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SEED DORMANCY ALLEVIATION OF *GREWIA TENAX* (FORSSK.): A WILD FRUIT TREE SPECIES OF PAKISTAN

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Abstract

Grewia tenax (Forssk.) Fiori is a fruit shrub and grows wild in arid and semi-arid tropics of Asia and Africa. The species is highly valuable for the rural populations because of its edible fruit and fodder for livestock. Species has immense potential for re-vegetation of degraded lands, as it has ability to withstand soil salinity and drought. Wild stands of the species are sparse which is supposed to have some kind of seed dormancy. Seeds of *G. tenax* were subjected to different combinations of heat and cold seed stratification treatments in two consecutive experiments. A positive correlation (r^2 = 0.97) was observed between total emergence and weeks of seed exposure to constant dry heat at 40°C from 0 to 4 weeks. Maximum germination (70%) was achieved, when seeds were exposed to dry heat at 40°C for 4 weeks as compared to control (20%). Seeds exposed to constant heat for 4 weeks also took only 4 and 5 days to reach 1st and 50% emergence, respectively as compared to untreated seeds, which took 10 and 14 days to reach 1st and 50% emergence, respectively. Moreover, emergence spread lasted only 4 days as compared to untreated seeds with 21 days. Our results indicate that seeds of *G. tenax* possess a limited physiological dormancy which can be overcome by heat stratification

Key words: Seed Dormancy, Germination, Stratification, Grewia, Multipurpose fruit.

Introduction

Increasing average temperatures combined with rise in rainfall variability and recurrent drought spells (Houghton *et al.*, 2001; Walsh & Ryan, 2000; Easterling *et al.*, 2000) are affecting wild stands of sensitive plant species in arid and semi-arid tropical areas of the world. Under these circumstances, indigenous multipurpose fruit tree species can play an important role to combat land degradation and minimize the risks of food scarcity (Teketay, 1996). *G. tenax* is one of those underutilized indigenous fruit tree species that grows wild in many arid and semi-arid tropical regions of the world (Bredenkamp, 2000) and has potential to rehabilitate degraded land and overcome malnutrition in rural population. Despite its ability to grow on wide array of soil and withstand harsh climatic condition, wild stands of the species are often threatened by overuse. Many of perennial plants species have developed mechanisms for seed survival under unfavorable climatic conditions (Dalling *et al.*, 2011). Dormancy is one way that enables seeds to survive (Salazar *et al.*, 2011., often for a number of years in the soil seed bank (Graeber *et al.*, 2012; Mark & Ooi, 2012) until conditions are suitable for germination. On the other hand seed dormancy is considered a big hurdle to the effective use of many species (Adams *et al.*, 2011; van Klinken *et al.*, 2013) in land revegetation programs (Merritt *et al.*, 2007).

There is little information on seed dormancy and germination characteristics of *G. tenax*. The purpose of this study was to identify kind of seed dormancy in *G. tenax* and to find out dormancy breaking treatment to enhance its germination.

Materials and Methods

Seeds of *G. tenax* were collected from wild stands in the surrounding of Dera Ismail Khan District (31° 48' N; 70° 37' E) of Pakistan and shifted to Witzenhausen, Germany. Seeds were extracted from the pulp and stored in paper bags at room temperature until the start of experiment. Before sowing seeds were disinfected by soaking in sodium hypochloride solution (2%) for 15 minutes followed by three rinses with distilled water (Saied *et al.*, 2008). Two successive experiments were conducted to identify appropriate seed treatment to improve their germination.

In the 1st experiment, treatments were untreated seeds (T_0), constant heat exposure of the seeds to 40°C (T_1), constant cold exposure of the seeds to 4°C (T_2) and alternate heat and cold exposure of the seeds to 4 and 40°C (T_3). Seeds were treated for one week before sowing. On the basis of the results of the 1st experiment, 2nd experiment was launched in which seeds were subjected to constant heat exposure at 40°C for 0 (T_0), 1 (T_1), 2 (T_2), 3 (T_3), 4 (T_4), 5 (T_5) and 6 weeks (T_6) before sowing.

The pretreated seeds were sown separately in a silica sand-based medium contained in inverted pyramid cells of 50-cell plastic trays, placed in plastic pans. Seeds were allowed to germinate under 30/25°C (day/night) temperature and 50% relative air humidity. To minimize evaporation, each plastic tray was covered with a thin transparent plastic sheet with minute holes to facilitate gas exchange. Each experiment was composed of 8 replications with 8 seeds per experimental unit.

Emergence was assessed daily for a period of 30 days. A seed was considered germinated when the hypocotyls hook emerged above the surface. The number

of days to the 1st emergence (E_{1st}), 50% emergence (E_{50}), emergence spread (Es) and total emergence percentage (E_t) were recorded. At the end of the experiment seedlings were harvested and cleaned of sand. After taking fresh weight (FW) of the seedlings, samples were oven dried at 70°C for 48 hours for dry weight (DW).

The SPSS statistical package (SPSS Inc. Chicago, USA) was used to analyze experimental data using one way analysis of variance (ANOVA). Means were separated by a Tukey-test (p = 0.05).

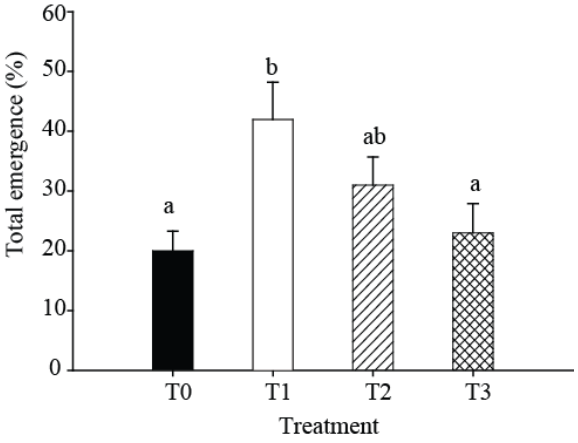

Fig. 1. Effect of different pre-sowing seed stratification treatments on total emergence (E_t) percentage of *G. tenax* seeds, where T_0 = control, T_1 = constant heat exposure, T_2 = alternate heat and cold exposure and T_3 = constant cold exposure. Bars show means of eight replicates and letters indicate significant difference among treatment means (p<0.05; Tukey test).

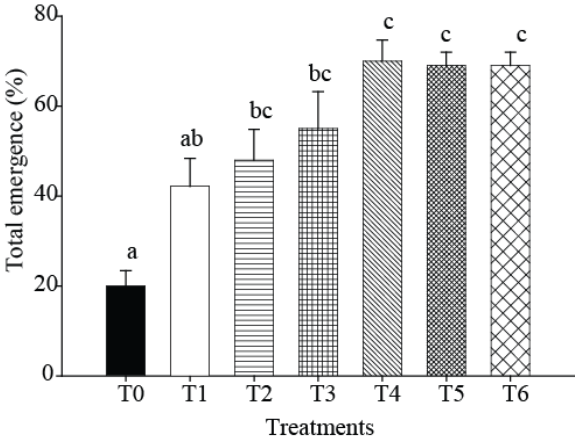

Fig. 2. Effect of different pre-sowing heat stratification periods on total emergence (E_t) percentage of *G. tenax* seeds, where T_0 = control and T_1 –T_6 correspond to seeds exposed to constant heat at 40°C for 1, 2, 3, 4, 5, and 6 weeks, respectively. Bars show means of eight replicates and different letters indicate significant difference among treatment means (p<0.05; Tukey test).

Results

The results of the 1st experiment showed that constant heat exposure of seeds at 40°C for one week (T_1) significantly improved (p<0.05) total emergence (E_t) from 20% (control) to 42% (Fig. 1). At the same time, the number of days to 1st emergence (E_{1st}), 50% emergence (E_{50}) and emergence spread (E_s) were reduced slightly (data not shown). Alternate cold and heat exposure (T_2) and constant cold exposure (T_3) of seeds for one week before sowing did not yield any significant increase in total emergence (Fig. 1).

On the basis of results of the 1st experiment, seeds were subjected to increasing duration of constant heat to identify the most effective period of heat exposure. A positive correlation (r^2 = 0.97) in total emergence percentage was observed with increasing duration of heat exposure from 1 to 4 weeks ($T_1 – T_4$) at 40°C (Fig. 3).

Constant heat exposure for 4 weeks prior to sowing significantly improved (p<0.05) the total emergence (E_t) of seeds up to 70% as compared to the untreated control (20%; Fig. 2). It also significantly reduced (p<0.05) mean days to 1st emergence (E_{1st}), 50% emergence (E_{50}) and emergence spread (E_s). All these three parameters showed negative correlation when duration of heat exposure increased from 1 to 4 weeks ($T_1 – T_4$) at 40°C (Fig. 3). Seeds exposed to constant heat for 4 weeks, started to emerge (E_{1st}) after 4 days of sowing as compared to the control seeds which showed 1st emergence after 10 days. These seeds also achieved 50% emergence (E_{50}) after 5 days of sowing while control seeds took 14 days. At the same time, emergence spread (E_s) in these seeds that is duration between emergence of the 1st and the last seedlings lasted only 4 days as compared to untreated seeds with 21 days (Table 1). Further increases in heat exposure duration did not yield any significant improvement in total emergence (E_t), days to 1st emergence (E_{1st}), 50% emergence (E_{50}) and emergence spread (E_s). Constant heat exposure for 4 to 6 weeks before sowing also significantly increased fresh and dry weight of the seedlings (Table 1).

Discussion

Since only a few weeks of heat stratification significantly improved germination percentage and rate, seeds of *G. tenax* were physiologically dormant, which is the most common form of dormancy found in seeds (Baskin & Baskin, 1998; Baskin & Baskin, 2003; Dalling *et al.,* 2011). Our results are in agreement with the findings of Turner *et al*. (2006), who reported breaking of seed dormancy by exposure to heat in *Acanthocarpus preissii*. Four weeks of continuous heat exposure also broke the physiological dormancy of *G. fascicularis* (Hoyle *et al.,* 2008; Mangan *et al.,* 2010).

After-ripening changes in seeds can also occur at room temperature but can be enhanced by exposure to heat. The molecular mechanisms of after-ripening in seeds are not well understood (Finch-Savage & Leubner-Metzger, 2006; Graeber *et al.,* 2012) but are thought to be non-enzymatic reactions removing inhibitors, membrane alterations within the seed and protein degradation (Bell, 1999; Finch-Savage & Leubner-Metzger, 2006; Mark & Ooi, 2012). Exposure of seeds to low or high temperature has been shown to stimulate germination by inducing physical and physiological changes within the seeds and breaking dormancy (Willis & Groves, 1991; Bewley & Black, 1994; Copeland McDonald, 2004; Baskin *et al.,* 2005; Adams *et al.,* 2011).

Table 1. Effect of different pre-sowing heat stratification (at 40°C) treatments on days to first emergence (E_{1st}), 50% emergence (E_{50}), emergence spread (E_s) , fresh weight (FW) and dry weight (DW) of *G. tenax*.

Heat exposure at 40°C (weeks)	E_1st (days)	E50 (days)	Es (days)	FW (g)	DW (g)
Control	10 ± 1.5 a	14 ± 2.8 a	21 ± 2.5 a	1.3 ± 0.2 a	0.7 ± 0.4 a
1	08 ± 0.4 ab	12 ± 0.5 ab	14 ± 0.8 ab	2.1 ± 0.3 ab	1.0 ± 0.4 ab
2	08 ± 0.4 ab	11 ± 0.5 ab	10 ± 0.8 ab	2.6 ± 0.3 bc	1.3 ± 0.4 bc
3	06 ± 0.3 bc	07 ± 0.6 bc	08 ± 0.8 b	2.7 ± 0.2 bc	1.3 ± 0.3 bc
4	04 ± 0.2 c	05 ± 0.2 c	04 ± 0.4 b	3.3 ± 0.2 c	1.8 ± 0.3 c
5	04 ± 0.2 c	05 ± 0.2 c	04 ± 0.4 b	3.1 ± 0.2 bc	1.7 ± 0.2 bc
6	04 ± 0.2 c	05 ± 0.2 c	04 ± 0.4 b	3.1 ± 0.2 bc	1.7 ± 0.2 c

Values show means of 8 replicates ± S.E. different letters indicate significant difference among treatment means ($p<0.05$; Tukey test)

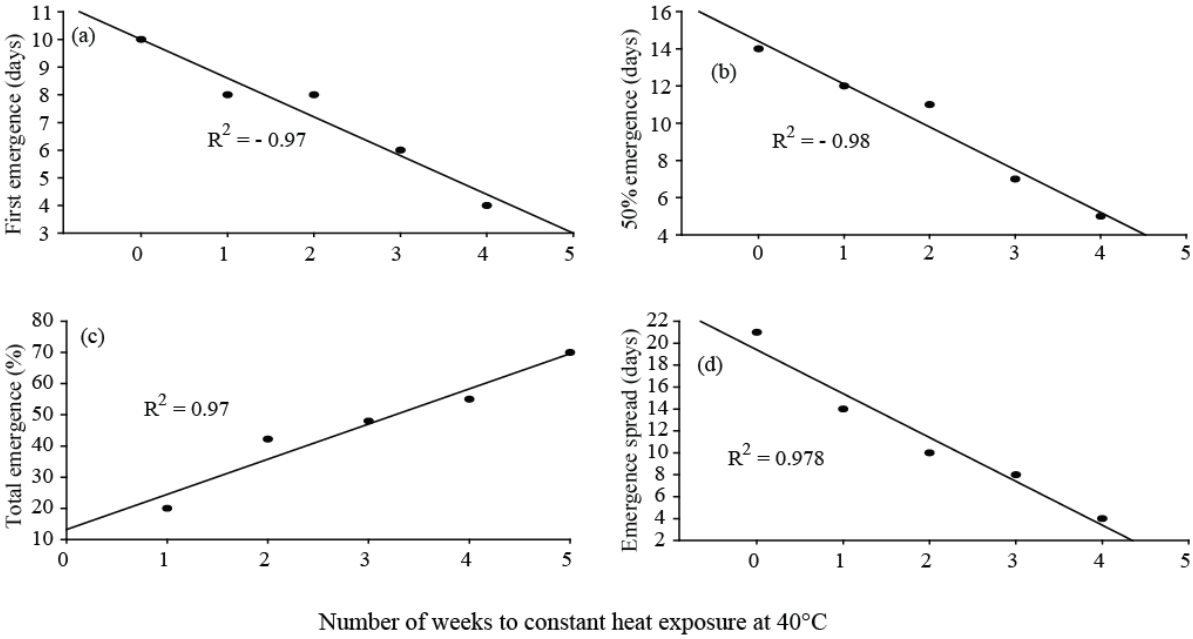

Fig. 3. Correlation between duration of constant heat exposure and days to first emergence (a), days to 50% emergence (b), days to total emergence (c) and emergence spread (d) of *Grewia tenax* seeds

It is well documented that seeds of many tropical plant species require either cold or warm stratification to germinate, depending on a number of factors especially environmental conditions during seed development and storage time (Hartmann *et al.,* 1997; Srivastava, 2002; Copeland & McDonald, 2004; Graeber *et al.,* 2010.). Factors such as relative humidity, temperature, soil moisture and internal seed morphology and embryo size also influence a seed's lifespan and ability to germinate (Copeland & McDonald, 2004; Jayasuriya *et al.,* 2009). Seeds with under-developed or immature embryos may not germinate even under highly favorable germination conditions (Baskin & Baskin, 2001; Srivastava, 2002; Nambara *et al.,* 2010).

Conclusion

Seeds of *G. tenax* appear to exhibit physiological dormancy like some forb species of semi-arid tropical Queensland (Hoyle *et al.,* 2008) and *Acanthocapus preissii*, a common perennial herb of Western Australia (Turner *et al.,* 2006). Germination of *G. tenax* seeds can be enhanced up to considerable extent by pre-sowing heat stratification treatment i.e. constant heat exposure seeds to 40°C for 4–6 weeks. Findings of this study have important implications for the use of *G. tenax* for revegetation programs of degraded arid lands.

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