Preliminary Evaluation of Different Seed Dormancy Breaking Methods in Wild Tuber Cowpea (*Vigna Vexillata*)

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ABSTRACT

Effectiveness of different seed dormancy breaking methods in *Vigna vexillata* seeds were evaluated. Two germination tests were done at 1 and 4 weeks after seed collection. A randomized completely block design (RCBD) with 8 seed dormancy breaking methods was used. The methods were; pre-chilling (PRC) in 5°C for 1 week, preheating (PRH) at 35°C for 1 week, sealed polythene envelops (SPE), gibberellic acid (GA_3) 0.05%, potassium nitrate (KNO_3) 0.2%, sulfuric acid scarification (H_2SO_4) and mechanical scarification (MS) and a control. The H_2SO_4 and MS had highest germination percentage. There was a significant difference in germination between seeds sown at 1 and 4 weeks after seed harvesting. Sowing *V. vexillata* seeds at 4 weeks after harvesting had higher germination percentage than 1 week after harvesting. Therefore H_2SO_4 and MS were effective in breaking seed dormancy at least 4 weeks after harvesting. It is important to repeat the experiment and perform viability tests prior to sowing as this study assumed that all seeds were viable.

Keywords: *Dormancy, Germination, Scarification, Mechanical, Storage, Viability, Wild seeds.*

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Highlights of this paper

- The study evaluated the effectiveness of 8 seed breaking methods on *Vigna vexillata* seeds.
- Seeds that were stored for 4 weeks had higher germination percentage than 1 week after seed harvest. H_2SO_4 and MS were the most effective methods in breaking the dormancy at 4 weeks after seed harvest.

1. INTRODUCTION

Vigna vexillata (wild tuber cowpea) belongs to the family Fabaceae and represents an intermediate form between *Vigna unguiculata* (cowpea) and *Vigna radiata* (mungbean) [1]. *Vigna vexillata* is a twining vine or prostate herb which is different from the usual cowpea because it forms a root tuber. This plant is mainly distributed in tropical Africa, Asia and Australia [2]. In Zimbabwe, the plant is wildly growing in most parts of Manicaland, Mashonaland East, Mashonaland Central, Masvingo and areas around Harare. It has been domesticated and is commercially produced in countries such as North-eastern and Southern India, Australia, Ethiopia, South Africa as well as Sudan [3] but unfortunately it has not been domesticated in Zimbabwe.

Vigna vexillata is an annual with good seedling vigour and wet season growth. This wild tuber cowpea has long pods which are normally covered with brown hairs which help to repulse pod bearing insects. Ecologically, it has a wide adaptability on poor soils, tolerance of water logging and spreading vigour [4]. Normally it is an excellent pioneer for poor lands as a cover crop, green manure or erosion control crop. In Africa and Australia it is highly regarded as a pasture crop [5]. Also its foliage has been established as highly palatable to herbivores and thus it is good for livestock feed. *Vigna vexillata* is a legume and has great potential to enriching soils nitrogen through the biological nitrogen fixation and can be used in many tropical cropping systems. *Vigna vexillata* has high above-ground biomass production that can help in soil conservation by reducing the rate of splash erosion. It is a potential fodder crop due to the high biomass production [5].

In India, the seeds have been used as pulses while the roots as a tuber [2]. *Vigna vexillata* is said to be characterized by significantly higher protein content than some known *Vigna* species which include *Vigna luteola*, *Vigna reticulata*, *Vigna oblongifolia*, *Vigna unguiculata dekindtiana*, *Vigna racemosa* and *Vigna ambacensis*. Grant, et al. [2] agrees that, *Vigna vexillata* seeds comprise approximately 25% of protein in wild accessions.

The tuber has a soft easily peeled skin and creamy edible tasty flesh which can be eaten raw just like sweet potatoes. Chandel, et al. [6] reiterates that *V. vexillata* is of great importance as the tubers contain approximately 15% of protein which is greater than in sweet potatoes and yams. However, there is no documented research on this crop in Zimbabwe. Therefore, if domesticated, the crop can solve the malnutrition problem especially in weanlings. Regardless of being a potentially multi-use crop, there are no documented efforts aimed at domesticating the *V. vexillata* in Zimbabwe. *Vigna vexillata* naturally propagates by seed, making seed dormancy is a major issue during its domestication. Much research has been done aimed at breaking dormancy of hard seeded temperate legumes, but there is still limited work on tropical and subtropical plant species [7]. Wild plant seeds are inherently dormant and may take long to germinate. International Seed Testing Association (ISTA) [8] noted that seeds of many wild species are most likely to have hard seed coats, therefore pretreating to enhance water imbibition during germination is necessary. In an attempt to domesticate a plant, seed germination tests are of critical importance. Therefore, this study was to evaluate the effectiveness of different methods for breaking seed dormancy in *V. vexillata*.

2. MATERIALS AND METHODS

2.1. Site Description

The experiment was done at the Department of Research and Specialist Services (DR&SS) in Seed Services germination laboratory, Harare Zimbabwe. DR&SS is situated about 4 km north of the Harare City Centre along 5th street extension. The area receives an average rainfall of 885 mm per year [9] falling between mid-October and mid-April. Mean monthly temperatures range from 12.3°C in June to 20°C in October [9]. The soils belong to the Fersiallitic group of the Kaolinitic order [10]. The germination cabinets had cool fluorescent artificial light inside with constant uniform temperature of 25%.

2.2. Experimental Design

Randomized complete block design (RCBD) with the model $Y_{ij} = \mu + \alpha_i + \beta_j + e_{ij}$ where Y_{ij} is the observation of the i^{th} treatment and j^{th} replicate, μ is the overall mean, α_i is the effect of the i^{th} treatment effect, β_j is the effect of the j^{th} block and e_{ij} is the usual NID (o, σ^2) random error term was used in this experiment with 8 methods of breaking dormancy which included; mechanical scarification (MS), concentrated sulphuric acid scarification (H_2SO_4), gibberellic acid (GA_3), sealed polythene envelopes (SPE), preheating (PH), pre-chilling (PC), potassium nitrate (KNO_3) and control which had no pretreatment . Four replicates of 25 seeds per each treatment were used and were 4 blocks (germination trays) per each treatment. The 8 trays were randomly placed on shelves in the germination cabinet ensuring adequate spacing and to avoid interference.

2.3. Seed Collection

Mature pods of *V. vexillata* were collected from the wild in Strathavern wetland, Herbarium and Botanic Gardens in Harare, Zimbabwe. The pods were collected in the last week of October 2014. The pods were picked at their physiological maturity stage and about to shatter though most pods were still closed. Then the pods were sun dried until a homogeneous moisture content was achieved and kept in khaki padded envelops. The envelopes were put in a cool dry place before use.

2.4. Seed Dormancy Breaking Methods Used in the Study

Method 1 treatment with potassium nitrate (KNO_3): A 0, 2% solution of potassium nitrate (KNO_3) was prepared by dissolving 2 g of KNO_3 in 1 L of water to saturate the germination substrate at the beginning of the test. Water was used to moisten thereafter.

Method 2 mechanical scarification (MS) with sandpaper: The seeds were sandpapered to improve permeability to moisture and gasses. Care was taken to scarify the seed coat immediately above the tip of the cotyledons in order to avoid damaging the embryo.

Method 3 scarification with concentrated sulphuric acid (H_2SO_4): The seeds were soaked in concentrated sulphuric acid (98%) for 5 min until the seed coat became pitted. After digestion, seeds were thoroughly washed in running water before sowing.

Method 4 pre-chilling (PRC): The replicates for germination were placed in contact with the moist paper in petri- dishes and kept at a low temperature of 5°C for 7 days. The seeds were then placed into the germination sand trays and later taken to the germination cabinet as was done to all the other treatments. Care was taken to avoid freezing.

Method 5 preheating (PRH): The non-imbibed seeds of the replicates for germination were heated at a temperature of 35°C with free air circulation for a period of up to 7 days before they were placed into germination trays with sand and later placed into the germination cabinet.

Method 6 sealed polythene envelopes (SPE): Germination trays were placed in sealed polythene envelopes and placed into the germination cabinet.

Method 7 use of gibberellic acid (GA_3): The germination sand was moistened with 0.05% solution of GA_3, which was prepared by dissolving 500 mg GA_3 in 1 L of water. The germination trays were then placed into the germination cabinets as with other treatments.

Method 8 control: The seeds were randomly placed in the germination trays containing sterilized moist sand without any pretreatment and then taken to the germination cabinet.

2.5. Sand Media Preparation

Sand which was free from fertilizer and chemicals contamination was graded and sterilized in-order to remove impurities and kill microbes. The sand was then sieved through a 0.8 mm × 0.05 mm (mesh) before use to achieve homogeneous soil aggregates. The pH of sand ranged from 6.0 to 7.5. The sand was then mixed with water at a rate of 2500 g soil to 250 ml of water per tray. The sand and water were thoroughly mixed by a shovel. Then sand was placed in germination tray and leveled using a depth determining slicer. A planting board was placed on top of the soil in the germination tray and clean seeds were sown. The seeds were dropped in each hole of the planting board which was later removed and the seeds covered with the sterilized sand. The trays were then covered with polythene and then taken to the germination incubator using a trolley.

2.6. Germination Test

The collected wild seed lot of *V. vexillata* was divided into two, the first lot was pre-treated with the dormancy breaking methods as described above in the first week of harvesting. The second lot was stored for four weeks after harvest before sowing. The germination tests were carried based on the standards of the International Seed Testing Association (ISTA) [11]. The seeds were sown in controlled environmental conditions in a laboratory cabinet germinator. Each germination test consisted of 800 seeds in total and 100 seeds were used for each treatment method. The 100 seeds were further divided into three replicates of 25 seeds each. Then sown in germination trays which were later covered with polythene bags to retain moisture and the seeds placed in the germination cabinet according to the experimental design. Seed germination conditions were constant for both sowing periods. The temperature in the germination cabinet was kept also constant at 25°C with an artificial light.

2.7. Data Collection

Percentage germination was recorded after 10 days of sowing. The un-germinated seeds were counted per each plot and a tetrazolium test performed to determine their viability. Normal germinated seedlings, fresh ungerminated and dead seeds in each category were calculated and recorded. A seed was considered germinated when the tip of the radical had grown out of the cotyledons. Dead seeds were recorded as the number of seeds that were neither hard, nor produced any part of a seedling, but were soft, discolored and mouldy at the end of the test period. Some seeds were dormant and failed to germinate under the ideal conditions of the germination, and remained clean, firm and showed potential (positive results from the tetrazolium test) to develop into a normal seedling.

2.8. Data Analysis

An analysis of variance (ANOVA) was run to determine the effectiveness of the 8 used methods in breaking seed dormancy in *V. vexillata*. Prior to the ANOVA analysis, the counting data was transformed using square root transformation in-order to conform to the ANOVA assumptions. GenStat version 10 was used in the data analysis.

3. RESULTS AND DISCUSSION

3.1. Effects of the Different Methods of Breaking Seed Dormancy on Germination

There were significant difference among the seed dormancy breaking methods (P< 0.001). The MS and H_2SO_4 had highest percentage (90 %) at both 1 & 4 weeks after seed harvesting Figure 1.

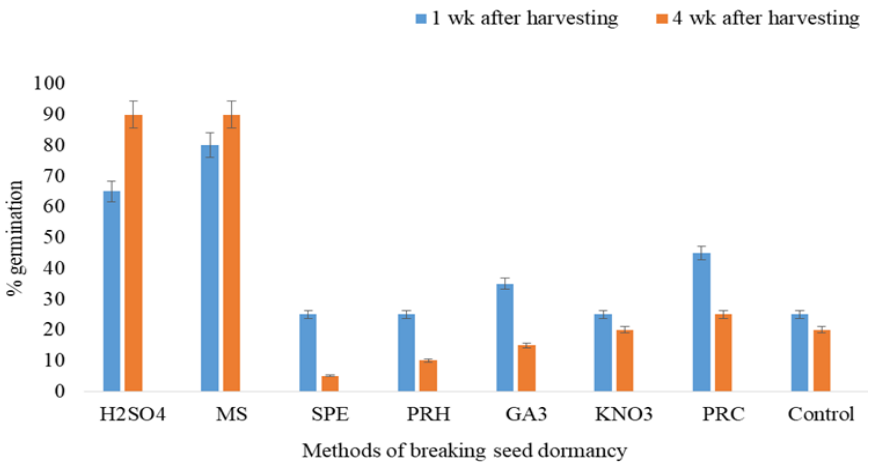

Figure-1. Effects of different methods of breaking seed dormany on germination.
Source: Experiment conducted in the Seed Testing Laboratory, Department of Research and Specialist Services, Harare, Zimbabwe; Bars shown – standard error.

The percentage germination was the least (5 %) on seeds treated with SPE after 4 weeks of harvesting Figure 1.

3.2. Fresh Ungerminated Seeds in the First and Second Germination Test

There was a significant difference among the seed dormancy breaking methods (P< 0.001) at both 1 and 4 weeks after seed harvesting Figure 2.

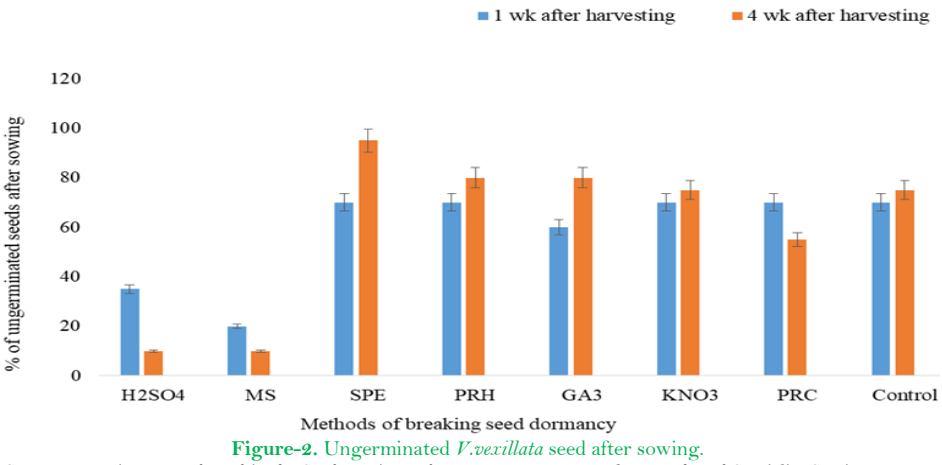

Figure-2. Ungerminated *V.vexillata* seed after sowing.
Source: Experiment conducted in the Seed Testing Laboratory, Department of Research and Specialist Services, Harare, Zimbabwe; Bars shown – standard error.

The highest (95 %) and least (10 %) ungerminated seeds were in the SPE and MS at 4 weeks after harvesting respectively.

3.3. Effects of Different Methods of Seed Breaking on Viability after Sowing

Most treatments except the control had dead seeds which significantly differ ((P< 0.001) among the seed dormancy breaking methods used) Figure 3.

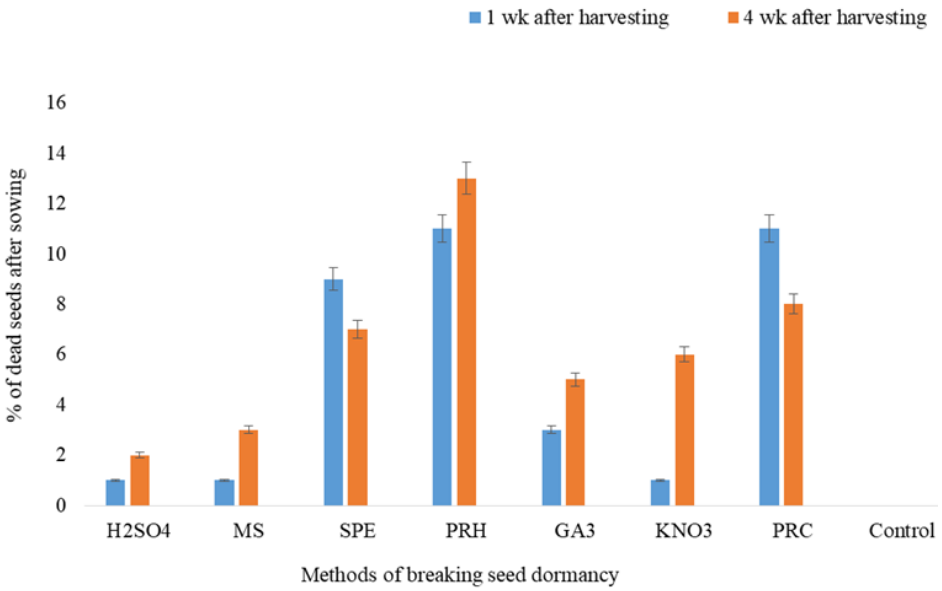

Figure-3. Effects of different methods of breaking seed on seed viability after sowing.
Source: Experiment conducted in the Seed Testing Laboratory, Department of Research and Specialist Services, Harare, Zimbabwe; Bars shown – standard error.

The highest (13 %) number of unviable seeds was obtained from PRH of seeds 4 weeks after harvesting. The least (1 %) number of unviable seeds were observed in the H_2SO_4, MS and KNO_3 at 1 week after harvesting (Figure 3).

4. DISCUSSION

The results showed that pretreating *V. vexillata* with H_2SO_4 and MS promoted germination most. H_2SO_4 and MS showed were effective in breaking seed dormancy at both 1 and 4 weeks after harvesting. This could be due to high effects of these methods in breaking and softening the seed coats. Similar observations were made by Aliero [12] when seed dormancy was effectively broken by the concentrated sulphuric acid and sandpaper scarification. On the other side, the potassium nitrate was not effective in breaking seed dormancy which is contrary to findings by Previero, et al. [13] who concluded that potassium nitrate was very effective in breaking dormancy of many legume species. The reason for these differences is unknown from this study but the potassium nitrate was ineffective breaking dormancy and this could be an area for further studies. The GA_3 performed lower than Tigabu and Oden [14] findings who noted that GA_3 was very effective in breaking seed dormancy.

There were least un-germinated seeds treated with H_2SO_4 and MS. This could be attributed to high imbibition of water after cracking of seed coat that resulted to high germination percentage. The pretreatment with PRH and PRC had more number of dead seeds than other treatments. The results agreed to Aliero [12] who recommended use of both concentrated sulphuric acid and mechanical scarification in breaking seed dormancy. Preheating and pre-chilling had high number of dead seeds which could be due to stress induced to the embryo during the heating

and cooling prior to the germination tests. High percentage of ungerminated seeds in SPE and PRH at 1 week after harvesting suggested that the seeds may require a longer period of storage after harvest before sowing. The results agree to findings by Wang, et al. [15] who observed that *V. vexillata* can best germinate after storage of 3 to 8 years in storage under sealed aluminum foil bags at 8°C after drying to 5%.

5. CONCLUSION AND RECOMMENDATIONS

H_2SO_4 and MS were effective in breaking dormancy of *V. vexillata* seeds at both 1 and 4 weeks after harvesting. Therefore *V. vexillata* seeds can germinate well at least 4 weeks in storage after harvesting. It is of paramount importance to repeat the experiment and performing viability test prior to sowing because this study assumed that all seeds were viable before sowing which may not be the case. There is need for further research on times taken to germination and evaluating the effects of seed dormancy breaking methods on domesticated *V. vexillata* seeds from other countries.

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