition to incubating scarified (with sand paper) and non-scarified seeds in light (continuous light from fluorescent tubes 36 μmol m^{-2} s^{-1}) scarified and non-scarified seeds also were incubated in darkness in a growth chamber maintained at 35±1°C for 18 days.

2. **Temperature**: Physically scarified seeds were germinated at temperatures of 25, 30, 35 and 40°C in light. Germination conditions and the rest of the procedure were the same as outlined above.

3. **Salinity**: Mechanically scarified seeds were germinated in a series of concentrations (1 to 8 bar) of NaCl, prepared in accordance with Meiri *et al.*, (1971). Germination conditions were the same as described above.

4. Moisture stress: Moisture stress was developed using polyethylene glycol (PEG) 4000 (mol. wt.). Mechanically scarified seeds were germinated in a series of PEG 4000 concentrations to provide osmotic potentials of 1 to 8 bars. PEG solutions of different osmotic potential were prepared in accordance with Lawlor (1970). Germination conditions were the same as described above.

Results

Fecundity: Number of fruits (pods)/plant was highest in the old-field population and lowest in the natural shrub community (Table 1). Fecundity and average seed output weight were also highest in the old-field habitat and lowest in the natural shrub community. The three populations differed significantly (p at the most 0.05) in fecundity, as well as in average seed output weight per plant.

Table 1. Seed characteristics and fecundity of three different populations of *Rhynchosia minima*.

Reproductive characteristics	Populations		
	Old-field	Vacant lot	Natural shrub community
Number of fruits/plants	149.2±12.81	125.7±15.14	46.2±8.99
Percentage of 2-seeded fruits	81.4	74.7	76.2
Percentage of 1-seeded fruits	18.6	25.3	23.8
Average seed output	270.65	219.59	81.40
Average seed weight (mg)	15.75±0.213	15.89±0.235	16.30±0.210
Average seed output weight/plant (mg)	4262.73	3489.3	1326.82

Seed weight variation: Mean seed weight was lowest in old-field population and highest in the natural shrub community (Fig. 1 & Table 2). Population from old-field and natural community showed significant difference in mean seed weight ($p<0.05$, Mann-Whitney U-test). Coefficient of variation was low for all three populations. Old-field and natural community populations exhibited significant positive skewness of seed weight distribution.

Table 2. Seed weight statistics of three populations of Rhynchosia minima.

Reproductive characteristics	Populations		
	Old-field	Vacant lot	Natural shrub community
Mean seed weight (mg)	15.75±0.213	15.89±0.235	16.30±0.210
Coefficient of variation (%)	19.14	20.92	18.30
Coefficient of skewness (g1)	0.279±0.171	0.169±0.171	0.363±0.171
Coefficient of kurtosis (g2)	2.502±0.342	2.434±0.342	-0.108±0.342
t for skewness	1.624*	0.987	2.114*
t for kurtosis	-1.4007	-1.651	-0.317

*$p<0.05$

Dormancy:

1. **Scarification:** Chemical scarification with 1N HCl for 2 and 5 minutes significantly increased ($p<0.01$) the germination percentage over the untreated seeds. Physical scarification (Ps) with sand paper gave higher germination percentage than that obtained following chemical scarification (Fig. 2).

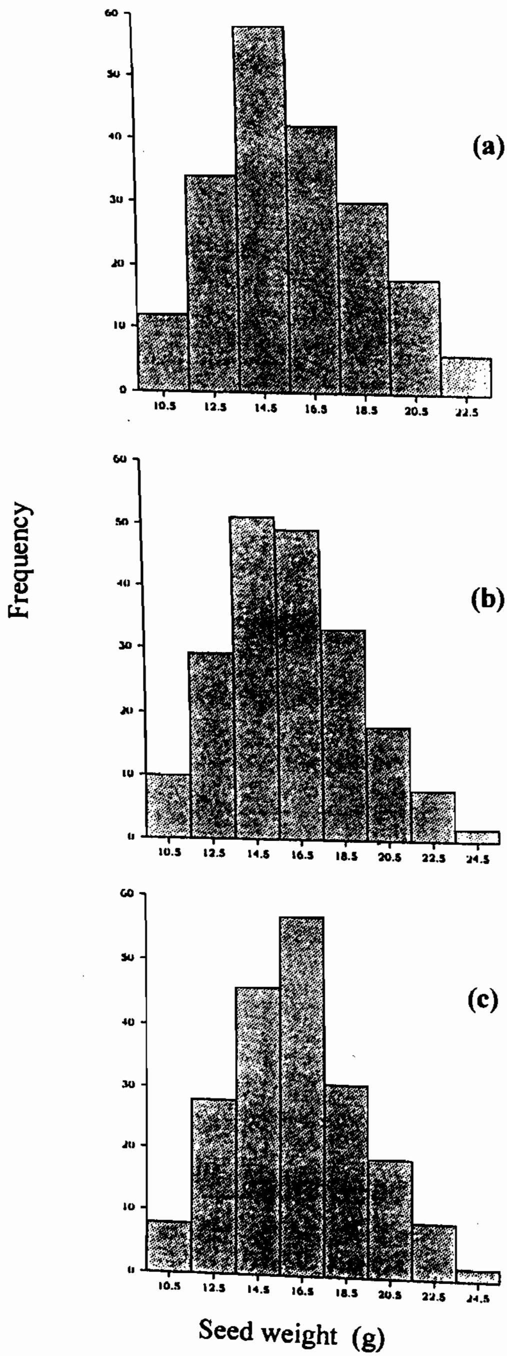

Fig. 1. Distribution of seed weight in the three populations of *Rhynchosia minima*.
(a) old-field, (b) vacant lot, and (c) natural shrub-dominated community.

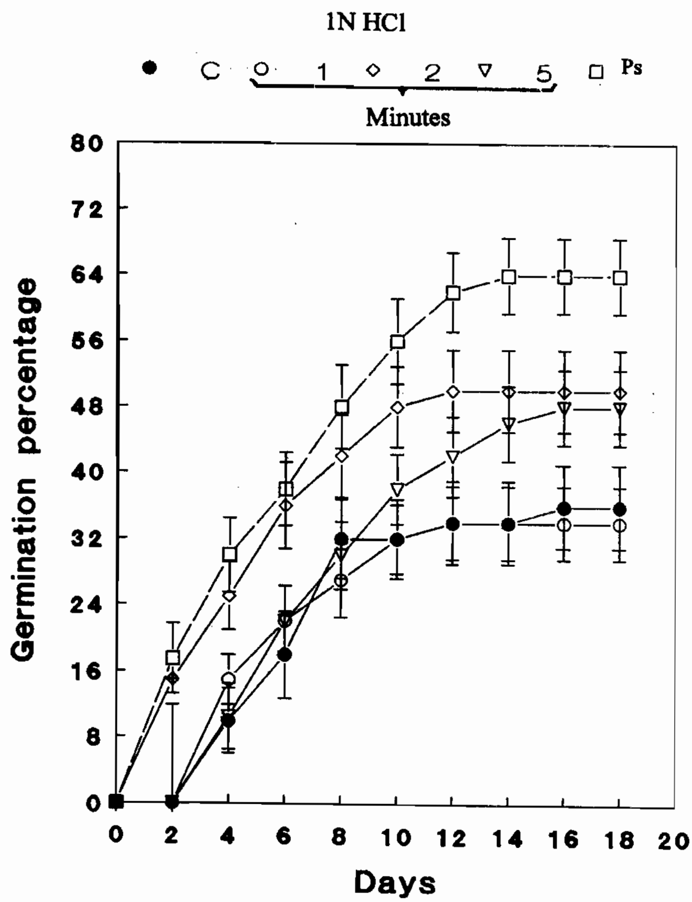

Fig. 2. Effect of physical and chemical scarification on germination of *Rhyncosia minima*. C, control; 1, 2, 5 min. 1N HCl treatment; Ps, physical scarification.

2. **Hot water treatment:** Treatment with hot water (80°C) for 2 min. ($p < 0.05$) and 5 min. ($p < 0.001$) significantly elevated germination percentages over the controls (Fig. 3).

3. **Dry heat treatment:** Germination was increased significantly ($p < 0.001$) when seeds were subjected to dry heat (50° and 70°C) treatments compared with the untreated seeds (Fig. 4). However, no significant difference was observed in the germination percentage of seeds treated at 50° and 70°C (Fig. 4).

Water uptake: At a temperature of 30°C, the rate of imbibition of scarified seeds was greater than that of unscarified seeds (Fig. 5). Comparison of the imbibition curves shows that the rate of imbibition was highest during the first 4h with imbibition being essentially completed by 10h.

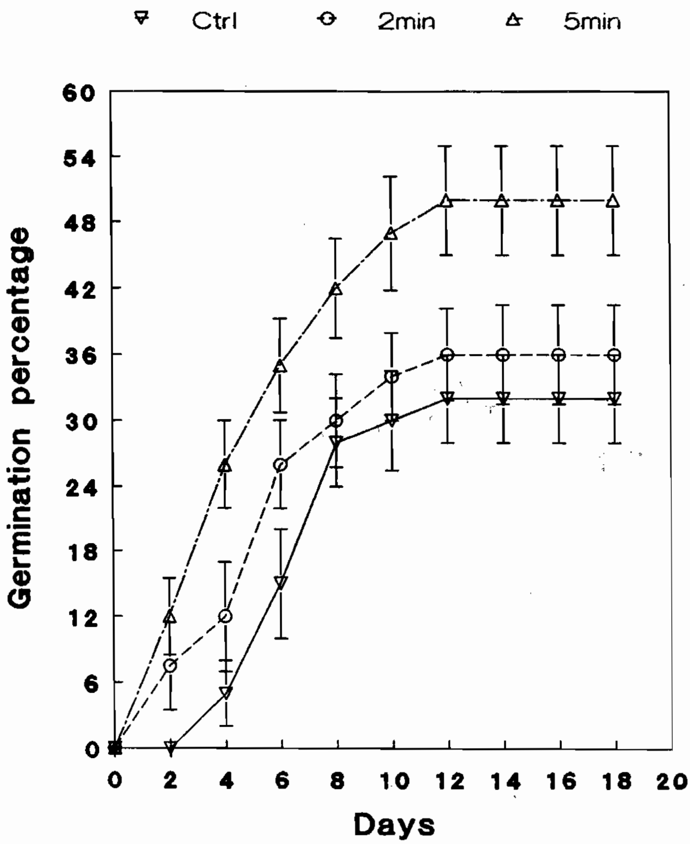

Fig. 3. Effect of hot water treatment on germination of *Rhyncosia minima*. Ctrl., control; 2 min., 5 min. boiling water treatment.

Effect of environmental factors:

1. **Light and dark:** Percentage of germination was significantly ($p < 0.01$) greater in light than in darkness. Scarification with sand paper improved germination both in light and in dark, but significantly greater germination occurred in light ($p < 0.001$) (Fig. 6).

2. Temperature: Both rate and final percentage of seed germination were markedly influenced by temperature regimes (Fig. 7). At 25°C and 30°C germination was delayed and the final germination percentage was low. Highest final germination percentage was obtained at 35°C.

Effect of salinity: At 1 bar salinity slight stimulation of germination was observed while at concentrations of 6, 7 and 8 bar NaCl salinity significant inhibition of germination was found (Fig. 8). Inhibitory effect increased with an increase in concentration from 6 onwards.

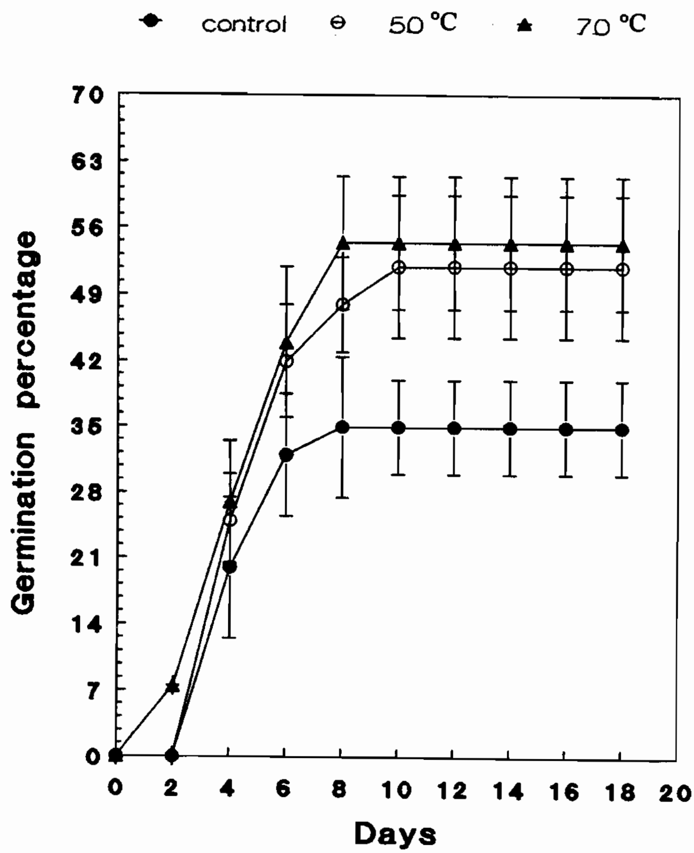

Fig. 4. Effect of dry heat treatment on germination of *Rhyncosia minima* at 50 and 70°C.

Effect of moisture stress: Moisture stress produced using PEG 4000 had no significant influence on final germination percentage upto 6 bar but at 7 and 8 bar germination percentage was significantly reduced ($p<0.01$) (Fig. 9). At isoosmotic concentrations, in general PEG 4000 had lesser inhibitory effect compared to NaCl salinity.

Discussion

Among the three populations of *Rhynchosia minima* studied, significant differences were recorded in fecundity. Since the ability of a species to colonize and perpetuate in a habitat depends to a great extent on its seed output and reproductive capacity, *R. minima* exhibits differential success in different habitats. *R. minima* showed highest fecundity and average output weight in old-field habitat where it has highest density and dominates the vegetation.

Both, between populations and within-population variation in seed size was demonstrated. Seed size variation has important demographic consequences. Variation in seed weight and size ensures an efficient segregation in seed dispersal (Venable & brown,

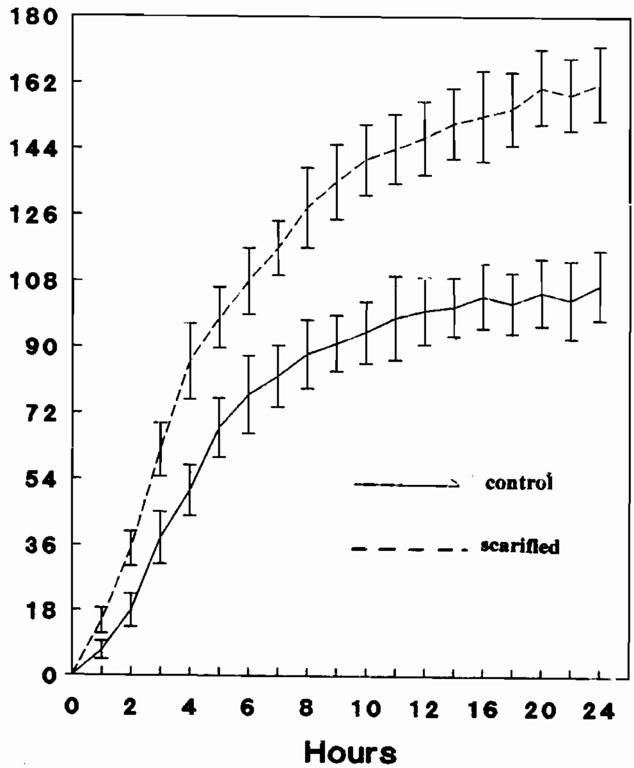

Fig. 5. Water uptake as increase in fresh weight as a percentage of the initial weight of control and scarified seeds of *Rhyncosia minima* as a function of time. Ctrl., control; Scar., physically scarified seeds.

1988). Germination or emergence percentage has been shown to be closely related to seed weight (Bhat, 1973; Weis, 1982; Zhang & Maun, 1990; Stamp, 1990; Aiken & Springer, 1995). Higher germination and/or emergence force in natural populations by virtue of heavier seeds is an asset in that seeds can emerge through a covering soil layer relatively more easily than lighter seeds. Bhat (1973) showed an inverse relationship between seed weight and seed coat impermeability, which also seems to be true for *R. minima*. Seed size variation also has other ecological implications, including effects on seedling establishment (Schall, 1980; Gross & Werner, 1982; Winn, 1984; Zhang & Maun, 1990; Mian & Nafziger, 1994), competitive ability (Harper *et al.*, 1970; Gross & Werner, 1982; Gross, 1984; Wilson, 1994) and even the reproductive ability of adult plants (Stanton, 1984; Wulff, 1986a,b).

It is therefore of survival advantage to a population to have variable seed weight, consequently variable intensity of seed coat dormancy, thereby ensuring erratic or non-quasi-synchronous germination. Such non-quasi-synchronous emergence of *R. minima* seedlings has been observed in the field.

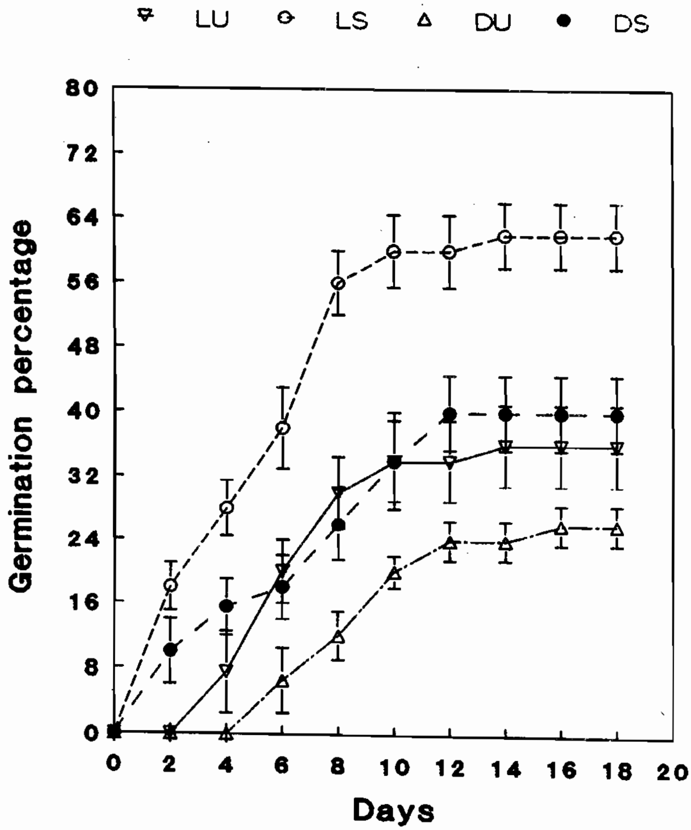

Fig. 6. Effect of light and dark on germination of *Rhyncosia minima*. LU, unscarified seeds in light; LS, scarified seeds in light; DU, unscarified seeds in dark; DS, scarified seeds in dark.

About 65% of the *R. minima* seeds exhibited innate dormancy due to a water impermeable seed coat which inhibited the process of water imbibition. Physical dormancy is common among members of Leguminosae (Martin *et al.*, 1975; Pavone & Reader, 1982; Mayer & Poljakoff-Mayber, 1989; Palma *et al.*, 1995; Teketay, 1996) and imparts survival value in that impermeable seeds are capable of remaining dormant but viable for long periods of time. Chemical scarification, hot water and dry heat treatment all significantly increased the germination percentage, but scarification with sand paper yielded the highest final germination percentage. Similar results have also been reported by Khan *et al.*, (1984), Auld (1986), Mahar *et al.*, (1993), Palma *et al.*, (1995) and Teketay (1996) for various leguminous seeds. It has been shown that acid scarification acts on the cuticle of *Acacia senegal* seeds (Palma *et al.*, 1988). However, in other legumes, e.g., *Prosopis falcata*, the obstacle to germination is found at a deeper level, namely the palisade layer (Palma *et al.*, 1995). Martin *et al.*, (1976) obtained increased germination percentages in seven out of 18 legumes by giving seeds a dry heat treatment and suggested that dry or moist heat modified substances in the seed coat making it permeable to water and thereby breaking the dormancy.

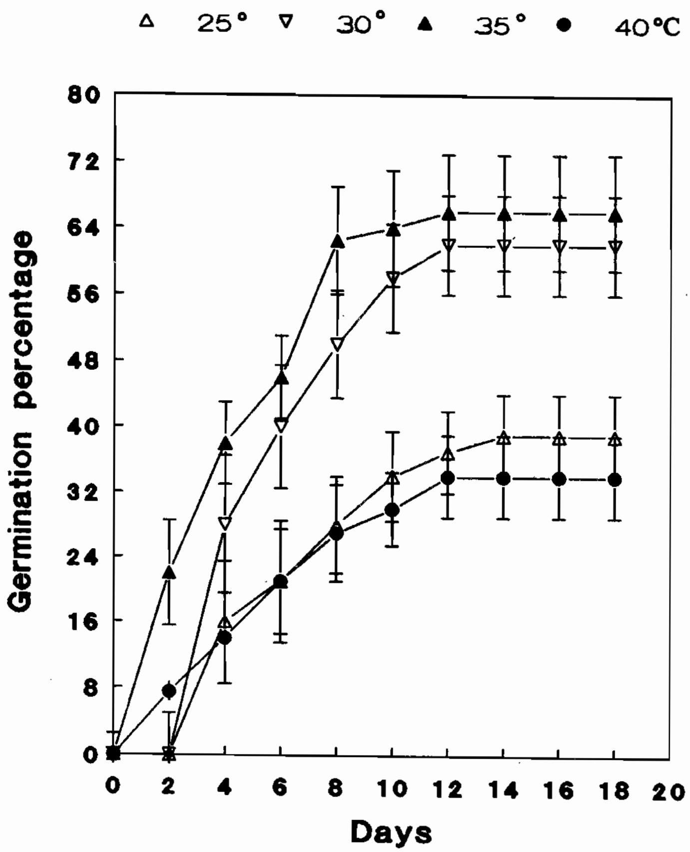

Fig. 7. Effect of temperature regimes on germination of *Rhyncosia minima*.

Both scarified and unscarified seeds of *R. minima* gave remarkably higher germination percentage in light compared to dark. The seeds appear to be light requiring since *R. minima* is a colonizing species that invades old fields and other disturbed areas where the vegetation is removed, or it occupies gaps created in the natural vegetation. The frequent emergence of the seedlings of *R. minima* in open habitats particularly after disturbance suggests their requirement for light. However, it seems that the light requirement slowly decreases with the length of storage period (personal observations).

R. minima germinated over a wide range of temperature regimes (25-40°C). Seeds appear to be adapted to high temperature as they gave substantial germination (26.6%) at 40°C. The optimal temperature for germination is apparently 35°C. Mahar *et al.*, (1993) also obtained highest germination percentage at 35°C in *Indigofera oblongifolia*, a legume common in the study sites. Mahmud & El-Sheikh (1978) and Cony & Trione (1996) working with a desert legume *Prosopis chiliensis* found optimum germination temperatures between 30 and 40°C. High temperature requirement for germination of *R. minima* seems to be well-correlated with summer temperatures as its seeds germinate after the summer rainfall, when day times temperatures in the region are within the

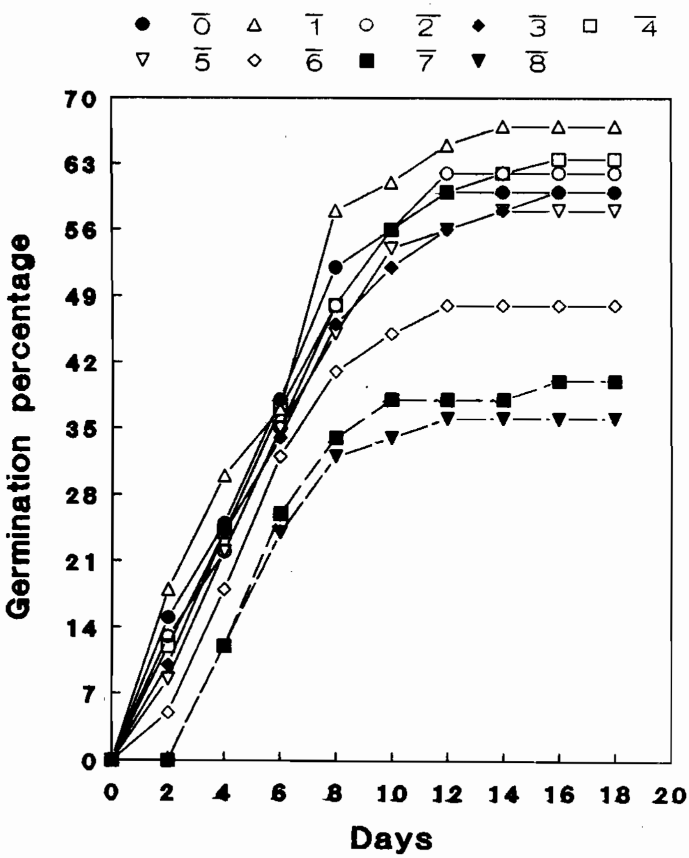

Fig. 8. Effect of salinity levels on germination of *Rhyncosia minima*.

range of 30-40°C (Chaudhri, 1961). No doubt other seeds become permeable as they are exposed to high soil temperatures in field during dry season. Thus by the time "wet" season begins a high percentage of the seeds may be permeable (Quinlivan, 1966, 1968).

NaCl salinity at low osmotic potentials (6 to 8 bars) severely affected germination of *Rhynchosia minima* indicating its low tolerance to salinity. The adverse effects of salinity on germination are well known and even the germination of drought resistant species is affected by moderate levels of salinity (Myers & Morgan, 1989; Ismail, 1990; Blits & Gallaghar, 1991; De Villiers *et al.*, 1994). Although reduced germination in saline medium is mostly attributed to osmotic effect there is also evidence for ionic toxicity (Redman, 1974; Bliss *et al.*, 1986; Myers & Morgan, 1989). However, seeds of *R. minima* germinate after rains, thus they germinate when salinity has been lowered.

Slightly less severe effect on germination was observed when seeds were subjected to moisture stress (simulated soil matric water potentials) using PEG-4000 as compared to salinity. Reduction in germination rate or final germination percentage due to simu-

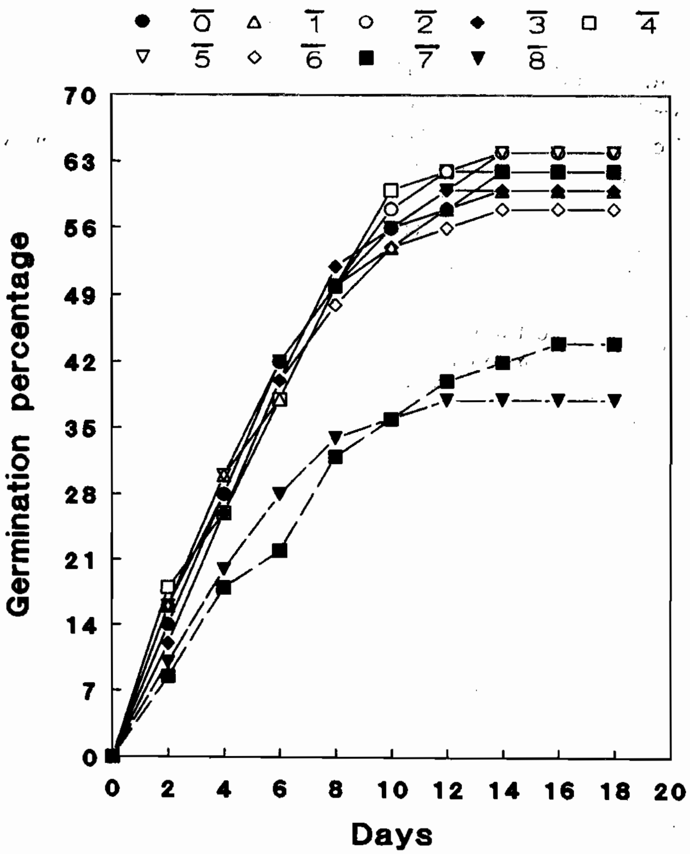

Fig. 9. Effect of moisture stress (created by PEG-4000) on germination of *Rhyncosia minima*.

lated moisture stress has also been reported for various species (Sharma, 1973; Mott, 1974; Zhao & Harris, 1992; Blank *et al.*, 1994) and progressively greater reduction occurs with the lower levels of osmotic potential (Sharma, 1973; Blank *et al.*, 1994). Evans & Etherington (1990) suggested that in non-saline soils (such as the soils where *R. minima* is commonly found) changes in soil water content mainly affect the matric potential as compared to soil solution potential. Therefore, a slight decrease in matric potential has a much greater effect in delaying imbibition and germination (Williams & Shakewick, 1971; Hadas & Stibbe, 1973; Hadas & Russo, 1974). On the other hand, McWilliams & Phillips (1971) found that the effect on germination of a reduced osmotic potential caused by PEG was the same as that of an equal decrease in matric potential, provided that the soil's capacity to supply water at a specific potential was not limiting, and that good seed/soil contact was achieved. Kaufmann & Ross (1970) and McWilliams & Phillips (1971) also showed that where other factors, such as the resistance of the seed coat, impose additional resistance to water movement between soil and the imbibing tissues of the seed, then the equivalence between osmotic and matric potentials no longer holds and impermeable seeds, like that of *R. minima*, would be subjected to much lower matric potential. The lack of tolerance of moisture stress at 6 to 8 bars during germination ensures that no germination takes place after light showers. Under the arid conditions of the study area, evaporation is high after rainfall and thus the need for the soil surface to remain near field capacity (or sufficiently higher matric potential) means that germination must be preceded by a heavy rainfall. Field observations over the years suggest at least 25-30 mm rainfall for the germination of annuals like *R. minima* (personal observations). Under such rainfall conditions suitable soil moisture regime for germination prevails for at least 3-5 days during which annuals emerge. Furthermore, surface run-off water accumulates in the lower-lying drainage channels or depressions creating so called 'safe sites' for seeds to germinate and enable some proportion of the plants to survive to maturity, even in the summers in which little or no further rainfall occurs.

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(Received for publication 16 December 1999)