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DOES SEED HARDNESS BREAKING TECHNIQUES SHOW DIFFERENT EFFECT ON GERMINATION: A TEST OF THREE OKRA VARIETIES?

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Abstract

Okra (*Abelmoschus esculentus* (L) Moench) being among the most important crops grown in northern Nigeria is supposed to be produce in high quantity, but germination of this crop is faced with different challenges due to seed dormancy. This study was conducted in order to compare three techniques employed in breaking seed dormancy of three varieties of *A. esculentus*. The study was carried out at Botanical Garden, Gombe State University in 2016. Three varieties of *A. esculentus* namely, Chalawa, Dan Beru and El-Muazu were used. Three treatment techniques employed were seeds soaked in 80% concentrated H_2SO_4 for three minutes, seeds soaked in 80 °C hot water for 12 hours, seeds soaked in water at room temperature for 12 hours and seeds that were not treated (control). Results showed that 80 % concentrated H_2SO_4 for three minutes was the most effective in breaking of *A. esculentus* seed dormancy. Although hot water at temperature 80 °C for 12 hours decreased percentage of germination than seeds soaked in water at room temperature, this was restricted to Chalawa and Dan Beru varieties. The results of this study suggest that percentage of germination among the three varieties of *A. esculentus* differs. Therefore, the long period of *A. esculentus* seed dormancy occurring under natural conditions which is at least ten days; can be shortened to a few days (two days) by subjecting seeds to 80 % concentrated H_2SO_4 for 3 minutes and water at room temperature for 12 hours particularly for Chalawa and Dan Beru varieties respectively. Our study highlights the need for careful examination of seeds to detect dormancy and the need for proper application of the right treatment for each variety as suggested by the variation in response to treatment by El-Muazu.

Keywords: Dormancy, Seed treatment, Okra, Germination.

Introduction

Abelmoschus esculentus is a tropical to sub-tropical crop and is sensitive to frost; low temperatures, water logging and drought conditions, and the cultivars from different countries have certain distinct adaptive characteristics specific to the country to which the crop is grown (Kochlar, 1986). The crop is cultivated from August to September in north eastern Nigeria, where this study was carried out. Immature fresh and green seed pods are consumed as vegetable. It offers mucilaginous consistency after cooking. Often the extract obtained from the fruit is added to different recipes like soup, stews and sauces to increase the consistency (Lim, 2012; Jain *et*

al., 2012; Maramag, 2013). Okra seeds powder is also a good substitute for aluminium salts in water purification (Valenti *et al.*, 1989). A mature *A. esculentus* seed is a good source of oil and protein known to have superior nutritional quality (Kochklar, 1986). *Abelmoschus esculentus* seed oil is rich in unsaturated fatty acids such as linoleic acid, which is essential for human nutrition and wellbeing. This vegetable (fruit and leaves) is rich in vitamins, calcium, potassium and other minerals. Its mature fruit and stems contain crude fibre, which is used in the paper industry (Kochlar, 1986).

Propagation of *A. esculentus*, just like most tropical crops, can be limited by several factors among which are low rate of germination, seedling establishment and recruitment. While many studies focused on yield or seed production as indicators of reproductive success, there is need to consider early stages of crop reproductive failure due to some inhibitors such as seed dormancy. Delayed germination as a result of dormancy often distorts the reproductive circle and cause farmers to sometimes replant (Collen *et al.*, 2015). Poor and delayed seed germination due to dormancy is one of the major challenges in the propagation of *A. esculentus*. This is exacerbated by the fact that *A. esculentus* plants unlike other crop plants are mainly propagated by seeds. Dormant seeds often do not germinate under normal conditions and require manipulation through mechanical, thermal or chemical means to eliminate the limiting factor which is often a hard seed coat. This is crucial considering the fact that most viable seeds with high yield potential may be limited at this stage resulting in waste of time, resources and energy. It is therefore less likely to measure reproductive success for these naturally inhibited seed varieties or species. Plant reproduction is a cycle that can only be assessed when seed germinates, develop to mature plant and produce fruits with potentially viable seeds (fecundity). Fecundity (reproductive success) is affected by several factors, key among them being; low levels of seed viability or seed dormancy. The presence of a hard, water-impermeable seed coat and embryo dormancy has made natural regeneration of some crops very difficult for farmers (Duan *et al*., 2004). It could be that the seeds exhibit some form of dormancy, possibly associated with the seed coat. This is the first factor to be checked if seeds do not germinate after-ripening period (Debeaujon *et al*., 2000). The capacity of many seeds to germinate is determined by the seed dormancy. By establishing a permeability barrier, vital materials such as water required for imbibitions and subsequent radical emergence are not limited. This barrier imposed by the seed coat also obstructs gaseous exchange, particularly oxygen uptake required for respiration; and/or the outward diffusion of endogenous germination inhibitors (Koornneef *et al.*, 2002; Yasseen *et al*., 1994). Typical characteristics of hard seeds are seed coats that are permeable to water but not to gases or vice versa (Debeaujon *et al*., 2000; Kikuchi *et al*., 2006; Manz *et al*., 2005).

Scarification has been one of the methods traditionally used to break seed dormancy (Fariman *et al*., 2011; Garcı´a-Gusano *et al*., 2004; Purquerio *et al.*, 2010; CAN *et al*., 2009). In order to accelerate this

process, the application of H_2SO_4 and hot water to remove seed coat has been used in many species (Joshi *et al*., 1998; Aliero 2004; Duan and Pant, 2010). Some form of scarification is usually needed to make hard seed coats permeable to water or gases. This can be accomplished by means of mechanical, thermal or chemical treatments (Debeaujon *et al*., 2000; Mohammadi *et al*., 2012). Collen *et al*., (2015) reported that soaking seeds of wild okra (*Corchorus olitorius*); in hot water at 80 ^oC for 10 minutes seem to be an effective combination of treatment and duration for enhancing germination with about 95 % for wild okra. It was observed that hot water a form of thermal scarification (Debeaujon *et al*., 2000), breaks physical (seed coat) dormancy in seeds by causing cracks in seed coats without altering the anatomy of the micropyle. Hot water may also have other influences such as causing thermal shock to the embryo, or leaching of inhibitors (Debeaujon *et al*., 2000). Song *et al*., (1996) reported that seeds of *Desmathus* spp. were damaged and no germination resulted when they were soaked in boiling water at 100 ^oC for 15 minutes. On the other hand, a study indicated 100 % germination of fodder legume seeds soaked in boiling water at 100 ^oC, but the exposure time was relatively short (3–6 minutes) (Auld *et al*. 1988). Considering the confounding effects of variable factors such as type of treatment (soaking, heating and chemical scarification), optimum duration of treatment, and varietal features of seeds, further research is needed to determine what factors are crucial across a broad spectrum of seeds and species with regards to their dormancy. This approach will enable the development of a technique that is applicable to a wide range of seeds of various cultivars and species.

Abelmoschus esculentus can only be propagated from seeds and its production by both communal and commercial farmers in northern Nigeria is very low due to seed dormancy. Since physical dormancy in *A. esculentus* results in poor germination and negatively affect yield. We hypothesized that: i) the use of chemical (Sulphuric acid) for scarification and stratification time for breaking *A. esculentus* seed dormancy is an effective method to enhance *A. esculentus* seed germination. ii) Hot water treatment will enhance germination of *A. esculentus* seed.

Methodology

Study area

The study was conducted at the Botanical Garden, Gombe State University; Gombe. The University is located between latitude 10^o 18.6' N and longitude 11^o 26.3' E (Figure 1).

Experimental treatments

The experimental treatments were (i) seeds soaked in 80% concentrated sulphuric acid (H_2SO_4) for 3 minutes, (ii) seeds soaked in hot water at 80°C for 12 hours, (iii) seeds soaked in water at room temperature for 12 hours and (iv) control seeds─neither chemical nor water was used. Replication was done twice for each treatment.

Figure 1: Map of the University.

A total of 360 mature *A. esculentus* seeds comprising one hundred and twenty (120) seeds from each variety (Chalawa, Dan Beru and El-Muazu) were collected for this study at the Seed Council, Government Residential Area (GRA), Gombe and the Federal Ministry of Agriculture, Gombe, Gombe State, Nigeria. Forty (40) seeds of each variety were obtained and sub-divided into four (4) with ten seeds in each division. Seeds were sterilised with Mankuzeb fungicide to avoid fungal infection before use in the experiment. Physical viability observation was carried out prior to setting up the experiment. Okra seeds were soaked in a bucket three quarter filled with water and floating seeds were discarded as not viable seeds. The viable seeds were found at the bottom of the bucket and were used in this experiment. For each of the three varieties, 10 seeds were placed in beakers labelled with masking tape and concentrated Sulphuric acid was added to each variety in different beaker. A stop watch was used to measure time of exposure to H_2SO_4. After exposing the seeds for 3 minutes, seeds were then removed from the acid. The removed seeds were immediately washed thoroughly with water to neutralize the acid. The seeds were then dried under shade condition before use for the germination tests.

In the second treatment; ten okra seeds of the three varieties in different beakers were soaked in hot water at 80 ºC for three minutes. In the third treatment ten okra seeds of the three varieties were soaked in a beaker containing water at room temperature for a period of 12 hours. All seeds of these treatments were dried under shade condition then used for germination tests and in the fourth, ten okra seeds of the three varieties were not exposed to any treatment (control). The seeds were then sowed in the field. The experiment was replicated three (10 x 4 x 3) times. Data

collection on germination commenced on the next day.

Effects of treatment on Germination

Germination was determined starting with day one and was terminated at day two for each treatments and the control. Seeds were regarded as germinated when the radical appeared at least equal to half length of the seed. Germinated seeds were counted and their numbers were recorded daily.

Data collected on germination of the three treatments and control were analysed using SPSS version 15.0 to determine the percentage of the different treatments on germination of the three varieties we followed the formula of International rules for seed testing (ISTA, 2009).

Results

Results indicate that less than a quarter (< 90 seeds) of the total number of okra seed varieties used in this experiment germinated. In fact, only 84 of 360 seeds germinated, corresponding to 23.3% germination after two days of planting.

Germination success among *A. esculentus* varieties differed with treatments. This was more profound for seeds treated with 80 % Concentrated H_2SO_4 across the three varieties (9, 3 and 24 seeds germinated for *Chalawa, Dan Beru and El Muazu* seed varieties respectively) (Table 1).

Table 1: Varieties of *A. esculentus* used and the treatments applied with total number of germinated seeds

Variety	Number of seeds planted	Treatment	Day one	Day two	Total seeds germinated	% germination
Chalawa	30	80% Conc. H_2SO_4	0	9	9	30
Chalawa	30	Hot Water (80° C)	0	6	6	20
Chalawa	30	Soaked (Room Tempt)	0	15	15	50
Chalawa	30	Control	0	0	0	0.0
Total	**120**				**30**	
Dan Beru	30	80% Conc. H_2SO_4	0	3	3	16.7
Dan Beru	30	Hot Water (80° C)	0	3	3	16.7
Dan Beru	30	Soaked (Room Tempt)	0	12	12	66.6
Dan Beru	30	Control	0	0	0	0.0
Total	**120**				**18**	
El Muazu	30	80% Conc. H_2SO_4	0	24	24	66.7
El Muazu	30	Hot Water (80° C)	0	9	9	25.0
El Muazu	30	Soaked (Room Tempt)	0	0	0	0.0
El Muazu	30	Control	0	3	3	8.3
Total	**120**				**36**	

Tempt = Temperature
Conc. = Concentration

Altogether, seeds soaked in concentrated H_2SO_4 for three (3) minutes achieved the highest germination success, which in this study was 40 % (i.e. 36 out of 90 seeds planted). Seeds soaked in water at room temperature for 12 hours trailed with (27 out of 90 seeds) i.e. 30 % germination success; followed by hot water treatment

with 18 seeds, corresponding to 20 % germination success. Untreated seeds used as control had the lowest germination success with only 3.3 % (i.e. three seeds out of 90 seeds) germinating.

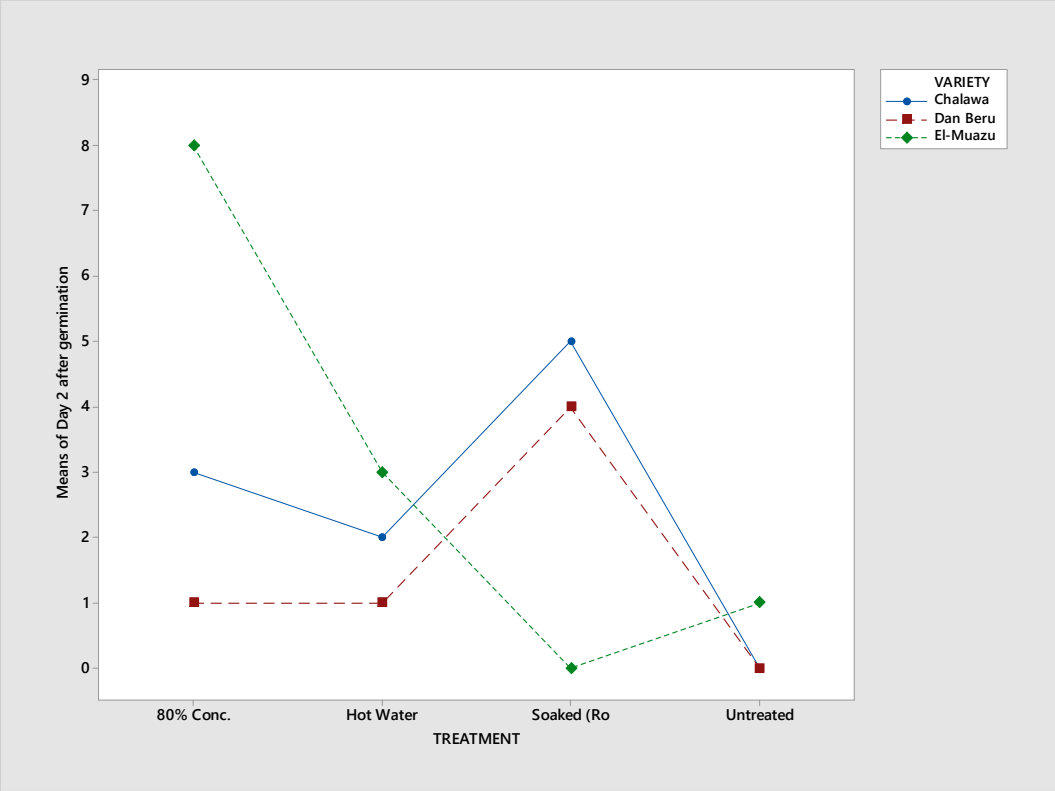

Figure 2: Interaction plot of day 2 after germination between Variety and treatment effects of the different Okra varieties.

Similarly, of the 120 seeds for each of the three *A. esculentus* varieties making a total of 360 seeds on the whole; 30 Chalawa seeds, 18 Dan Beru and 36 El Muazu seeds germinated regardless of whether seeds were treated or not. However, treatment with H_2SO_4 accounted for the total number (40 %) of germinated seeds for Chalawa and Dan Beru varieties; suggesting that treatment was obligate for germination for these two varieties. On the contrary, treatment H_2SO_4 accounted for 66.7 % (26 of 36 seeds germinated) for El Muazu. El Muazu seeds were the only *A. esculentus* variety that germinated under the control (i.e. seeds not exposed to treatment) method with 8.3 % (i.e. 3 of 36 seeds) germination.

Discussion

The response of *A. esculentus* varieties to treatment could be used as a probable indication of the remote factors responsible for its dormancy. Integument

breaking or softening, for instance, is needed to remove dormancy imposed by seed coat hardness or impermeability. The broad range in germination period is likely due to the inherent differences in morphological/physiological constraints of *A. esculentus* varieties.

Based on this notion, we can say that our three okra varieties differ in their morphological and physiological make-up. This assertion is drawn from the way in which each variety responded to the various treatments applied. For instance the strong inhibitory effect of seed coat on seed germination might have been more profound for El Muazu considering the level of germination success (24 out of 30 seeds) recorded when this variety was scarified with concentrated H_2SO_4 (Table 1). Scarification with H_2SO_4 has proven to be quite successful for treatment of exogenous dormancy where mechanical and thermal measures have failed (Garcı´a-Gusano *et al*., 2004; Purquerio, 2010).

Similarly, Dan Beru and Chalawa varieties responded well to thermal treatment (i.e. when seeds were soaked in water at room temperature), but displayed a drop in germination success when treated with hot water at 80 ºC. This drop in number of germinated seeds is probably an indication of intolerance to high temperatures (Table 1). It seems, probably soaking seeds in hot water causes damage to the embryo. Hot water at temperature of 80 ºC instead of giving more seed germination than at room temperature, but produced lower percentage of germination than soaked seeds at room temperature. Our results emphasize the need to test each method adequately to identify the optimum range and duration of treatment (see Popstov A.V. 1976).

The hardy nature of El Muazu seeds as opposed to Chalawa and Dan Beru is further strengthen by its apparent tolerance of hot water at 80 ºC, resulting in the most successful germination under this treatment across all three varieties (Table1). Furthermore, the effectiveness of thermal scarification with water at 80 ºC for seeds with hard coat has been reported by Auld *et al.*, (1988). Subjecting seeds to water at this temperature causes cracks in the seed coat enhancing gaseous and water flow for effective germination. However, there are reports of micropyle destruction and embryo shock when seeds with permeable coats were treated with water at this temperature (80 ºC) (Auld *et al*., 1988).

Generally, El Muazu variety was the most responsive in this study. Apart from displaying suitable response to chemical and thermal scarification, it was the only variety among used varieties that germinated in the control (absence of treatment). This study suggests that treatment could be obligate for successful germination of Chalawa and Dan Beru as these were the only varieties where treatment accounted for 100 % germination success. Although the information available through this experiment may not be sufficient to infer total dependence of Chalawa and Dan Beru varieties to dormancy treatment, further research may shed more light on this interesting observation.

While we know for a fact that germination success varies with treatment, as suggested by the results of this study, it must be

noted that this largely depends on the type of seed, its viability and cultivation under adequate ecological/ environmental conditions. Our results therefore could be limited by a host of factors, which may include but not restricted to soil type, adequate moisture, sowing depth and time of exposure to treatment.

Conclusion

This study indicates that the presence of hard seed coat limits the effectiveness of osmo-conditioning or hot water treatment on some okra seed varieties germination and that soaking of *A. esculentus* seeds in 80 % concentrated Sulphuric acid (H_2SO_4) for 3 minutes is the most effective in breaking okra seed hardness, followed by soaking the seeds in water at room temperature for 12 hours. However, the effectiveness of these treatments depends on the variety of *A. esculentus*.

Recommendation

Further studies to determine growth performance is likely to complement this work in order to achieve high yield of this vegetable.

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