

Short Communication

DEVELOPMENT OF SUITABLE PROPAGATION SYSTEMS FOR *Tephrosia* spp. IN SRI LANKA

A.I.S. PRIYADARSHAN¹, S.P. SENANAYAKE^{1*}, M.P. JAYATILLEKE² AND S. RAJAPAKSE³

¹ **Department of Botany, University of Kelaniya, Sri Lanka.**

² **Department of Science, Technology and Engineering, Western College of Idaho, USA.**

³ **Department of Molecular Biology & Biotechnology, University of Peradeniya, Sri Lanka.**

ABSTRACT

Genus *Tephrosia* (Fabaceae) commonly known as ‘Pila’ in Sri Lanka. Some *Tephrosia* spp. are used in many traditional therapeutic preparations in Sri Lanka and also in other countries. Increased use of *Tephrosia* in traditional therapeutic systems has developed a great demand for these species. Thus production of planting materials for commercial cultivation has become a timely requirement. Therefore, this research was conducted to determine suitable propagation systems for four selected *Tephrosia* spp.; *T. purpurea*, *T. villosa*, *T. noctiflora* and *T. pumila*. Even though seeds of *Tephrosia* spp. showed high viability, lower germination percentage was observed on wet filter paper as well as in six different potting media [M₀ (Coir: dust: Sand; 1:1:1), M₁ (Coir dust: Sand: Top soil; 1:1:1), M₂ (Coir dust: Sand: Top soil: Compost; 1:1:1:1), M₃ (Coir dust: Sand: Top soil: Compost: Cow dung; 1:1:1:1:1), M₄ (Coir dust: Sand: Compost; 1:1:1), M₅ (Coir dust: Sand: Top soil: Cow dung; 1:1:1:1)] during a period of one week. Water impermeability of seed coats was identified as the main reason for the seed dormancy in *Tephrosia* spp. and soaking seeds in Con. H₂SO₄: H₂O; 3:1 for 25 minutes and rubbing seeds on sandpaper for 2 minutes were determined as suitable dormancy breaking methods.

* Corresponding author Email: priyangi@kln.ac.lk

Variation was observed in the growth performance of *Tephrosia* spp. in six different potting media. Growth performances of seedlings of *Tephrosia* spp. in hydroponic solution and soil medium were observed and soil medium was selected as the most suitable medium for the growth of *Tephrosia* species based on their growth performances.

Keywords: *Tephrosia* spp., Potting media, Seed dormancy breaking methods, Propagation systems

INTRODUCTION

The genus *Tephrosia* (Vern: Pila) belongs to family Fabaceae and is distributed in warm temperate and tropical areas throughout the world and 10 species are found in Sri Lanka. *Tephrosia* species are perennial shrubs or herbs with high seed production (Dasanayake *et al.*, 1991). Several species of *Tephrosia* are commonly used as ingredients in traditional therapeutic preparations and well known for their anticancer properties and healing ability of wounds. Jayaweera (1981) has recorded that the whole plant of *T. purpurea* and *T. villosa* are commonly used in disorders in liver, spleen and kidney and also as an anthelmintic in children in Sri Lanka, India, Vietnam, Nigeria and West Africa. Seeds of many species of the family Fabaceae exhibit dormancy which is primarily due to water impermeability of the seed coat (Baskin and Baskin, 2014). Seed pre-treatments can be used to improve seed germination. Acid scarification is one of the best treatments for seeds with hard, impermeable seed coats (Baskin and Baskin, 2014). The concentrated sulphuric acid treatment has been widely used to improve seed germination of several hard seed coat species (Dole and Wilkins, 1999; Hassen *et al.*, 2005). Scarification of seeds with sulphuric acid (64% v/v) for 30 to 60 mins has been shown as useful to improve seedling emergence (Ali *et al.*, 2011). According to Hassen *et al.*, (2005), scarification by sandpaper appears to be the best method for maximising germination with small amount of seeds whereas immersing the seeds in concentrated sulphuric acid for 20 minutes followed by soaking in water for 24 h is better for large amount of seeds.

Due to the increasing popularity of *Tephrosia* spp. as ingredients in many traditional therapeutic systems, establishment of propagation systems for commercial cultivation is required to ensure the continuous supply of authenticated material to meet the demand. Therefore, the objective of this research was to establish suitable propagation systems for selected *Tephrosia* spp.

METHODOLOGY

Establishment of suitable propagation systems for *T. purpurea*, *T. villosa*, *T. noctiflora* and *T. pumila*

Seeds of *T. purpurea*, *T. villosa*, *T. noctiflora* and *T. pumila* were collected from different localities in Sri Lanka. Tetrazolium test was carried out to determine the seed viability of each seed lot of the species. Germination of seeds on wet filter paper as well as in different potting media: M₀- Coir: dust: Sand; 1:1:1, M₁- Coir dust: Sand: Top soil; 1:1:1, M₂- Coir dust: Sand: Top soil: Compost; 1:1:1:1, M₃- Coir dust: Sand: Top soil: Compost: Cow dung; 1:1:1:1:1, M₄- Coir dust: Sand: Compost; 1:1:1 and M₅- Coir dust: Sand: Top soil: Cow dung; 1:1:1:1, was observed. Four replicates of twenty five seeds were used in each treatment of each experiment.

Different seed dormancy breaking methods (Table 1) were applied to determine a suitable seed dormancy breaking treatment for *Tephrosia* spp. Then germination of treated seeds on wet filter paper as well as in different potting media was monitored. Experiments were conducted in the plant house, Botanical garden, University of Kelaniya under ambient temperature (~ 30 °C) and light conditions Shoot height and percentage leaf production was measured weekly for 2 months to evaluate the growth performance of plants grown in different substrates. Percentage leaf production was calculated according to the following formula;

$$\text{Leaf Production (\%)} = \frac{\text{No. of leaves increased in a month}}{\text{Total No. of leaves at the end of the month}} \times 100$$

One-way analysis of variance (ANOVA; $\alpha = 0.05$) was performed to compare the effectiveness of the treatments. Tukey's mean comparison test was performed to determine the significant differences among treatments. Data were arcsine transformed prior to the analysis (Sahu, 2013).

One batch of two month old seedlings (6 replicates) was grown in the most suitable potting media, selected based on the growth performance, for each *Tephrosia* spp. and another batch was grown in a hydroponic solution (Albert solution). Growth performance of seedlings was observed by measuring the shoot height and leaf production after period of one month as growth parameters.

One-way analysis of variance (ANOVA; $\alpha = 0.05$) was used to compare the treatments and Tukey's mean comparison test was performed to determine the differences among treatments.

Table 1: Seed dormancy breaking methods and treatments

Seed dormancy breaking method	Treatments
Mechanical damage of the seed coat	Crushing with sand for 2 min.
	Rubbing in sandpaper (No 120) for 2 min.
Hot water soaking	Soaking in hot water (80°C) for 5 min.
	Soaking in hot water (80°C) 2 min. and immediately transferring to cool water
Oven drying	Keeping in oven (40°C) for 5 min. and 10 min.
	Keeping in oven (60°C) for 5 min. and 10 min
	Keeping in oven (80°C) for 5 min. and 10 min
Scarification with Concentrated sulphuric acid	Soaking in Con. H ₂ SO ₄ for 10 min, 25 min. and 45 min.
	Soaking in Con. H ₂ SO ₄ : H ₂ O; 3:1 for 10 min., 25 min. and 45 min.
	Soaking in Con. H ₂ SO ₄ : H ₂ O; 1:1 for 10 min., 25 min. and 45 min.
	Soaking in Con. H ₂ SO ₄ : H ₂ O; 1:3 for 10 min., 25 min and 45 min.

RESULTS AND DISCUSSION

Even though seeds of *T. noctiflora*, *T. purpurea*, *T. villosa* and *T. pumila* have shown high viability in the wet filter paper during a period of one week, all the species have very low performance in the germination. It clearly indicates the presence of seed dormancy which prevents the germination of seeds and the necessity of developing effective propagation system. Seeds of *Tephrosia* spp. soaked in Con. H₂SO₄: H₂O; 3:1 for 25 minutes or rubbed on sandpaper for 2 minutes have exhibited a significantly higher seed germination percentages after a week in different potting media compared to the control.

Further, seeds of *Tephrosia* spp. soaked in Con. H₂SO₄: H₂O; 3:1 for 25 minutes or rubbed on sandpaper for 2 minutes have exhibited higher seedlings survival, shoot height and leaf production in different potting media after two months compared to the control.

Based on the evaluation of the seed germination rate and the growth performance of the seedlings after undergoing the treatments for seed dormancy breaking and potting media were selected as depicted in table 2 for each *Tephrosia* spp.

It was evident in the present findings that the seedlings grown in the potting media; M₃ for *T. purpurea*, M₄ for *T. noctiflora*, M₂ for *T. villosa* and *T. pumila* have shown the

highest shoot height, leaf production and shoot to root ratio compared to hydroponic solution.

Table 2: Suitable seed dormancy breaking methods and potting media for *Tephrosia* spp.

Species	Suitable seed dormancy breaking treatments	Suitable potting medium
<i>T. purpurea</i>	Soaking in Con. H ₂ SO ₄ : H ₂ O; 3:1 solution for 25 min. Rubbing on sandpaper for 2 min.	M ₃ (Coir dust: Sand: Top soil: Compost: Cow dung; 1:1:1:1:1)
<i>T. noctiflora</i>	Soaking in Con. H ₂ SO ₄ : H ₂ O; 3:1 solution for 25 min Rubbing on sandpaper for 2 min.	M ₄ (Coir dust: Sand: Compost; 1:1:1)
<i>T. villosa</i>	Soaking in Con. H ₂ SO ₄ : H ₂ O; 3:1 solution for 25 min. Rubbing on sandpaper for 2 min.	M ₂ (Coir dust: Sand: Top soil: Compost; 1:1:1:1)
<i>T. pumila</i>	Soaking in Con. H ₂ SO ₄ : H ₂ O; 3:1 solution for 25 min. Rubbing on sandpaper for 2 min.	M ₂ (Coir dust: Sand: Top soil: Compost; 1:1:1:1)

Based on the evaluation of the seed germination rate and the growth performance of the seedlings after undergoing the treatments for seed dormancy breaking and potting media were selected as depicted in table 2 for each *Tephrosia* spp.

It was evident in the present findings that the seedlings grown in the potting media; M₃ for *T. purpurea*, M₄ for *T. noctiflora*, M₂ for *T. villosa* and *T. pumila* have shown the highest shoot height, leaf production and shoot to root ratio compared to hydroponic solution.

According to the findings of the present study, mechanical (rubbing on sandpaper) scarification and application of sulphuric acid were found to be the effective in inducing the seed germination of *Tephrosia* spp. Aliero (2004) has reported that, seed dormancy is usually associated with the factors of the protective coverings of the seed coat or the enclosed embryo. Mechanical or chemical scarification alleviates the coat imposed dormancy, physical dormancy, caused by water impermeable seed coats or physiological dormancy, caused by high mechanical resistance of the coats of seeds as these treatments can disrupt the seed coat and allow the seeds to imbibe or reduce the resistance of the coats to protrude radicle (Baskin and Baskin, 2014). Thus from the results of present experiments it can be concluded that the seeds of all four *Tephrosia* species have seed coat imposed seed dormancy. Moreover, can suggest that those *Tephrosia* spp. also have

physical dormancy as in the most of the other tropical *Tephrosia* spp. which was noted by Jayasuriya et al. (2013),

Clear variation in growth performances was observed in different species with respective the different potting media and that indicated their adaptability in various natural environmental conditions. Soil condition and other prevailing ecological conditions would have impact on the successful distribution of these species even within the same climatic zone. Further experiments are needed to be carried out to evaluate the distribution of these species in Sri Lanka related to the different climatic, ecological and soil conditions and in terms of their adaptability for these environments.

CONCLUSIONS

Seeds soaked in Con. H₂SO₄: H₂O; 3:1 solution for 25 min. and rubbed on sandpaper for 2 min. were determined as the suitable seed dormancy breaking treatments for *T. purpurea*, *T. villosa*, *T. noctiflora* and *T. pumila*, based on the germination rate. Evaluation of the growth performance of *Tephrosia* spp. has shown that soil medium is more effective compared to the hydroponic system for the growth of *Tephrosia* spp. These findings provide necessary information in recommending suitable propagation systems for *Tephrosia* spp. to ensure continuous supply of authenticated raw material for commercial production in herbal industry.

ACKNOWLEDGMENT

Authors wish to acknowledge the financial assistance given by the National Science Foundation, Sri Lanka (Research grant: NSF RG/2011/NRB/06).

REFERENCES

- Ali, H.H., Tanveer, A. and Nadeem, M.A., 2011. Methods to break seed dormancy of *Rhynchosia capitata*, A summer annual weed, *Chilean journal of Agriculture research*, **71**: 23-28.
- Aliero, B.L., 2004. Effects of sulphuric acid, mechanical scarification and wet heat treatments on germination of seeds of African locust bean tree, *Parkia biglobosa*, *African Journal of Biotechnology*, **3**: 179-181,
- Baskin, C.C. and Baskin, J.M., 2014. *Seeds; Ecology, Biogeography, and evaluation of dormancy and germination*, Academic press, California, USA, pp. 5- 164.

- Dasanayake, M.D. and Fosberg F.R., 1991. *A Revised Handbook to the Flora of Ceylon*, vol VII, Amerind publishing Co pvt Ltd, New Delhi, pp. 235-237.
- Dole J.M. and Wilkins H.F., 1999. *Floriculture principles and species*, Prentice-Hall, Inc, USA, pp. 3-26.
- Hassen, A., Rethman, N.F.G. and Niekerk, W.A.V., 2005. Research note: effect of different seed treatment options on dormancy breaking, germination and emergence of *Ziziphus mucronata* (buffalo thorn) seed, *Tropical Grassland*, **39**: 124-128.
- Jayasuriya, K.M.G.G., Wijetunga, A.S.T.B., Baskin, J.M., Baskin, C.C., 2013. Seed dormancy and storage behavior in tropical Fabaceae: A study of 100 species from Sri Lanka. *Seed Science Research* **23**: 257-269.
- Jayaweera D.M.A., 1981. Medicinal plants used in Ceylon Part 3. National Science Council of Sri Lanka, pp 249, 251.
- Karuppusamy S., Muthuraja M. and Rajasekaran K.M., 2011. Antioxidant Activity of selected lesser known edible fruits from Western Ghats of India, *Indian Journal of Natural Products and Resources*, **2**: 174-178.
- Sahu, P.K., 2013. *Research Methodology: A Guide for Researchers In Agricultural Science, social science and other related fields*, Springer publication, India, 1st edit. Vol. XIII, pp. 211 – 216.