Breaking Seed Dormancy in a Forest Plant: *Grewia damine* Gaertn

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ABSTRACT

Seeds of Grewia damine are dormant and vegetative propagation is difficult which act as an obstacle for the introduction of this species for commercial or conservation processes. Therefore, the present study was conducted to identify the seed dormancy breaking treatments to improve seed germination of G. damine. The following dormancy breaking treatments were applied: control (intact seeds); scarified seeds with sand paper; hot water treatment (100 ^{0}C) for 5, 10 and 15 minutes; refrigeration at 2-5 ^{0}C for 1, 2 and 3 weeks; Conc. H_2SO_4 or HNO_3 immersion for 5, 10 and 15 minutes. Germination parameters were measured and the data were subjected to ANOVA in Minitab 15. A significantly high ($p < 0.05$) germination percentage (93%) and other germination parameters (GS= 13.628, GMT= 4.956, GI= 5.556 and $T_{50\%}$= 5.447) were recorded in the treatment of scarification with a sand paper followed by the treatments with Conc. H_2SO_4 for 15 minutes and boiling water for 5 minutes where germination was 55 %. While significantly low germination of 22 % was recorded with Conc. HNO_3 for 15 minutes. The results suggests that seeds of G. damine possess a physical dormancy. None of the untreated seeds of G. damine germinated within the study period and scarification with a sand paper proved to be the most effective method of breaking seed dormancy. Hence seeds could be used by the nurserymen after breaking dormancy to produce plant material at large scale.

KEYWORDS: Grewia damine, Native, Physical dormancy, Scarification, Seed germination

Introduction

The use of native plants in ground-level landscaping is promoted by both aesthetic and scientific arguments. The aesthetic arguments are either culturally based, being that native plants are part of our cultural heritage (MacDonagh *et al*., 2006) or design driven, as native plants blend into the surrounding landscape (Kiers, 2004). Scientific arguments are based on maintenance requirements, habitat creation and the potential for exotic plants to become invasive (Butler *et al*., 2012).

According to Kramer (2013), the widespread replacement of native vegetation with exotic ornamental plants in managed landscapes is a growing problem for the organisms that depend on native plants for food, shelter and breeding. Native plants share an evolutionary history with regional insects and other organisms (Wilde *et al*., 2015). Hence, landscaping primarily with exotic plant species would be expected to be detrimental to insect herbivores that have adapted to native plant hosts (Tallamy, 2004). Recently concluded research work on birds and insects back this argument (Burghardt *et al*., 2008; Burghardt and Tallamy, 2013; Chong *et al*., 2014). Nevertheless, exotic plants dominate esthetically-managed landscapes in many parts of the world.

To support biodiversity in urbanized areas, the increased use of native plants has been encouraged by naturalists and a niche market has already established in developed countries for native plants that provide wildlife support, local adaptability and aesthetics (Wilde *et al*., 2015). Native plants make up approximately 13% of the total sales of the nursery industry in the United States (Hall *et al*., 2011). Native plants can often be more expensive than exotic plants, due to small scale production (Norcini, 2006). Stakeholder surveys have found that the availability of native plants is a major limitation to increasing their use in landscaping (Hooper *et al*., 2008; Brzuszek and Harkess 2009; Kauth and Perez 2011).

Sri Lanka, has been identified as one of the 25 biological hotspots of the world (Myers *et al*., 2000) and the floristic wealth of the country could be tapped to introduce wild plants into the landscape industry (Yakandawala and Adikari, 2014). In Sri Lanka, some studies were conducted on aesthetics and propagation of native plants such as *Lawsonia inermis* (Yakandawala and Adikari, 2014) and *Osbeckia octandra* (Yakandawala *et al*., 2013). In this context, *Grewia damine* Gaertn. (Malvaceae) commonly known as 'Damunu' was identified as a native plant which has a potential to be promoted as an ornamental plant in the landscape industry. It is a medium sized tree distributed in all the three major climatic zones of the country (Robyns and Meijer 1983). It produces small, attractive yellow color flowers which attract a diversity of wild bees and red colored fruits attract birds (Unpublished). Hence, it could be promoted as a potential native plant that could be used to attract wildlife.

In addition to its potential landscape value, it has been used as a medicinal plant. Root and fruits of *G. damine* were used in the powdered form and employed in prevention of the osteoporosis (Pereira *et al*., 2002). The wood is tough and used for making oars, shafts and gunstocks, while the bark is good for making string (Robyns and Meijer, 1983). Its timber is used as wood and in tool handles; bark provides fiber and its fruits are edible (Ashton *et al*., 1997). Sticks were used to support vegetable and betel cultivation.

According to literature, propagation of G. *damine* has not been attempted before and vegetative propagation by air layering or with stem cuttings has been proved unsuccessful (unpublished). Fresh seeds of his species has exhibited zero germination, suggesting that its seeds may possess some type of seed dormancy. Further, seed germination under natural conditions is observed to be low. According to Mozhgan *et al*., (2013), seeds of natural plants do not exhibit uniform germination in natural ecosystems as they must survive unfavorable conditions.

Dormancy is one way that enables seeds to survive (Salazar *et al.,* 2011) often for a number of years in the soil seed bank until conditions are suitable for germination (Graeber *et al.,* 2012; Mark and Ooi, 2012). 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). Therefore, the present study was conducted to identify the seed dormancy breaking treatments that could be applied to improve seed germination of *G. damine*. Hence with the availability of propagules, it could be introduced as a potential landscape plant.

Materials and Methods

The study was conducted in the Faculty of Agriculture and Plantation Management, Wayamba University of Sri Lanka. Mature fruits were collected from Kaudulla area in Polonnaruwa district and immediately after collection, fresh seeds were isolated from the berries, separated from unripe seeds, and the pericarp was washed away with clean water. After drying for one week in shade under the room temperature, uniformly-sized seeds were used in different seed dormancy breaking experiments (Table 1).

To study seed germination, 25 seeds were placed in Petri dishes on two layered filter paper (Whatman no.1) moistened with distilled water. The Petri dishes were arranged in a Completely Randomized Design (CRD) with four replicates. As the control, seeds were set for germination without any treatment. The seeds were recorded as germinated if the radical has emerged at least 2 mm beyond the seed coat. Germination counts were made every day for 60 days. The experiment was repeated twice.

Data Recording

Total germination (TP), germination speed (GS), germination mean time (GMT) germination index (GI) and the time taken for 50% germination ($T_{50\%}$) were calculated to evaluate the germination using following formula as described in Seedling Evaluation Handbook of Association of Official Seed Analysts (AOSA. 1991).

Table 1. The different treatments used to break dormancy of *G. damine* seeds

Treatment	Description
T1	Control
T2	Scarification with a sand paper (P 320)
T3	Hot water treatment (100 ^{0}C) for 5 min
T4	Hot water treatment (100 ^{0}C) for 10 min
T5	Hot water treatment (100 ^{0}C) for 15 min
T6	Refrigeration at a temperature of 2-5 ^{0}C for 1 week
T7	Refrigeration at a temperature of 2-5 ^{0}C for 2 weeks
T8	Refrigeration at a temperature of 2-5 ^{0}C for 3 weeks
T9	Conc. H_2SO_4 treatment for 5 min
T10	Conc. H_2SO_4 treatment for 10 min
T11	Conc. H_2SO_4 treatment for 15 min
T12	Conc. HNO_3 treatment for 5 min
T13	Conc. HNO_3 treatment for 10 min
T14	Conc. HNO_3 treatment for 15 min

Total germination (TG): TG = (SNG/SN0) x100

SNG = number of germinated seeds;
SN0 = number of experimental seeds with viability

Germination speed (GS): $GS = \Sigma D \times n / \Sigma n$,

n = number of germinated seeds at each day;
D = number of days after the start of the experiment.

Germination index (GI): The germination index (GI) was calculated as described in the Association of Official Seed Analysts (AOSA, 1983) by following formula.

$GI = \sum (Gt/Tt)$,

Gt = number of seeds germinated on t^{th} day, Tt = number of days up to t^{th} day.
Mean germination time (MGT): $MGT = \Sigma TiNi / \Sigma Ni$,
Ni = number of the newly germinated seeds during time of Ti.
The time taken for 50% germination ($T_{50\%}$): $T_{50} = t_i + \{ (N/2) - n_i \} (t_i - t_j) / n_i - n_j$

N= final number of germination and n_i, n_j cumulative number of seeds germinated by adjacent counts at times t_i and t_j when $n_i < N/2 < n_j$.

Data Analysis

The data were subjected to ANOVA in Minitab 15. Significant differences between treatments were determined using LSD test at the 0.05 probability level.

Results

It was revealed from the present study that none of the untreated seeds of *G. damine* germinated within 60 days (Table 2). However, germination resumes with four dormancy breaking treatments *viz.*, scarification with a sand paper, hot water treatment (100 ^{0}C) for 5 minutes, Conc. H_2SO_4 treatment for 15 min and Conc. HNO_3 treatment for 15 min.

A significantly high germination of 93% (Figure 1) was recorded in the treatment of scarification with a sand paper (mechanical scarification) within 14 days, followed by the treatments with Conc. H_2SO_4 for 15 minutes and boiling water for 5 minutes where germination was 55 %. While significantly low germination of 22 % was recorded with Conc. HNO_3 for 15 minutes. In the treatment with scarification, seeds initiated germination within two days after the treatment while in other treatments it was three days (Figure 2).

Apart from the germination percentages, all the other germination parameters were also significantly high ($p < 0.05$) in mechanical scarification (GS= 13.628, GMT= 4.956, GI= 5.556 and $T_{50\%}$= 5.447) (Table 2 and Figure 2). This was followed by treatments with Conc. H_2SO_4 for 15 minutes (GS= 4.783, GMT= 8.979, GI= 1.733 and $T_{50\%}$= 7.422) and boiling water for 5 minutes (GS= 5.147, GMT= 8.489, GI= 1.836 and $T_{50\%}$= 7.687).

Treatment with Conc. HNO_3 for 15 minutes recorded significantly low germination speed and germination mean times compared to above two treatments. No seeds were germinated in hot water treatment beyond 5 minutes, any low temperature treatments or in acid treatments less than 15 minutes (Table 2).

Table 2. Effect of different seed treatments on breaking dormancy of *G. damine* seeds

Treatments	GS	GI	MGT	T50
T1	-	-	-	-
T2	13.63 ± 0.36 [a]	5.56 ± 0.33 [a]	4.96 ± 0.29 [a]	5.70 ± 0.41 [a]
T3	5.15 ± 0.40 [b]	1.84 ± 0.13 [b]	8.49 ± 0.12 [b]	7.69 ± 0.03 [b]
T4	-	-	-	-
T5	-	-	-	-
T6	-	-	-	-
T7	-	-	-	-
T8	-	-	-	-
T9	-	-	-	-
T10	-	-	-	-
T11	4.78 ± 0.18 [b]	1.77 ± 0.04 [b]	8.98 ± 0.47 [b]	7.42 ± 0.43 [b]
T12	-	-	-	-
T13	-	-	-	-
T14	1.61 ± 0.53 [c]	0.69 ± 0.15 [c]	9.30 ± 0.54 [b]	7.74 ± 0.59 [b]

Figures not sharing the same letters in the same column differ significantly at $p < 0.05$

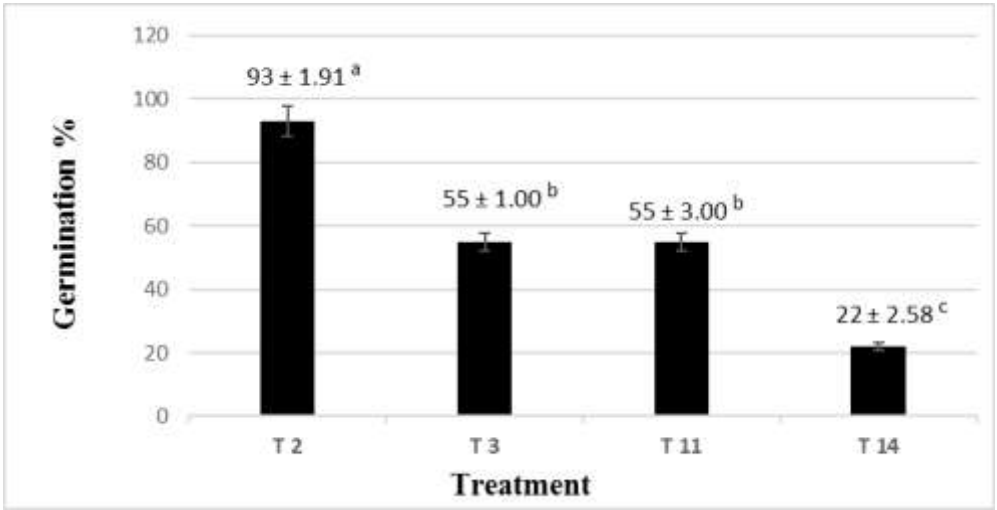

Figure 1. Germination percentage of *G. damine* seeds under different treatments

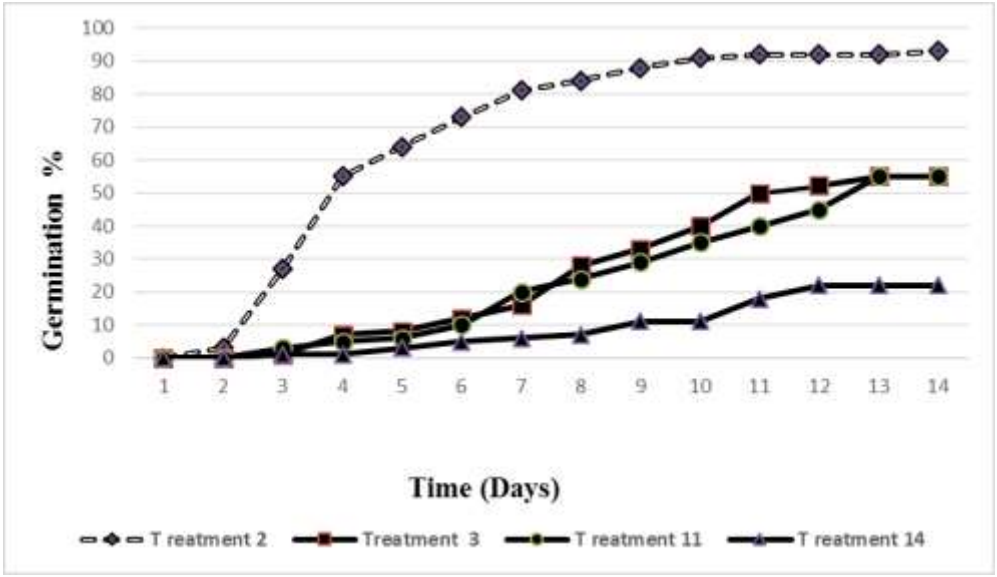

Figure 2. Effect of different treatments on seed germination of *G. damine* on consecutive days

Discussion

Seed dormancy is an innate seed property that defines the environmental conditions in which the seed is able to germinate. It is determined by genetics with a substantial environmental influence which is partially mediated by the plant hormones (Mayer and Poljakoff-Mayber, 1989). A completely non-dormant seed has the capacity to germinate over the widest range of normal physical environmental factors possible for the genotype (Baskin and Baskin, 2004). Strong seed dormancy exists in many wild and weedy plant species and in semi-domesticated crops (Finkelstein *et al*., 2008). Dormancy enables seeds to avoid germination during periods that are only ephemerally favorable. By possessing seeds with various degrees of dormancy, plants can also distribute their offspring across time and against unpredictably variable environments (Venable, 2007; Poisot *et al*., 2011).

In *G. damine* seed has a very thick seed coat probably to protect the seed as it travels through the digestive tract of bird dispersers. The presence of a thick, tough seed coat could be a mechanical barrier to germination and this could lead to the physical dormancy. Even though it is an efficient mechanism that guarantees the survival and subsequent spread of *G. damine*, it is also a factor that limits its propagation. Physical dormancy is caused by a water impermeable seed or fruit coat with one or more palisade cell layers (Van Staden *et al*., 1989; Baskin and Baskin, 2004). Seeds germinate when an impermeable seed coat or fruit wall is breached. During the present study, different physical seed dormancy breaking methods were studied as each methods has its own advantages and disadvantages, including cost

and practicality. Out of which, physical, chemical and hot water scarification led to the breaking of dormancy in *G. damine* seeds.

Total germination (maximum percentage germination), is a widely used index in seed germination studies (Correa *et al*., 2000). The total germination index only depends on final results, hence it interprets for germination capacity of seeds under any treatment. In the present study, physical scarification with a sand paper recorded the significantly high germination percentage of 93% (Figure and 1Table 2) compared to other successful treatments. Further, all the other germination parameters studied were also significantly high with physical scarification (Table 2) with a sand paper. Scarification is any treatment that results in the rupturing or weakening of the tegument, permitting the passage of water and the initiation of germination (Cavalheiro, 2007). Several studies have been made with the mechanical scarification as an efficient dormancy breaking method to provoke seed germination (Rolston, 1978). The physical scarification allows water and air to enter into the seed and stimulate germination which ended up with the elongation of the embryonic axis (Holdsworth *et al*., 2008).

Acid treatments for 15 min with Conc. H_2SO_4 and Conc. HNO_3 also break the dormancy and record germination of 55 % and 22 % respectively but at significantly low levels compared to physical scarification. According to Eira *et al*., (1993), in nature, enhancement of water up taking process can also occur through the action of acids during seed digestion by dispersing animals and the action of soil microorganisms. However, prolong soaking periods were not tested during the present study as according to Schmidt, (2000), time of immersing in the acid is critical since long soaking periods can excessively burn the seed coat and damage the embryo.

Hot water treatment is another frequently used method to overcome dormancy, whereby the seeds are soaked in hot water depending on the species and seed coat thickness for a specific period of time (Tadros *et al*., 2011). In the present study, hot water treatment at 100 ^{0}C for 5 minutes recorded 55 % germination. However, immersion in hot water for more than 5 minutes might have caused a high quantity of damaged or dead seeds hence germination was not observed. Under in-situ conditions, in natural areas the high soil temperature may be responsible for the permeability of the water to the seeds to break the dormancy (Baskin *et al.*, 2000).

According to previous studies, other species in the family Malvaceae have recorded an exogenous physical dormancy caused by an impermeable seed coat (Halse and Mishaga 1988; Baskin and Baskin 1998; Baskin *et al*., 2000). Being a member of the same family, *G. damine* also recorded a similar phenomenon, and to break dormancy, out of the methods used, scarification with a sand paper can be recommended as the best method.

A major challenge to increase the use of native species in landscape industry is providing native plants that are both ecologically functional and economically

viable (Wilde *et al*., 2015). According to McKay *et al*., (2005), attention should also be paid to genetic diversity and local adaptability of the plants. As *G. damine* is naturally occurring in all the three major climatic zones of the country and as there is no barrier for seed propagation with the current findings, propagation of *G. damine* via seeds could be used as a cost-effective method by the nurserymen to produce the necessary plant material for landscape or habitat restoration projects in home gardens or other public open spaces.

Conclusions

The results of the present study suggests that seeds of *G. damine* possess a physical dormancy as germination resumes with dormancy breaking treatments. The scarification with a sand paper proved to be the most effective method of breaking seed dormancy as it recorded significantly high germination percentages and other germination parameters. Hence seeds could be used by the nurserymen after breaking dormancy to produce plant material at large scale.

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