Effect of pre-planting seed treatment on dormancy breaking and germination of *Indigofera* accessions

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Abstract

A factorial treatment combination of 7 different accessions of *Indigofera* (*I. cryptantha* 7067, *I. brevicalyx* 7517, *I. arrecta* 7524, *I. spicata* 8254, *I. vohemarensis* 8730, *I. trita* 10297 and *I. spicata* 10299) and 3 different seed treatments [untreated or control, scarified and immersed in boiled water (water boils at 93°C at this elevation, 1327 m)] were evaluated. Treatment of seed enhanced dormancy breaking in most accessions with scarification being more effective than boiled water treatment in 6 accessions, but not in the case of *I. vohemarensis* 8730. In 5 accessions (*I. cryptantha* 7067, *I. brevicalyx* 7517, *I. arrecta* 7524, *I. spicata* 8254 and *I. vohemarensis* 8730), scarification improved the total germination percentage, though it simultaneously resulted in higher seed mortality of *I. brevicalyx* 7517, *I. arrecta* 7524 and *I. vohemarensis* 8730 than in the control. In 4 accessions (*I. brevicalyx* 7517, *I. arrecta* 7524, *I. vohemarensis* 8730 and *I. trita* 10297), boiled water treatment improved germination percentage without causing any significant risk of seed mortality in the last 3 species.

Introduction

Indigofera species show great promise as grazing forages for ruminants. Typical of Leguminosae, *Indigofera* species are high in protein. Their ability to tolerate drought, floods and salinity makes them agronomically desirable (Skerman 1982). Their deep-rooted growth form, ability to respond to small rainfall events and resistance to herbivory make them a potentially valuable cover crop and forage species for semi-arid and arid areas. Strickland *et al*. (1987) report that about 50% of the species in the genus are toxic to some degree, but only 30% are palatable. The forage toxicity and feeding value of *Indigofera brevicalyx* and *I. vicioides* have been reported to be similar to that of *Medicago sativa* (lucerne), while higher toxicity and lower feeding values have been reported for *I. spicata* (Strickland *et al.* 1987).

Germination, emergence and establishment of legumes depend on the interaction of biological, environmental and management variables. In semi-arid and arid conditions, which prevail in parts of Ethiopia, seedling emergence and establishment are constrained mainly by the irregular distribution of rainfall within a season. Apart from this, seed size, weight, dormancy and integument thickness have significant effects on the emergence and establishment of seedlings from soil seed banks under natural conditions (Carren *et al*. 1987; Veenendaal *et al*. 1996; Sy *et al*. 2001). The extent of seed coat dormancy needs to be within acceptable levels for range reseeding projects to be profitable, while uniform germination is probably more beneficial in the case of sown pastures.

Poor germination was experienced in more than 50% of *Indigofera* accessions received from the International Livestock Research Institute (ILRI) gene bank for a characterisation study being conducted in Pretoria. The major cause was dormancy associated with hard seed (Abubeker, unpublished data). Although different pre-planting treatments are reported to be effective for breaking hard seed dormancy in different legume species (Hanna 1973; Grant 1979; Dell 1980; Buttler *et al.* 1982; Ramamoorthy and Rai 1990), little has been documented in the case of *Indigofera* species.

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From the accessions that exhibited a poor germination rate, 6 species were selected at random and included with an accession showing an acceptable level of germination in the present study. The aim of this study was to compare the suitability of pre-planting seed scarification and treatment with boiled water as practical techniques to break seed dormancy and enhance germination of different *Indigofera* species.

Material and methods

The seeds of the *Indigofera* species studied were received from the ILRI gene bank and were collected from forage seed production sites of ILRI at Zeway and Soddo, in Ethiopia. A total of 7 accessions of 6 *Indigofera* species were randomly selected for use in this study, 6 from those species known to have poor germination (*I. cryptantha* 7067, *I. brevicalyx* 7517, *I. arrecta* 7524, *I. spicata* 8254, *I. vohemarensis* 8730 and *I. trita* 10297) and 1 (*I. spicata* 10299) with reasonable germination. A factorial combination of these 7 accessions and 3 seed treatments (untreated or control, seed scarified and seed treated with boiled water) were evaluated in a completely randomised design with 3 replications.

About 2 gm of seed from each accession were subjected to either mechanical scarification (rubbing the seeds between sandpaper) or boiled water treatment (placing seeds in boiled water and leaving them until the water cooled). At Pretoria water boils at 93°C because of the elevation (1327 masl). After treatment, 50 seeds from each treatment were placed in petri dishes fitted with moist filter paper. These were placed in a growth cabinet set to 12-hour light/12-hour dark and day/night temperature of 30/20°C. Seeds were adequately watered throughout the experimental period with distilled water. Germination counts were made every 3 days for 15 days. Seeds were considered germinated when the radicle had emerged through the integument; germinated seeds were removed after each count. At the end of the test, seeds that had not germinated were categorised into hard and dead components by touching and piercing with a needle. Hard seeds could not be pierced with the needle.

The percentages of germinated, hard and dead seeds were subjected, after arcsine transformation, to analysis of variance using Proc GLM of SAS (1989). When Fisher's F values were significant at $P < 0.05$, the analysis was continued by comparing the means using Tukey's test at the threshold of $P < 0.05$. Arcsine-transformed means were back-transformed for presentation.

Results and discussion

There was a significant ($P < 0.05$) interaction between *Indigofera* accession and effect of seed treatment, suggesting that any effects of treatment on dormancy breaking, germination rate or seed mortality should be assessed separately for each accession. Hence, means for individual species are presented.

Hard seed breakdown

In all accessions, the percentage of hard seed remaining at the end of the germination test was significantly higher ($P < 0.05$) in the control seeds than in those either scarified or treated with boiled water (Table 1). The integument of the seed of many leguminous species is resistant to the penetration of water (Skerman 1982), *e.g. Cassia obtusifolia* (Sy *et al*. 2001) and *Acacia senegal* (Danthu *et al.* 1992). This results in poor germination caused by hard seed coat dormancy, which can be overcome in most accessions by treating seed to reduce the impermeability of the integuments (Elberse and Breman 1989).

Table 1. Percentage of hard seed after incubation of seeds treated in different ways in a growth cabinet for 2 weeks.

Species/accessions	Percentage of hard seeds		
	Untreated seed (control)	Seed treatment	
		Scarification[1]	Boiled water[2]
I. cryptantha 7067	88 Auv[3]	0 Cx	72 By
I. brevicalyx 7517	95 Du	0 Fx	11 Ea
I. arrecta 7524	75 Gr	0 Ix	28 Hz
I. spicata 8254	85 Juv	1 Lx	60 Ky
I. vohemarensis 8730	95 My	0 Nx	0 Nb
I. trita 10297	79 Ov	0 Qx	7 Pa
I. spicata 10299	55 Rw	0 Tx	17 Sza

[1] Seed rubbed with sandpaper.

[2] Seed immersed in boiled water and left until the water cooled down.

[3] Means within rows followed by the same uppercase letter or within columns followed by the same lower case letter are not significantly different ($P > 0.05$).

Scarification broke hard seed dormancy to a significantly (P < 0.05) greater extent than boiled water treatment in all accessions, except *I. vohemarensis* 8730. This is probably due to scarification fracturing the seed testa and allowing rapid imbibition of water, while the effect of boiled water treatment is mainly through rupturing of the seed coat by ejecting the strophiolar plug and cracking the testa (Argel and Paton 1999). This leads to water imbibition over a relatively longer period of time, than with a fractured seed testa. The high proportion of hard seed observed in the control treatment is similar to that in a previous pilot study, which reported a high proportion of hard seed from the non-germinating components at the end of the germination test (Abubeker, unpublished data). Hard seededness is an important trait that enhances survival of a species to the next generation by ensuring sequential germination of seeds from the soil seed bank in semi-arid and arid areas, which are often characterised by extreme and high climatic variability. However, from the perspectives of sown pasture, rangeland reseeding and pasture renovation, a higher proportion of hard seed in the seed lot could impact negatively on targetted levels of rapid establishment.

Germination and mortality

The total percentage germination and mortality of seeds from the different pretreatments and accessions are presented in Tables 2 and 3, respectively. In 5 accessions (*I. cryptantha* 7067, *I. brevicalyx* 7517, *I. arrecta* 7524, *I. spicata* 8254 and *I. vohemarensis* 8730), scarification significantly (P < 0.05) increased the total germination percentage compared with the control, though it simultaneously resulted in significantly (P < 0.05) higher seed mortality of *I. brevicalyx* 7517, *I. arrecta* 7524 and *I. vohemarensis* 8730 accessions than in the control treatment. This agrees with the results of Hopkinson and Paton (1993), who reported increased laboratory germination of *Stylosanthes scabra* cv. Seca seed following scarification, with a slightly increased risk of causing seed death.

In 4 accessions (*I. brevicalyx* 7517, *I. arrecta* 7524, *I. vohemarensis* 8730 and *I. trita* 10297), boiled water treatment significantly (P < 0.05) increased germination but increased seed mortality in *I. brevicalyx* 7517 only. Similar increases in the germination of *Centrosema pubescens* seed have been reported following immersion in boiling water for a period of 1 second to 20 minutes or leaving it to cool down (Phipps 1973).

Table 2. Percentage of seeds which germinated over 2 weeks from seed treated in different ways.

Species/accessions	Percentage of germinating seeds		
	Untreated seed (control)	Seed treatment	
		Scarification[1]	Boiled water[2]
I. cryptantha 7067	3 Ap[3]	73 Br	11 Aw
I. brevicalyx 7517	2 Cp	41 Dr	47 Duv
I. arrecta 7524	10 Epq	49 Fr	57 Ftuv
I. spicata 8254	11 Gpq	73 Hr	26 Gvw
I. vohemarensis 8730	2 Ip	56 Jr	89 Kt
I. trita 10297	15 Lpq	46 LMr	73 Mtu
I. spicata 10299	31 Oq	53 Or	43 Ouv

[1] Seed rubbed with sandpaper.
[2] Seed immersed in boiled water and left until the water cooled down.
[3] Means within rows followed by the same uppercase letter or within columns followed by the same lower case letter are not significantly different (P > 0.05).

Table 3. Percentage of dead seeds remaining after 2 weeks incubation in a growth cabinet for the different seed treatments.

Species/accessions	Percentage of dead seeds		
	Untreated seed (control)	Seed treatment	
		Scarification[1]	Boiled water[2]
I. cryptantha 7067	9 Al[3]	27 Am	17 An
I. brevicalyx 7517	3 Bl	59 Cm	43 Cn
I. arrecta 7524	15 Dl	51 Em	15 Dn
I. spicata 8254	5 Fl	26 Fm	14 Fn
I. vohemarensis 8730	3 Gl	44 Hm	11 Gn
I. trita 10297	6 Il	54 Jm	21 IJn
I. spicata 10299	15 Kl	47 Km	39 Kn

[1] Seed rubbed with sandpaper.
[2] Seed immersed in boiled water and left until the water cooled down.
[3] Means within rows followed by the same uppercase letter or within columns followed by the same lower case letter are not significantly different (P > 0.05).

Conclusions

As a practical technique to overcome poor germination, associated mainly with hard seed dormancy, the present study found considerable variation among the accessions in terms of their response to seed treatment options before planting. An effective treatment method should significantly improve germination rate of the seed lots without causing a significant increase in the mortality of potentially viable seeds. This has been successfully achieved in *I. cryptantha* 7067 and *I. spicata* 8254 by scarification. In contrast, improved germination rates of *I. vohemarensis* 8730, *I. arrecta* 7524 and *I. trita* 10297 were obtained without significant seed mortality by boiled water treatment. The effects of the 2 treatment methods are similar for both *I. brevicalyx* 7517 and *I. spicata 10299.* Either technique can be used to increase germination in the case of *I. brevicalyx* 7517, but with significant mortality, while neither technique seems appropriate in the case of *I. spicata* 10299, which has a lower proportion of hard seed (54%) than other accessions (>75%).

Previous studies with *Leucaena leucocephala* report that manipulation of hot water temperature is more effective than immersion time in breaking hard seededness while minimising seed mortality (Oakes 1984). Further improvements in germination could be expected in the case of *I. brevicalyx* 7517 by determining optimum hot water temperature below boiling point (93°C at the elevation of Pretoria) and/or identification of optimum immersion time.

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