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Response of Seed Treatments on Seed Germination in Wild *Crotalaria* Species

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Abstract

Laboratory trials were carried out to evaluate the effect of concentrated sulphuric acid (98%) and hot water on the germination capacity of four wild and one cultivated species of *Crotalaria* viz *C. laburnifolia* L, *C. pallida* Aiton, *C. medicagenea* Lam, *C.retusa* L and *C. juncea* L. Hot water treatment at 80°C for 5 min. was found to be most effective in breaking hardseededness in *C. laburnifolia.* The percentage of hard seeds declined sharply in *C. medicaginea,* and *C. retusa* when soaked in water at 70°C for 15 min. In *C. pallida* soaking seeds in hot water at 60°C for 30 min enhanced germination. Acid scarification was also found to be effective in breaking the dormancy but had undesirable effects and resulted in maximum of abnormal seedlings and dead seeds. Subjecting seeds to dry heat and mechanical scarification was found ineffective in removing hardseededness.

INTRODUCTION

Crotalaria (family Fabaceae) consists of some 600 species out of which about 170 species are found in India. It is distributed throughout tropical and sub-tropical regions and to a lesser extent in temperate areas. *Crotalaria* species. are used as source of fibers, forage, medicine, silage, green manure, cover crops and ornamentals. Wild species are sources of desirable genes in any crop improvement program but the precise use of these wild species as such or in future breeding cannot be determined until they are collected and grown and their compatibility with cultivated strains assessed. Keeping this in view wild species of *Crotalaria* were collected under National Agricultural Technology Project (Plant Biodiversity).

While testing the viability of seeds of *Crotalaria* species for long term conservation in the National Gene Bank (NGB), it was observed that seed of some species exhibited dormancy/ hardseededness ranging from 99-100%. In nature, hardseededness is of significant ecological benefit as it helps to overcome unfavorable conditions but in agriculture hardseededness can be disadvantageous, as the lack of germination prevents uniform plant establishment in the field. Seed dormancy hampers viability testing and seed regeneration for germplasm managers, plant breeders and other researchers as it can often interfere with the results of germination tests designed to estimate the percentage viability of accessions. Many wild and weedy crop relatives, which may possesses extremely valuable genetic diversity, display dormancy characteristics. Relatively little attention has been paid to wild plant species, whose seeds are generally, not traded or commercially tested. These taxa pose a special challenge to the managers of germplasm collections (Widrlechner,1997) Dormancy is not a problem in *C. juncea,* a cultivated species but the seeds of wild species viz., *C. pallida*, *C. medicaginea* ,*C. retusa* and *C. laburnifolia* show hard seeds. The purpose of this study was to evaluate the efficacy of the sulphuric acid and hot water treatment in breaking hardseededness in four wild *Crotalaria* species

MATERIALS AND METHODS

Physiologically mature seeds of *C. pallida* (IC374409), *C. medicaginea* (IC282178), *C. retusa* (IC410889) and *C. laburnifolia* (IC311758) and *C. juncea* (IC282646) were received from CRIJAF, Barrackpore, India for long term conservation in the NGB. The seed viability was confirmed by using 2, 3, 5 –Triphenyl Tetrazolium

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Eds. M.L. Chadha et al.
Acta Hort. 752, ISHS 2007

Chloride (TTC) test using randomly selected 25 seeds in two replicates. Seed morphological characteristics such as seed length, width, seed colour and surface texture were recorded in the above mentioned species. Seed of the wild species exhibited 90-100% dormancy due to hard seed coats. Thus the the following treatments were given during 2005.

Acid Scarification

Sub samples of seeds were immersed in 10 ml of concentrated H_2SO_4 for 10, 20, 40, 60 min., and 50 and 25 % H_2SO_4 for 15 and 30 min. each. After the treatment, seeds were thoroughly washed in running tap water for 2-3 h and seed were dried by filter paper before plating.

Hot Water Treatment

Seeds were soaked in hot water at 60° and 70°C for 15 min., and 30° and 80°C for 5 and 10 min.. Germination test was performed using 3 replicates of 100 seeds each by keeping seeds between papers at 25 °C temperature in a germinator. Observations were recorded as per ISTA Rules 2003. The seeds were also subjected to other treatments such as mechanical scarification by sand paper and dry heat treatment at 65°C for17 h. Analysis of variance was performed in completely randomized design (CRD) using MSTAT C software and critical difference values were calculated at 5% probability level.

RESULTS AND DISCUSSION

Results of Tetrazolium test confirmed that viability of seeds selected for the dormancy breaking treatments was 100 % as the seeds showed deep red embryonic axis and cotyledons. However we could observe only 5-10 % germination in a standard germination test, emphasizing the presence dormancy in these species. In cultivated species *C.juncea* the germination was 100%. The *Crotalaria* species showed variation in seed morphological characteristics (Table 1.). The mean length and breadth and 100 seed weight was recorded to be highest in *C. juncea* and lowest in *C.medicaginea.* Several investigators working with different crops have employed various treatments for breaking hard seededness i. e. H_2SO_4 treatment as softening impermeable seeds (Quinlivan, 1971), in breaking hard seededness (Tomar and Promila Kumari,1991, Srinivasan et al., 1997). Similarly enhanced germination was recorded in *Abutilon indicum* with hot water scarification (Gupta et al., 2001).

Amongst different scarification treatments to break the hard seed dormancy in *C. laburnifolia* hot water treatment for 5 min. at 80°C resulted in 68% germination whereas concentrated H_2SO_4 treatment for 60 min resulted in 65% germination. The percentage of hard seeds declined sharply in *C. medicaginea,* and *C. retusa* with increase in temperature i.e. 70°C for 15 min. although 10% seeds in *C. medicaginea* and 27% seeds in *C. retusa* remained hard after this treatment. With further increase in soaking time the number of hard seed % was reduced but the % of abnormal and dead seeds increased (Table2). There were negligible differences in germination between conc. H_2SO_4 for 20 min. and hot water at 70 ^{0} C for 15 min. In *C. pallida* soaking seeds in hot water at 60°C for 30 min. enhanced germination up to 81% followed by conc. H_2SO_4 treatment for 40 min.(72%) (Table 3). However acid scarification increased germination percentage but has undesirable effects and results in abnormal seedlings and dead seeds (Table3).The optimum dormancy breaking treatment varied with the species though in general concentrated H_2SO_4 treatment for 60 min. and hot water treatment at 70^0 C for 15 min. were effective in all the four species. The differences in seed coat colour and the associated phenolic compounds may be linked to the differential response of seeds to dormancy breaking treatment.

From the present study it can be concluded that hot water treatment for different durations was sufficient for breaking hard seeds in wild *Crotalaria* species while retaining the maximum viability resulting in normal seedlings. On the other hand H_2SO_4 can be damaging at the same time increases the % abnormal seedlings and dead seeds.

These treatments can be employed by genebank staff for routine seed testing during seed conservation and regeneration programmes.

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Tables

Table 1. Morphological characteristics of wild *Crotalaria* species.

Botanical identity	National identity	Seed size (mm) Length	Width	Seed colour	100 seed wt. (gm)	Surface texture
C. laburnifolia	IC – 311758	4	3	Brownish	3.36	Smooth
C. medicaginea	IC – 282178	2	1.5	Dark grey	0.35	Smooth & shiny
C. juncea	IC – 282646	6	4.5	Greenish	3.76	Smooth & shiny
C. pallida	IC – 374409	3	2	Greenish	0.7	Very smooth & shiny
C. retusa	IC - 410889	2	1.5	Very light brown	0.52	Smooth & shiny

Table 2. Effect of hot water treatment on percentage normal, abnormal seedlings, hard seeds and viability of four wild C*rotolaria* species

Treatment	Normal					Abnormal					Hard					Viability*				
	Cl	Cp	Cr	Cm	Mean	Cl	Cp	Cr	Cm	Mean	Cl	Cp	Cr	Cm	Mean	Cl	Cp	Cr	Cm	Mean
60 °C 15 min	0.5	76	42	32.5	37.7	0	0	0	0	0	99.5	24	58	67.5	62.2	100	100	100	100	100
60 ° C 30 min	11	82	49	44.5	46.7	0	0	0	0	0	89	18	44.5	55.5	51.7	100	100	94	100	98.5
70 ° C 15 min	59	57	82	70	67.2	0	0	0	0	0	41	23	16.5	30	27.7	100	81	99	100	95
70 ° C 30 min	64	36	72	64	59.1	0	0	7.5	0	1.9	36	16	13	27	23.1	100	53	85	91	82.2
80 ° C 5 min	68	22	51	52	48.2	0	8.5	21.5	20.0	12.5	32	14	0	19.5	16.4	100	36	51	71	64.6
80 ° C 10 min	49	18	23	39	32.2	22	12.5	20.5	30	21.2	26	5.5	0	8	9.9	75	23	23	47	42.1
control	-	9	2	4.5	4	0	0	0	0	0	0	91	97.5	95.5	71.0	100	100	100	100	100
	35.9	43	46.1	43.8		3.1	3	7.1		7.1	46.2	27.5	32.8		43.3	96.4	70.0	78.8	87.1	
CD 5%	Species 1.50; Treatment 1.99					Species 1.17; Treatment 1.55					Species 1.50; Treatment 1.99					Species 1.20; Treatment 1.61				
CD 5% S x T	3.99					3.11					3.96					3.21				

*Normal seedlings +hard seeds

*Normal seedlings +Hard seeds *C. laburnifolia (Cl) C. pallida,* (Cp) *C. medicagena(Cr) C. retusa (Cr)*

Table 3. Effect of acid scarification on treatment on percentage normal, abnormal seedlings, hard seeds and viability of four wild

Table 3. Effect of acid scarification on treatment on percentage normal, abnormal seedlings, hard seeds and viability of four wild *Crotolaria* species.

Treatment	Normal					Anormal					Hard					Viability*				
	Cl	Cp	Cr	Cm	Mean	Ci	Cp	Cr	cm	Mean	Cl	Cp	Cr	Cm	Mean	Cl	Cp	Ccr	cm	`Mean
25% /15min.	0	27.5	21	20	17.2	0	7	0	0	1.75	100	65.5	91	97.5	81	100	93	100	100	98.25
25% min. /15min	0	35	33	24	23.1	0	9.5	0	0	2.37	100	55.5	78.5	80	74.5	100	90.5	100	100	97.62
50% min. /15min	10.5	37.5	61	33.5	35.7	0	10	2	0	3	89.5	49	67	75.5	60.1	100	86.5	97	100	95.87
50%/30 min.	50.5	42.5	82.5	40	53.9	0	11	2.5	0	3.4	49.5	42.5	35.5	66.5	41.5	100	85	96.5	100	95.37
100%/10 min.	0	44	33.5	40.5	29.5	0	4.5	6.5	7	4.5	100	50.5	14	60	63.9	100	94.5	87	92	93.37
100%/20 min.	26.5	59	67	75.5	57	0	14.5	8.5	8.5	7.9	67.5	17.5	53.5	51.5	26.1	94	76.5	77.5	84,.5	83.12
100%/40 min.	57	71	75	70	68.4	6.5	19	11.5	15	13	30.5	0	10.5	9	8.5	87.5	71.5	75	73.5	76.87
100%/60 min.	65	64	66.5	63.5	64.7	6.5	23	16	9.5	13.7	16	0	0	3.5	4	81	64	66.5	63.5	68.75
Control	0	9	2.5	6	4.3	0	0	0	00	0	1 00	0	0	95.5	96	100	100	100	100	100
CD 5%	Species 1.27; treatment 1.91					Species 0.75; Treatment 1.12					Species 1.16;Treatment 1 .71					Species 1.21; Treatment 1.8				
CD 5% S x T	3.81					2.24					3.48					3.62				

*Normal seedlings +Hard seeds *C. laburnifolia (Cl) C. pallida,* (Cp) *C. medicagena(Cr) C. retusa (Cr)*