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Original article

Breaking seed dormancy in smooth loofah (*Luffa cylindrica* (L.) M. Roem.) using scarification and dry heat treatment

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ABSTRACT

Hard seed dormancy is a common problem with smooth loofah seed. Scarification, clipping and dry heat were used to break dormancy in smooth loofah seed at the Department of Horticulture, Kasetsart University, Bangkok, Thailand. A completely randomized design with 20 treatments was used involving: untreated seed (control), clipping, scarifying by scarifier at 40, 70 and 100 revolutions per minute (rpm) for 1 min, and dry heat at 60 °C, 70 °C and 80 °C for 1–5 h. After breaking dormancy, seed germination was tested in four replicates, with 50 seeds per replicate. The thickness of the seed coat was measured under a digital microscope. The results showed that clipped seeds gave the highest germination (100%) and decreased mean germination time (3.58 d). Scarified seed using a scarifier at 70 and 100 rpm for 1 min resulted in germination rates of 67.0–75.5%, which was higher than for seeds scarified at 40 rpm. The dry heat-treated seeds at 60 °C for 3–5 h and at 70 °C for 2–5 h had germination of 71.0–80.5%. The outer layer of seed coat scarified at 100 rpm for 1 min was thinner than those of un-scarified seed samples. Dry heat had no effect on the seed coat thickness, but affected cells of the inner seed coat as the sclerenchymous cells showed disordered characteristics and were non-uniform and seemed to have been torn off. Dry heat treatment and scarification significantly improved germination compared to the control treatment. However, 80% germination may not be considered as an effective method at a commercial scale where 100% germination is needed. Further investigation of more accessions that may have different seed coat thicknesses may be needed.

Introduction

Luffa cylindrica (L.) M. Roem syn *L. aegyptiaca* Mill, commonly called smooth loofah or sponge gourd, is a member of the Cucurbitaceae family. The plants are economically important in many parts of the world—for instance, in China, Korea, Japan, India, Central America, as well as in Thailand—for their young fruits, which are used as ingredients for food, while ripening fruits are also used to produce consumer goods such as cleaning materials and engine oil filters (Oboh and Aluyor, 2009). The seeds are composed of 46% oil and 40% protein (Siemonsma and Piluek, 1993).

Some cucurbit species have severe problems with seed dormancy and viable seeds cannot germinate even in favorable environments because of seed coat impermeability is considered a major mechanism causing hard seed dormancy (Bradbeer, 1988). Smooth loofah seed is considered hard-seeded with its thick seed structure and a hard seed coat; moreover, phenolic compounds including pectin or suberin on the surface of the seed coat restrict water uptake into the seed (Doijode, 2001). Physical dormancy is caused by one or more water-impermeable layers of palisades (Baskin and Baskin, 2004). In addition, Singh and Dathan (1998) found that the seed coat of smooth loofah is characterized by upright epidermal cells with rod-like thickenings and narrow, palisade-like osteosclereids which cause physical dormancy of smooth loofah seed. Seed dormancy is the most important factor limiting germination and there are various ways to break hard seed coat such as clipping, scarifying, and dry heat (Bradbeer, 1988).

One technique that has been widely and successfully used for breaking hard seed dormancy is scarification, which involves removing the seed coat or rubbing it with sandpaper or subjecting it to a temperature treatment (Bradbeer, 1988). Pinmanee et al. (2001) reported that cutting the bottom of the bitter gourd seed, but not removing the seed coat completely, increased germination from 40.5% to 70%, while germination was increased to 90% by removing the entire seed coat. The germination of watermelon seed

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has also been increased by removing the seed coat (Nerson, 2002). Loyma et al. (2009) reported that removing the seed coat of wax gourd seeds increased radicle emergence with scarification of 'Feang' and 'Fakkheaw' seeds by removing the outer testa and the outer and inner testa, significantly improving radicle emergence to 96.0% and 99.5%, respectively, of 'Feang' seeds and to 93.0% and 95.5%, respectively, of 'Fakkheaw' seeds, compared to the un-scarified seeds (83.0% for 'Feang' and 80% for 'Fakkheaw'). However, scarification may lead to embryo damage, abnormal seedlings, and dead seeds (Bradbeer, 1988).

Another technique for breaking hard seed dormancy is dry heat, which causes seed coat and perisperm dehydration and allows water and gases to enter the seed more quickly (Khan, 1980). The heat temperature for breaking dormancy in tropical and subtropical seeds is about 40–50 °C (ISTA, 2010). Sinviriyanon et al. (2011) reported that preheating at 70 °C could increase the seed germination of smooth loofah and decrease the mean germination time; however, the effect of preheating on seed germination depends on the accession. Moreover, a preheating treatment at 70 °C and 80 °C for 2, 4 and 6 h tended to increase the seed germination of smooth loofah but the germination was lower than 50% (Chamnongrit et al., 2011).

Seed dormancy is often a problem when planting smooth loofah as seeds have a low germination percentage and uniformity (Doijode, 2001). Therefore, the objective of this study was to determine the suitability of various techniques for breaking the dormancy of smooth loofah seed.

Materials and methods

Seed materials

Smooth loofah seeds of LF-01 were obtained from Chia Tai Co., Ltd (Bangkok, Thailand). Seeds were harvested and processed in September 2010. This study was conducted from October 2010 to January 2012 in a laboratory at the Department of Horticulture, Faculty of Agriculture, Kasetsart University, Bangkok, Thailand. The initial seed qualities tested were: 8.4% seed moisture content, 97.1 g per 1000 seed weight; 100% seed viability by tetrazolium test, 56.0% germination and 35.5% hard seed.

Methods of breaking seed dormancy

Seeds were treated in the laboratory to study methods of breaking dormancy. The experiment was arranged in a completely randomized design with 20 treatments. It consisted of two methods for breaking dormancy. The first method involved mechanical scarification and was carried out by clipping and scarification. Seeds were clipped opposite the embryo using a scalpel. Seeds were scarified using a scarifier at 40 revolutions per minute (rpm), 70 rpm and 100 rpm for 1 min. The second method was a dry heat treatment. Seeds were dried in a hot air oven at 60 °C, 70 °C and 80 °C for 1–5 h and then the seeds were kept in a desiccator for 30 min. Seeds after breaking dormancy were tested for quality in four replicates with 50 seeds per replicate.

Germination

Fifty seeds per replicate for four replicates were germinated in moist sand in a plastic box and kept in the germinator at 30 °C. First and final counts were done at 4 and 14 d after testing, respectively (ISTA, 2010). Normal seedlings were calculated as the germination percentage. The percentage of hard seeds was reported as that part of the germination percentage that remained viable with hard seed that could not absorb water.

Table 1
Germination and hard seed of smooth loofah by different methods of breaking dormancy.

Method of breaking dormancy	Germination (%)	Hard seed (%)
1. Untreated seeds (control)	56.0 ^{h†}	35.5 ^{a}
2. Clipping	100.0 ^{a}	0.0 ^{j}
3. Scarifier at 40 rpm for 1 min	63.0 ^{gh}	20.0 ^{bcd}
4. Scarifier at 70 rpm for 1 min	67.0 ^{efg}	23.5 ^{b}
5. Scarifier at 100 rpm for 1 min	75.5 ^{bcd}	16.5 ^{bcdef}
6. Dry heat at 60 °C for 1 h	66.0 ^{fg}	22.0 ^{bc}
7. Dry heat at 60 °C for 2 h	68.5 ^{defg}	19.5 ^{bcde}
8. Dry heat at 60 °C for 3 h	71.5 ^{cdef}	18.5 ^{bcdef}
9. Dry heat at 60 °C for 4 h	73.0 ^{bcdef}	17.5 ^{bcdef}
10. Dry heat at 60 °C for 5 h	80.5 ^{b}	12.5 ^{defgh}
11. Dry heat at 70 °C for 1 h	74.0 ^{bcdef}	17.5 ^{bcdef}
12. Dry heat at 70 °C for 2 h	78.0 ^{bc}	11.5 ^{efghi}
13. Dry heat at 70 °C for 3 h	80.0 ^{b}	11.0 ^{fghi}
14. Dry heat at 70 °C for 4 h	77.5 ^{bc}	7.5 ^{hi}
15. Dry heat at 70 °C for 5 h	75.0 ^{bcde}	13.0 ^{defgh}
16. Dry heat at 80 °C for 1 h	70.0 ^{cdefg}	15.5 ^{cdefg}
17. Dry heat at 80 °C for 2 h	26.5 ^{i}	4.0 ^{ij}
18. Dry heat at 80 °C for 3 h	22.5 ^{i}	4.0 ^{ij}
19. Dry heat at 80 °C for 4 h	21.0 ^{i}	8.5 ^{ghi}
20. Dry heat at 80 °C for 5 h	9.5 ^{j}	13.5 ^{defgh}
F-test	*	*
Coefficient of variation (%)	8.03	36.45

rpm = revolutions per minute.
* = significantly different at $p < 0.05$.
† = mean values in the same column followed by the same letter are not significantly different at $p < 0.05$ by Duncan's Multiple Range Test.

Mean germination time

Seeds were germinated as in germination test. The number of normal seedlings was counted from the day of the first count up to the day of the final count (14 d after testing). The mean germination time (MGT) was calculated using Equation (1) (Ellis and Roberts, 1981):

$$MGT = \sum nd/\sum n \quad (1)$$

Table 2
Mean germination time of smooth loofah seeds by different methods of breaking dormancy.

Method of breaking dormancy	Mean germination time (d)
1. Untreated seed (control)	5.17 ^{efg†}
2. Clipping	3.58 ^{h}
3. Scarifier at 40 rpm for 1 min	5.92 ^{cde}
4. Scarifier at 70 rpm for 1 min	5.45 ^{defg}
5. Scarifier at 100 rpm for 1 min	5.43 ^{defg}
6. Dry heat at 60 °C for 1 h	4.67 ^{g}
7. Dry heat at 60 °C for 2 h	5.38 ^{defg}
8. Dry heat at 60 °C for 3 h	5.37 ^{defg}
9. Dry heat at 60 °C for 4 h	4.95 ^{fg}
10. Dry heat at 60 °C for 5 h	4.87 ^{g}
11. Dry heat at 70 °C for 1 h	4.77 ^{g}
12. Dry heat at 70 °C for 2 h	5.75 ^{cdef}
13. Dry heat at 70 °C for 3 h	6.02 ^{cd}
14. Dry heat at 70 °C for 4 h	6.58 ^{bc}
15. Dry heat at 70 °C for 5 h	5.11 ^{efg}
16. Dry heat at 80 °C for 1 h	6.45 ^{bc}
17. Dry heat at 80 °C for 2 h	6.41 ^{bc}
18. Dry heat at 80 °C for 3 h	6.87 ^{b}
19. Dry heat at 80 °C for 4 h	8.16 ^{a}
20. Dry heat at 80 °C for 5 h	8.20 ^{a}
F-test	*
Coefficient of variation (%)	9.03

rpm = revolutions per minute.
* = significantly different at $p < 0.05$.
† = mean values in the same column followed by the same letter are not significantly different at $p < 0.05$ by Duncan's Multiple Range Test.

Table 3
Thickness of smooth loofah seed coat using different methods of breaking dormancy.

Treatment	Thickness of seed coat (mm)		
	Outer[a]	Inner[b]	Total
1. Untreated seed (control)	0.045 ^{A†}	0.211 ^{AB}	0.256 ^{A}
2. Scarifier at 100 rpm for 1 min	0.023 ^{B}	0.199 ^{B}	0.222 ^{B}
3. Dry heat-treated seed at 60 °C for 5 h	0.038 ^{A}	0.220 ^{A}	0.258 ^{A}
t-test	*	*	*
Coefficient of variation (%)	12.40	3.86	3.43

rpm = revolutions per minute.
*Significantly different at $p < 0.05$.
† = mean values in the same column followed by the same letter are not significantly different at $p < 0.05$ by Least Significant Difference.
[a] Outer seed coat composed of epidermal cell to hypodermis.
[b] Inner seed coat composed of sclerenchymatous main mechanical layer.

where n is the number of normal seedlings which germinated on day d and d is the number of days counted from the beginning of the test.

Measurement of seed coat thickness

Seeds from three treatments: 1) untreated seed (control), 2) scarified at 100 rpm for 1 min and 3) dry heat at 60 °C for 5 h were studied for seed coat structure. Seeds were cut longitudinally, and the thickness of the seed coat was measured under a digital microscope (Dino-lite; Taipei, Taiwan). The thickness of the outer seed coat was measured between the epidermal cell to the hypodermis and the inner seed coat was measured at the sclerenchymatous main mechanical layer.

Statistical analysis

All data were subjected to ANOVA according to the completely randomized design used in this study and means were compared using Duncan's Multiple Range Test.

Results

The effectiveness of the methods at breaking dormancy in smooth loofah seeds is shown in Table 1. There was a statistical difference in seed germination among the treatments. The clipped seeds gave the highest germination of 100% (nearly double the germination of the untreated seeds of 56.0%), followed by dry heat at 60 °C for 5 h and dry heat at 70 °C for 3 h (80.5% and 80%, respectively). However, dry heat-treated seeds at 80 °C for 2–5 h had low germination (9.5–26.5%) and most of the seeds were dead in these treatments (data not shown). Seeds scarified at 40 rpm, 70 rpm and 100 rpm for 1 min tended to increase in germination with rates of 63.0%, 67.0% and 75.5%, respectively, in line with the increased speed of the scarifier. Moreover, hard seeds ranged from 16.5% to 23.5% which was significantly lower than for the untreated seeds (35.5% hard seeds). However, scarifying seeds at higher speeds increased seed germination from 7.0% to 19.5% and the hard seed decreased from 12 to 19% when compared with the control.

Mean germination time

The mean germination time (MGT) of smooth loofah seeds by different methods of breaking dormancy is shown in Table 2. The clipped seeds gave the shortest MGT of 3.58 d, which was the fastest germination (Table 2) and had the highest seed germination rate of 100% (Table 1). Scarified seeds using a scarifier at 40 rpm, 70 rpm and 100 rpm for 1 min showed that scarification at higher speeds tended to decrease the MGT from 5.92 to 5.43 d, but this was not significantly different when compared with untreated seeds (5.17 d). The MGT of dry heat at 60 °C for 1–5 h was not significantly different either. However, dry heat-treated seeds at 60 °C for 1 h had the shortest mean germination (4.67 d). On the other hand, dry heat-treated seeds at 70 °C for 3–4 h, and at 80 °C for 1–5 h had the highest MGT of 6.02–8.20 days, which was the slowest speed of germination.

Effects of scarification and dry heat on seed coat structure

Scarified seeds using the scarifier at 100 rpm for 1 min had the lowest thickness of the inner, outer and total seed coat: 0.023, 0.199 and 0.222 mm, respectively (Table 3). However, there was no significant difference between the dry heat and untreated seeds. Dry heat-treated seeds at 60 °C for 5 h had the highest thickness of inner, outer and total seed coat, but the difference was not statistically different when compared with the untreated seeds (control). Fig. 1 shows the comparison of seed coat structure between untreated seed, scarified seed and dry heat-treated seed. In the untreated seed, the color of outer seed coat was black and the inner layer of seed coat had the cells arranged in an orderly fashion that prevented the imbibition of water (Fig. 1A). The seed coat of scarified seed was thinnest with the outer layer removed, but the inner layer of seed coat remained (Fig. 1B). Dry heat had no effect on the seed coat thickness, but affected the cells of the inner seed coat as the sclerenchyma cells appeared to be non-uniform and torn off (Fig. 1C).

Discussion

Hard seed dormancy is caused by an impermeable seed coat (Bradbeer, 1988). The seed coat structure of smooth loofah is thick

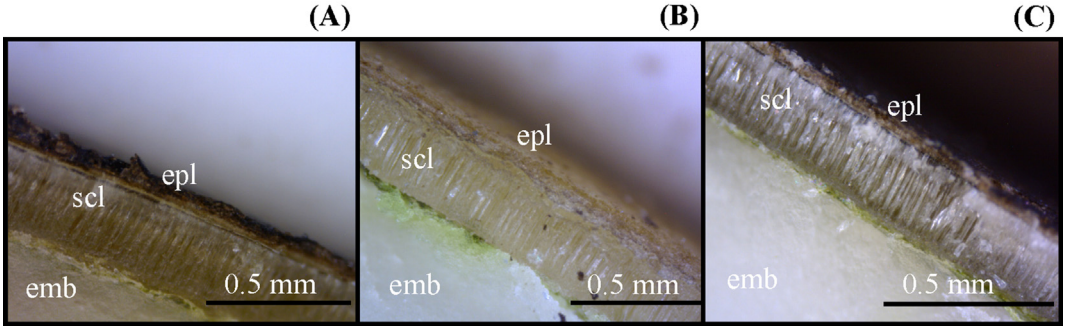

Fig. 1. Seed coat structure of smooth loofah: (A) untreated seed; (B) scarified seed using scarifier at 100 revolutions per minute for 1 min; (C) dry heat at 70 °C for 5 h (scale bar = 0.5 mm; emb = embryo; epl = epidermal cell; scl = sclerenchymatous main mechanical layer).

and hard, and has phenolic compounds including pectin and suberin on the surface of seed coat that restrict water uptake into the seed (Doijode, 2001). Physical dormancy is caused by one or more water-impermeable layers of palisades (Baskin and Baskin, 2004). In addition, Singh and Dathan (1998) found that the seed coat of smooth loofah is characterized by upright epidermal cells with rod-like thickenings and narrow, palisade-like osteosclereids which caused the physical dormancy of smooth loofah seed.

The clipped seeds had the highest germination percentage and germinated quickly within 3.58 d (Tables 1 and 2) because clipping the seed coat opens channels for air and water to uptake into the seeds faster than in other treatments. Clipping is not appropriate for use in large quantities in commercial applications because it is time and labor intensive, but it is suitable for breaking dormancy in small quantities of seeds (Wang et al., 2011). Seeds scarified at 100 rpm for 1 min had a germination rate of 75.5% (Table 1). Scarifying the seed coat at high speed damaged only the outer seed coat, but had no effect on the inner seed coat; however, the seed coat of scarified seeds was the thinnest (Table 3 and Fig. 1B). Mechanical scarification using a scarifier is a more practical method of scarification than clipping. Thus, a scarifier has direct commercial applications for increasing stand establishment. On the other hand, dry heat at 60 °C and 70 °C for 1–5 h and at 80 °C for 1 h had a higher germination percentage than untreated seeds. Dry heat had no effect on the seed coat thickness, but affected the cells of the inner seed coat as the sclerenchymous cells appeared to be non-uniform and seemed to be torn off (Fig. 1C). According to Ballard (1973) and Egley (1989), dry heat enhances seed germination because the structure of the seed coat is damaged in the palisade layer. High temperature promotes cracking of the seed coat (Corral et al., 1989). This structural alteration improves imbibition uptake, further increasing the germination percentage. However, dry heat at 80 °C for 2–5 h decreased seed germination compared with the control, probably due to the high temperature and long duration of dry heat which damaged the seeds. Similar results were reported when seeds were exposed to temperatures of 90 °C and higher for periods of three hours or more (Food and Agriculture Organization, 1983). A major advantage of the dry heat method is that it can be used to process large batches of seeds at once, making it a simple and time-saving process. Because of this, dry heat can also be developed as a practical guide for the seed industry. However, dry heat and scarification must be performed carefully using a cultivar with a thinner seed coat as otherwise, the seed could be damaged.

In conclusion, breaking dormancy by clipping had the highest germination of 100% and the lowest mean germination time of 3.58 d. Scarified seed using a scarifier at 70 and 100 rpm for 1 min produced a germination rate of 67.0–75.5% which was higher than in control seeds (56.0%). Dry heat at 60 °C for 3–5 h and dry heat treatment at 70 °C for 2–5 h resulted in germination rates of 71.0–80.5%. The study of the seed coat structure showed that the outer layer of a seed coat scarified at 100 rpm for 1 min was thinner than that of un-scarified seed. Dry heat had no effect on the seed coat thickness, but affected the cells of the inner seed coat, as the sclerenchymous cells showed disordered characteristics, were non-uniform and seemed to be torn off.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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