Preliminary studies on *Tephrosia* species: Effect of seed treatments on germination

O.J. Babayemi¹, I.O. Daniel^{2*}, M.A. Bamikole³, A. Ogungbesan¹ and B.O. Oduguwa⁴

¹ Department of Animal Science, University of Ibadan, Ibadan, Nigeria.

^{2*} Department of Plant Breeding and Seed Technology, University of Agriculture, Abeokuta, Nigeria.

³ Department of Animal Science, University of Benin, Benin city, Nigeria.

⁴ Research and Development Centre, University of Agriculture, Abeokuta, Nigeria.

Abstract

Germination tests were conducted on the seeds of three species of *Tephrosia* (*T. bracteolata*, *T. candida*, and *T. linearis*) to investigate potential seedling establishment and possible pre-treatments to improve seed germination. Pre-culture treatments were: i) No treatment (control), ii) rubbed in sand paper, iii) soaking in boiled water for 30 seconds, iv) for 3 minutes, v) for 5 minutes, vi) soaking in concentrated sulphuric acid, and vii) soaking in water for 24 hours. After treatment, a total of two hundred and fifty seeds (5 replicates each of 50 seeds) were cultured on moist filter paper for 15 days. Cumulative percentage germination, percentage dormant seeds and germination rates were calculated. Seeds of all the species of *Tephrosia* exhibited innate dormancy at varying levels, but most pronounced in *T. linearis*. Soaking seeds in boiled water for 30 seconds to 5 minutes resulted in higher seed germination ($p < 0.05$) than other pre-culture seed treatments in all the genotypes. Percentage hard seeds were also minimal in seeds treated this way, indicating effective dormancy alleviation in *Tephrosia* by these treatments. From the trial, soaking seeds in boiled water for 30 seconds is recommended as treatment of *Tephrosia* seeds to enhance germination and optimal potential seedling establishment.

Keywords: Germination, seed treatments, *Tephrosia* species, legume shrubs.

Introduction

Tephrosia species are leguminous shrubs widely dispersed throughout the tropics (Dutta, 1979). The plants are shrubs with erect stem ranging from 1.2m to 3m in height and had already being identified as a multi-purpose species, useful for soil fertility improvement and as a forage legume in agro-forestry systems (Kadiala *et al.*, 1996). In a typical agro-forestry system, *Tephrosia* serves as a source of stakes for crop production, and serves as a cover crop thus enhancing soil fertility and moisture conservation (Le-Houerou, 1980).

They also serve as forage, highly relished by ruminant animals. *Tephrosia* is reported to be highly rumen degradable and could increase

amino acid and nitrogen utilization efficiency and overall performance of livestock (Babayemi *et al.*, 2002).

The potentials of these species for soil improvement and livestock production are yet to be exploited because there is paucity of information especially on cultivation, establishment and management of *Tephrosia* as a forage crop. In the wild, the seeds scattered by explosion of pods and naturally establishes at the commencement of the rains, indicating that *Tephrosia* propagates by seeds. The emerged seedlings grow sporadically after the regular annual bush burning common in the tropics (Roder and Maniphone, 1993), suggesting seed dormancy alleviation with the

heat treatment. The purpose of the present study was then to determine pre-sowing treatments that will optimize the germination of *Tephrosia* seeds for cultivation.

Materials and Methods

The germination trials were conducted in March 2000 at the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria (7°30'N and 3°54'E).

Seed sources

Seeds of *T. bracteolata* and *T. candida* were sourced from the forage legume germplasm of International Livestock Research Institute (ILRI), Ibadan. *T. linearis* seeds were collected from the wild in a derived Savannah area of Nigeria between December, 1999 and January, 2000.

Seed treatments

Seeds of the 3 species were subjected to 7 pre-culture treatments, in a factorial complete randomised design. The treatments were (i) No treatment (control), (ii) rubbed in sand paper, (iii) soaking in boiled water for 30 seconds, (iv) soaking in boiled water for 3 minutes, (v) soaking in boiled water for 3 minutes, (vi) soaking in concentrated sulphuric acid for 30 seconds, and (vii) soaking in water at room temperature for 24 hours.

The seeds were sorted, counted and divided into 7 batches of 250 seeds for each species giving 21 seed batches (3 species x 7 treatments). Each batch to be subjected to soaking in boiled water or acid were placed in 1mm tubular wire mesh and then immersed. At the expiration of the treatment time, the seeds were withdrawn and cooled down rapidly under a running tap for 5 minutes according to Fulbright and Flenniken (1987). A seed batch was subjected to sand paper abrasion by placing them in wooden boxes lined on the sides with sand paper and then the seeds were rubbed with the wooden box cover until seeds were bruised on the seed coat. Two hundred and fifty bruised seeds were then counted from the rubbed seed lot. The last batch of seeds that

did not receive any pre-culture treatments served as the control.

Germination tests

After applying the treatments, each batch was divided into 5 replicates of 50 seeds each and then arranged in regular equidistant pattern on moistened filter paper in petri-dishes. Water was applied at 09.00h daily using a 5 ml syringe. The germination test lasted for 15 days; germination counts started 48 hours after seed culturing and continued on daily basis for 15 days. Seeds with visible radicle protrusion were scored as germinated. Unsprouted but hard seeds after culturing for 15 days were separated from rotten seeds and counted as dormant seeds. Seed germination rate was determined as the reciprocal of time to cumulative germination expressed in germination /day according to Daniel (1997):

$$GR = 1/t_n \cdot (\sum G_n)$$

Where G_n is number of seed sprouted at time t_n (in days).

Statistical analysis

The data generated were subjected to Analysis of Variance (ANOVA) tests of the effects of species, treatments and their interaction on the seed germination data. The statistical package used for the analysis was SPSS™. The ANOVA procedure was performed on transformed values to normalize the percentage data using arc-sine transformation table in Gomez and Gomez (1984). Time to total germination was also log transformed before ANOVA. The standard error of means generated from the ANOVA procedure was used to compare means.

Results

Effect of seed treatment on germination of *Tephrosia* seeds

Soaking seeds of the three *Tephrosia* species in boiled water for 30 seconds improved germination, minimised dormancy and increased seed germination rates significantly compared with untreated seeds and other seed treatments (Table 1).

Effect of seed treatments on the germination of *Tephrosia* species

Table 1 Effects of seed treatments on germination, dormancy and germination rates of *Tephrosia* seeds. Values are means of 15 replicates (5 replicates of each of the 3 species - *T. bracteolata*, *T. candida* and *T. linearis*). Standard errors of transformed means at $p=0.05$ are in brackets.

Treatments	Germination	Dormancy	Germination rates
Control %	30.4	34.3	5.4
*Transformed means	31.48(3.997)	33.29(5.310)	0.73(0.131)
Sand paper scarification %50.0		12.3	11.2
Transformed means	44.74(4.024)	17.62(2.960)	1.05(0.114)
Soaked in boiled water for 30 seconds %	68.0	0.5	24.6
Transformed means	58.82(4.671)	1.83(1.009)	1.39(0.096)
Soaked in boiled water for 3 minutes %	47.0	0.00	17.4
Transformed means	43.23(4.791)	0.00	1.24(0.120)
Soaked in boiled water for 5 minutes %	47.5	0.00	16.6
Transformed means	44.73(5.936)	0.00	1.22(0.105)
Soaked in sulphuric acid for 30 seconds %	41.5	11.60	7.4
Transformed means	39.21(4.053)	18.05(2.406)	0.87(0.135)
Soaked in water for 24 hours %	36.13	28.26	11.5
Transformed means	36.30(2.978)	29.50(4.822)	1.06(0.118)

*% sprouted (germination) and % hard (dormant) seeds were transformed to arc sin values while germination rates were transformed to log values (seed text for details of data transformation)

The significant increase in cumulative seed germination after soaking seeds in boiled water for 30 seconds was most pronounced in *T. linearis* (Table 2). It was also observed that there were no significant differences in the effects of soaking seed in boiled water for 30 seconds, 3 minutes and 5 minutes on cumulative seed germination in *T. linearis*. But

T. bracteolata seeds showed good germination responses that were comparable for seeds soaked in boiled water for 30 seconds, seeds rubbed in sand paper and seeds soaked in sulphuric acid. The germination of *T. candida* seeds remained the lowest with all the seed treatments (Table 2).

Table 2 *Effect of seed treatments on cumulative seed germination in different Tephrosia species. Values are means of 5 replicates. Standard errors of arc sin equivalent of mean percentage germination at $p=0.05$ are in brackets.*

Treatments	<i>T. bracteolata</i>	<i>T. candida</i>	<i>T. linearis</i>
Control %	55.20	13.60	22.40
Arc sin	48.00(2.929)	18.27(6.584)	28.17(1.169)
Sand paper scarification %	72.00	18.80	59.20
Arc sin	58.37(3.008)	25.43(2.199)	50.44(2.888)
Soaked in boiled water % for 30 seconds	70.40	41.60	92.00
Arc sin	57.11(1.493)	40.08(2.673)	79.28(5.145)
Soaked in boiled water % for 3 minutes	34.00	23.20	84.00
Arc sin	35.54(2.125)	27.59(4.791)	66.56(1.473)
Soaked in boiled water % for 5 minutes	25.20	26.80	90.40
Arc sin	29.54(3.600)	30.65(4.126)	74.00(4.305)
Soaked in sulphuric acid % for 30 seconds	61.20	17.60	45.60
Arc sin	51.63(5.192)	24.61(1.834)	41.38(7.008)
Soaked in water for % 24 hours	54.00	24.40	30.00
Arc sin	47.26(3.832)	28.79(4.237)	32.85(3.651)

Effect of seed treatment on dormancy of Tephrosia seeds

Total percentage dormant seed was significantly higher in untreated (control) seeds than all the pre-treated seeds of all the species (Table 1). Seeds of the three *Tephrosia* species differed significantly from one another in percentage dormant seeds before seed treatment (Table 3). In this regard, the seed of *T. linearis* was highest followed by *T. bracteolata* and *T.*

candida. Table 3 also shows that soaking seeds in boiled water for 3 and 5 minutes totally removed seed dormancy for the 3 species. Soaking seeds in boiled water for 30 seconds also removed dormancy from *T. linearis* and minimised it in the other two species to insignificant levels. Seed dormancy was still high and comparable in the control and in seeds that were soaked in cold water for 24 hours for the three species.

Effect of seed treatments on the germination of *Tephrosia* species

Table 3 Effect of seed treatments on seed dormancy in different *Tephrosia* species. Values are means of 5 replicates. Standard errors of arc sin equivalent of mean percentage dormancy at $p=0.05$ are in brackets.

Treatments		<i>T. bracteolata</i>	<i>T. candida</i>	<i>T. linearis</i>
Control	%	26.00	4.00	72.80
Arc sin		30.54(1.697)	10.70(1.156)	58.61(1.469)
Sand paper scarification	%	5.60	3.20	27.60
Arc sin		13.22(1.859)	7.98(3.23)	31.47(0.633)
Soaked in boiled water for 30 seconds	%	0.40	1.20	0.00
Arc sin		1.63(1.626)	3.93(2.469)	0.00
Soaked in boiled water for 3 minutes	%	0.00	0.00	0.00
Arc sin		0.00	0.00	0.00
Soaked in boiled water for 5 minutes	%	0.00	0.00	0.00
Arc sin		0.00	0.00	0.00
Soaked in sulphuric acid for 30 seconds	%	3.20	8.40	23.20
Arc sin		9.08(2.464)	16.51(1.784)	28.67(1.623)
Soaked in water for 24 hours	%	21.60	4.80	58.40
Arc sin		26.82(4.163)	11.92(2.222)	49.77(6.333)

Effect of pre-culture treatments on germination rate of *Tephrosia* seeds

All the pre-culture treatments except soaking seeds in sulphuric acid significantly improved seed germination rates above the control in all the three species of *Tephrosia* (Table 1). Seed germination rates were highest in *T. linearis* seeds soaked in hot water for 30 seconds but

not significantly different from seeds treated with hot water for 3 to 5 minutes (Table 4). In *T. bracteolata*, seeds rubbed with sand paper and seeds soaked in cold water showed comparable germination rates with seeds soaked in boiled water for 30 seconds and 3 minutes. The slowest germination rates were observed in *T. candida* seeds (Table 4).

Table 4 Effect of seed treatments on seed germination rates (% germination per day) in different *Tephrosia* species. Values are means of 5 replicates. Standard errors of log transformed means at $p=0.05$ are in brackets.

Treatments	<i>T. bracteolata</i>	<i>T. candida</i>	<i>T. linearis</i>
Control %	19.50	1.51	5.37
Log	1.29(0.105)	0.18(0.093)	0.73(0.131)
Sand paper scarification %	32.36	3.24	13.49
Log	1.51(0.020)	0.51(0.065)	1.13(0.057)
Soaked in boiled water % for 30 seconds	34.67	8.13	52.48
Log	1.54(0.015)	0.91(0.012)	1.72(0.070)
Soaked in boiled water % for 3 minutes	25.12	5.01	41.69
Log	1.40(0.110)	0.70(0.154)	1.62(0.010)
Soaked in boiled water % for 5 minutes	11.22	7.94	51.29
Log	1.05(0.117)	0.90(0.019)	1.71(0.073)
Soaked in sulphuric acid % for 30 seconds	19.05	1.99	11.22
Log	1.28(0.130)	0.30(0.094)	1.05(0.191)
Soaked in water for % 24 hours	33.11	3.72	12.59
Log	1.52(0.146)	0.57(0.052)	1.10(0.105)

Discussion

The significantly higher germinability observed in the seeds of *T. linearis* and *T. bracteolata* than *T. candida* corroborates the morphological differences that exist among the species; while *T. candida* is perennial, the other two species are annual. Babayemi *et al* (2002) also reported the slowest seedling growth rates at the early stages in *T. candida*. This disparity in seed physiology may not be confirmed as a species' characteristic from this study, since the seeds of each specie used in this trial differed in source and storage history, which might contribute to the physiology of each seed lot. However, provenance offers a possible explanation of the marked differences in seed germinability among the *Tephrosia* species; while *T. linearis* and *T. bracteolata* are common to West Africa, *T. candida* is exotic to the environment being a native of India (Tamo *et al.*, 1993).

The results showed low germinability and high percentage dormancy in untreated seeds of all the *Tephrosia* species, which strongly indicate innate dormancy of the seeds of the genus in the natural state. The germinability of the (control) seeds showed that just about one-third (34%) of the seeds would emerge without any pre-sowing treatments, necessitating the need for pre-treatment before sowing *Tephrosia* seeds. Shelton (1994) also reported low germinability (10% germination) in unscarified *Tephrosia* seeds. The present study showed that without seed pre-treatment 48%, 28% and 18% seedling establishment may be expected from *T. bracteolata*, *T. linearis* and *T. candida* respectively. Another evidence of innate seed dormancy in *Tephrosia* seeds was the slower germination rates of all the control seeds than the treated seeds.

Effect of seed treatments on the germination of *Tephrosia* species

Soaking *Tephrosia* seeds in boiled water proved very effective for seed germination enhancement, dormancy alleviation and increased rate of seed germination, which support reports of rapid germination and establishment of *Tephrosia* seeds after the yearly slash and burn activities on tropical arable lands (Roder and Maniphone, 1995). Heat appears to be essentially a dormancy alleviation treatment for *Tephrosia* seeds. From this trial, soaking seeds in boiled water for 30 seconds to 5 minutes generally minimized seed dormancy significantly in all *Tephrosia* seeds. The significantly high values of cumulative germination reported for seeds soaked in boiled water for 30 seconds suggests that the treatment offers optimal pre-culture seed treatments for *Tephrosia*. The treatment doubled the germination capacity of poorly performing *T. candida* seeds when compared with untreated seeds.

Conclusion

The results of this study pointed out that pre-sowing treatment is essential for *Tephrosia* seeds, especially soaking seeds in boiled water for between 30 seconds to 5 minutes. Optimum germination could be achieved by soaking seeds for 30 seconds. Moreover, the ability of this treatment to improve seed germination in low vigour *Tephrosia* seeds was evident. The treatment is therefore recommended as a pre-sowing treatment for *Tephrosia* seeds in the prevailing environment.

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References

- Babayemi, O. J., M. A. Bamikole, I. O. Daniel, A. Ogungbesan and A. Babatunde. 2002. Growth, nutritive value and dry matter degradability of three *Tephrosia* species. *Nig. J. Anim. Prod.* 29(2): 199-206.

- Daniel, I. O. 1997. Conservation of West African yam (*Dioscorea* sp.) germplasm: physiology of seed and pollen storage. Ph.D. Thesis, University of Ibadan, Nigeria.
- Dutta, A. C. 1979. Botany for degree student. Calcutta, Oxford University Press. Delhi pp. 728.
- Fulbright, T. E. & K. S. Flenniken. 1987. Temperature and scarification effects on germination of prostrate bundleflower seeds. *Journal of Range Management*. 4(2): 170-173.
- Gomez, K. A. & A. A. Gomez. 1984. *Statistical procedures for agricultural research*. Wiley-Interscience publication, John Wiley and Sons, Inc. New York, Chichester, Brisbane, Toronto and Singapore.
- Kadiata, B. D., K. Mulongoy, N. O. Isirimah, M. A. Amakiri. 1996. Screening woody and shrub legumes for growth, nodulation and nitrogen fixation potential two contrasting soils. *Agroforestry Systems*. 33(2) 137-152.
- Le-Houerou, H. N. 1980. *Chemical composition and nutritive value of browse in West Africa*. Browse in Africa. ILCA Addis Ababa, Ethiopia. pg 329-338.
- Roder, W. & S. Maniphone. 1993. Forage legume establishment in rice slash and burn systems. *Tropical Grassland*. 29(2): 81-87.

Shelton, H. M. 1994. *Establishment of forage tree legumes*. In: Forage legume in tropical agriculture. (Eds. R. C. Gutteridge & H. M. Shelton) Wallingford: CAB International, U. K. pg 133-142.

Tamo, M., J. Baumgartan, V. Delucchi, & H. R. Herren. 1993. Assessment of key factors responsible for pest status of the bean flower trips in West Africa. *Bulletin of Entomological Research*. 83(2): 251-258.

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