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# A COLD SHOCK DURING IMBIBITION IMPROVES GERMINATION OF *ACACIA NILOTICA* SEEDS

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**Abstract**

*Acacia nilotica* seeds collected from Cholistan desert, Bahawalpur, Pakistan, exhibited poor germination profiles both at 37°C and at natural day and night alternating temperature cycle (25/16°C). At 37°C an erratic germination of 40±10% was obtained with a spread of germination from 6 to 17±2 days, while at alternating temperature cycle (25/16°C) it was 37±7% and 7 to 20±3 days, respectively. A number of physico-chemical treatments failed to improve these germination profiles. Imbibition at 37°C for a specific period followed by a cold shock, a drop in temperature for 14 h, significantly improved germination of seeds.

## Introduction

Various physico-chemical treatments have been used to promote seed germination and they exert divergent effects in different species. Seed scarification by sulphuric acid and hot water showed retardation in seedling growth in *Tephrosia purpurea*, while water soaking treatment enhanced germination of *Arbus precatorius* (Singh *et al.*, 1984). Lettuce seeds treated with Sodium hypochlorite (NaOCl) showed 70% germination at 35°C while 0.01 N hydrochloric acid treatment followed by NaOCl treatment further improved germinability at higher temperatures (Drew & Brocklehurst, 1984). Acetone (8.0%) treatment followed by hot water treatment stimulated germination of cotton seeds (Abd-El-Rehim *et al.*, 1982) while gibberellic acid (200 ppm) showed stimulation in germination of *Lavandula angustifolia* seeds (Chavagnat, 1978). Thiourea is an effective agent for improving germination of dormant seeds.

*Atropa belladonna* seeds showed an increase in germination at 25 and 30°C after treatment with 2% thiourea (Choudhary & Kaul, 1973). Dhillon & Johnson (1962) found 3% hydrogen peroxide treatment for 24 h as the most effective chemical treatment to break dormancy resulting in an improvement in germination of western larch seeds. A cold shock to imbibed seeds has been found to be more effective in promoting germination of many non-tropical species (Abdalla & McKelvie, 1980). Temperature alternations, stratification, exposure to white light and pre-sowing treatments like low temperature priming have also been found to be effective (Toole, 1973; Thompson, 1974; Heydecker & Coolbear, 1977; Chavagnat, 1978; Thomas, 1981; Bewley & Black, 1985; Bray *et al.*, 1989.

Studies were carried out to examine the germination processes of the native species of Cholistan desert that spans approximately 26,000 sq. km. of the southern part of the

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**Table 1. Effects of various physico-chemical treatments on the germination of *Acacia nilotica seeds.***

| Treatment | Max. Germination % | Spread of germination (days) |
|---|---|---|
| No treatment control | 40(±10) | 6-17 (±2) |
| $KNO_3$ treatment | 30 | 4-9 |
| EtOH | 50 | 8-21 |
| Heating | 50 | 8-19 |
| Running water | 70 | 8-21 |
| Stratification | 40 | 5-11 |

Seed germination was followed at 37°C upto 30 days. Figures for spread of germination represent the day of first seed germination and the day when maximum germination was acheived.

Punjab province of Pakistan. This desert has very harsh summer climate with day temperature soaring up to 50°C, low humidity and uncertain, irregular rainfall that varies from 125 to 250 mm per annum (Chaudhry & Nasim, 1995). Germination performance of *Acacia nilotica* seeds at 37°C and at room temperature after various low temperature priming treatments showed that untreated control seeds were low germinating and although some priming treatments improved germination performance of these seeds slightly, it was concluded that priming alone is unable to generate any dramatic changes (Nasim *et al.*, 1995). The present study shows that a cold shock during imbibition can effectively improve germination performance of *Acacia nilotica* seeds.

## Materials and Methods

*Acacia nilotica* seeds harvested in 1992-93 were obtained from the Cholistan Institute of Desert Studies (CHIDS), Islamia University, Bahawalpur. The seeds were stored at room temperature. Moisture content determination and germination tests were performed using standard methods (Nasim *et al.*, 1982). Germination tests were performed at natural alternating day and night room temperature (25/16°) and 37°C, between the moistened layers of filter paper or in sterile sand moistened with distilled water. Protrusion of radicle was considered as a marker for germination (Nasim *et al.*, 1995).

To improve the germination of *A. nilotica* seeds following treatments were carried out:

i) ***Chemical treatments***: Seeds were soaked in absolute alcohol (5 min.) 3% sulphuric acid (overnight), conc. nitric acid (5 min.) 10 ppm indole acetic acid solution (overnight), 1% (w/v) potassium nitrate (5 min.) or acetone for 5 min. Treated seeds were thoroughly washed with distilled water and air dried before testing for their germination performance.

ii) ***Physical treatments***: Prior to the germination tests, seeds were subjected either to stratification, placed in boiling water for 1 min., and then kept in running water for further 30 min., kept in running water for 24 h or in an oven at 50°C for 9 h.

iii) ***Temperature shock treatment***: Seven sets of 20 seeds each were placed for germination at 37°C alongwith a control set. Each test set was allowed to imbibe at 37°C for a predetermined time period of 24,48,72,96,120,144 or 168 h. Seeds were then taken out of the incubator and left at 16-20°C (the room temperature at night) for 14 h and after this shock each set was placed back at 37°C for germination. In another experiment 5 sets of 20 seeds each were pre-treated either with 3% sulphuric acid, boiling water, running water, acetone, or heated at 50°C, followed by the temperature shock after 48-hours of imbibition at 37°C and then examined for germination.

## Results

*Acacia nilotica* seeds exhibited poor germination characteristics both at natural day and night alternating temperature cycle (25/16°C) and 37°C in Petri plates. It took 7 (±3) days for the first seed to germinate at 25/16°C cycle with 37 (±7)% seed germination after 19 (±3) days, while at 37°C the first seed germinated on the 6th (±2) day and maximum germination of 40(±10)% was found on the 17th (±2) day of the experiment. Viability of the seeds was tested in a regular-soil pot test at natural day and night alternating temperature cycle where 80% of the seeds germinated albeit with a spread of germination from 15-29 days. Various physico-chemical treatments were used to improve germination of these seeds. Running water treatment proved to be most effective showing 70% germination whereas Ethanol and heating treatment each showed 50% germination (Table 1).

A drop in the test temperature during earlier phases of germination significantly improved germination of *A. nilotica* seeds. (Fig.1 & 2). It is interesting to note that in each test set the first seed germinated within 24 h after the cold shock. The number of seeds germinating within 24 h after the shock increased with the length of the imbibition period prior to the shock (Fig.1b). A combination of 3% sulphuric acid-overnight treatment and temperature shock was found more effective in improving germination of the *A. nilotica* seeds than the temperature shock alone (Fig.2).

## Discussion

Variations in the germination performance of plant seeds with changing environmental temperatures are well documented. While some species can develop thermodormancy (Sankhla & Sankhla, 1973), others may prefer higher temperatures for rapid germination (Carpenter *et al.*, 1993). Alternating temperatures may promote germination in some species (Davis *et al.*, 1993) while others may exhibit maximum germination at artificially maintained constant temperatures (Thompson, 1973). Dormant seeds, in general, germinate over a very narrow temperature range. However, if the dormancy is removed the same seeds can germinate over a substantially wider temperature range. Although germination test in regular soil revealed that at lest 80% of the *A. nilotica* seeds were viable (data not shown), in our lab experiments these

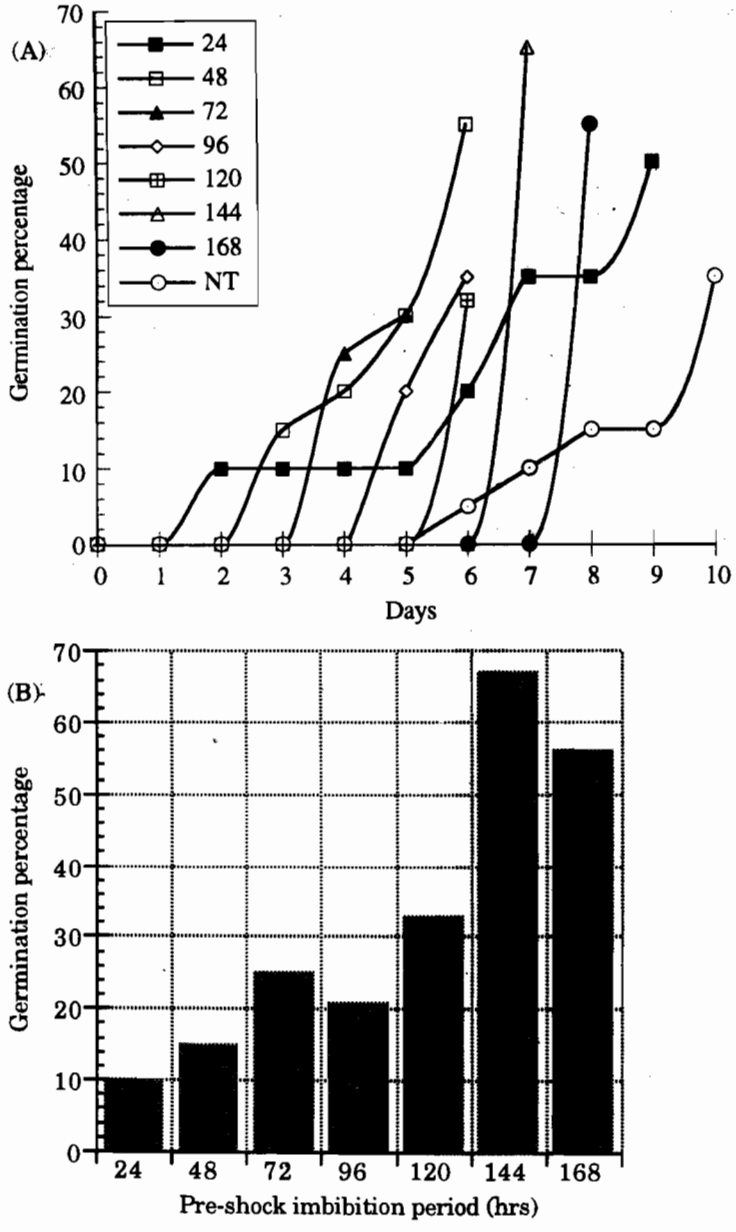


Fig.1. A) Effect of a cold shock on the germination performance of *Acacia nilotica* seeds imbibing at 37°C. Germination till day $T_{50}$ (the day when at least 50% germination was achieved) is shown. Figure legend 24, 48, 72, 96, 120, 144 and 168 are the pre-shock imbibition periods (in hours) at 37°C, NT is "No Treatment" control.

B) The number of *A. nilotica* seeds germianting within 24 hours after the cold shock is proportional to the length of the pre-shock imbibition period. The shock day has been taken as the zero day. The number of seeds (expressed in terms of germination percentage) germinating within 24 hours of the cold shock are plotted against the length of the pre-shock imbibition period (in hours)

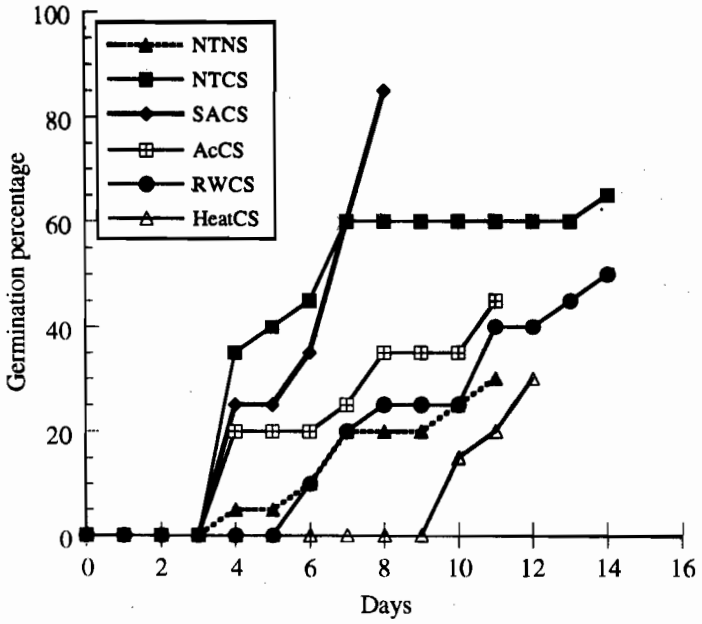


Fig.2. Some physico-chemical treatments which enhance the effect of cold shock on the germination performance of *Acacia nilotica* seeds. The seeds were given a specific pre-treatment combined with a cold shock and then tested for their germination performance at 37°C. NTNS: No. Treatment No Shock (negative control); NTCS: No Treatment but Cold Shock (Positive control); SACS: Sulphuric Acid treatment plus Cold Shock; AcCS: Acetone treatment and Cold Shock; RWCS: Running water treatment plus Cold Shock; Heat CS: Heating at 50°C in an oven for 9 hours and the Cold Shock.

seeds gave a maximum germination percentage of 40(±10)% at 37°C, suggesting that these seeds are dormant under our test conditions.

The dormancy in *A. nilotica* seeds may either be of a chemical nature (presence of a germination inhibiting compound) or of a physical nature (presence of coat-imposed dormancy). Selection of the treatments to break dormancy was, therefore, made to cover both these aspects. Although some chemical treatments did affect germination profiles of these seeds, physical treatments proved to be more effective (Table 1). The most effective treatment, however, was found to be a cold shock given during imbibition that improved germination percentage with a concomitant decrease in the spread of germination. The number of seeds germinating within the 24 h of the shock was found to increase with the pre-shock imbibition period, however, a shock after 48 h imbibition gave a very high germination of 80% in a relatively short period of 12 days. Pre-sowing treatment with 3% sulphuric acid resulted in a further improvement of the germination performance of these seeds (Table 2), as the germination percentage increased to ≥ 80% with the spread of germination decreasing from 3-12 days to 3-8 days. There are reports that *A. nilotica* seeds kept in cow dung for 24 h as a pre-sowing treatment take 6 days for the first seed germination and maximum germination is achieved in 33 days (Ashraf & Ahmed, 1994).

**Table 2. Effects of various pre-shock imbibition periods on germination performance of the *Acacia nilotica* seeds.**

| Pre-shock imbibition period (h) | Max. Germination % | Spread of germination (days) |
|---|---|---|
| 24 | 75 | 2-24 |
| 48 | 80 | 3-12 |
| 72 | 60 | 4-18 |
| 96 | 68 | 5-17 |
| 120 | 61 | 6-21 |
| 144 | 89 | 7-15 |
| 168 | 78 | 8-21 |

Seed germination was followed at 37°C upto 30 days. Figures for spread of germination represent the day of first seed germination and the day when maximum germination was acheived.

With the sharp temperature change in the imbibed state there might be some phase transition in the cell membranes affecting its permeability and hence the germination process or a drop in temperature might inhibit or retard an inhibitory reaction allowing certain germination related reactions to proceed albeit at a slower rate. Contrary to the later explanation a temperature drop might activate a reaction which helps germination to proceed and is otherwise inhibited at a high temperature. A temperature shock may be involved in the expression of particular protein (s) that might be involved directly or indirectly in the release of dormancy and hence the enhancement of the germination performance of these seeds. Cold shock and heat shock proteins are known to be involved in regulation of biological processes in various living systems including plants (Howarth & Ougham, 1993) and a possible involvement of similar proteins in regulation of *A. nilotica* seed germination needs further investigation.

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