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Effect of Dormancy Treatments on Seed Quality and Storability of *Stylosanthes hamata*

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ABSTRACT: *Stylosanthes hamata cv.* verano, which is an important forage legume, offers fodder for livestock, but its establishment in field is very poor because of hard seeded nature. Hence, it is important to reduce the hard seed content in a lot, for successful germination and uniform seedling emergence. In the present study, the germination and other parameters of *Stylosanthes hamata* seeds were tested under three types of dormancy breaking treatments. For physical treatment, hot water, hot air oven and smoke treatment were used. Mechanical treatment was performed by hand scarification using sandpaper and chemical treatment by sulphuric acid, sodium hydroxide, polyethylene glycol-6000 and ethrel solutions. The results revealed that, fresh seeds (control) recorded zero per cent germination initially, while mechanical scarification with sand paper recorded the highest germination (40.0 %) and seedling vigour index (335) initially. Similarly, at the end of eleven months of storage, the highest percentage of germination (73.5%) and vigour index (656) were obtained in mechanically scarified seeds (T_1) followed by acid scarification with conc. H_2SO_4 for 2 min. (43.8% and 376, respectively) along with the reduction of hard seededness. Maximum dead seeds (23.0%) were recorded in seeds soaked in hot water.

Key words: *Stylosanthes*, dead seed, hard seed, seed germination, seed storage

Stylosanthes hamata cv. Verano belongs to *Fabaceae* family and is an herbaceous annual (as well as short-lived perennial) with a non determinant, semi-erect habit and dichotomous branching pattern. *Stylosanthes hamata* was originally introduced to India from Australia in early 1970s' for the improvement of grasslands and is grown mostly in the rainfall range of 600-1100 mm. Stylosanthes was mainly used in India for re-vegetation of wastelands, where it reduces soil erosion and offers fodder for livestock [1]. Despite the great importance and useful characteristics, establishment of *Stylosanthes hamata* is difficult. One of the major constraints in successful stand establishment is hard seed. High hard seed content in a seed lot causes delayed or decreased seedling emergence. As a result stands become thin, sporadic and less competitive with weeds or undesirable species which in turn reduces not only N fixation but also lower yield and quality. Therefore, reduction of hard seed content in a seed lot is very important before planting. Hard seed coat dormancy is of ecological importance to the species. It can enhance the longevity of seeds and has a large impact on the survival and emergence of plants in the wild [2]. However, it affects the uniformity of germination of crops and poses a major challenge in seed testing and genebank operations, as dormant seed will not germinate even under optimum conditions. Seeds of many wild species of legume are most likely to have hard seed and pre treatments for dormancy breaking are required before testing and sowing [3]. Different degrees of hard seededness are achieved as the seed matures and loses moisture to reach equilibrium in accordance with the prevailing atmospheric humidity [4]. To overcome the hard seed nature different physical and chemical treatments have been developed to rupture, remove or dissolve the water impermeable

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seed coat. Many pre-treatments have been developed to rupture the hard seed coat for getting synchronized germination and also for assessing the true germination percentage in the seeds of commercial importance [5]. Therefore, an investigation on the effect of hard seed coat dormancy breaking treatments on germination and storability of *Stylosanthes hamata* was undertaken for enhancing the seed quality and to document the impact of these treatments on seed storability.

MATERIALS AND METHODS

An experiment on overcoming seed dormancy in *Stylosanthes hamata cv.* Verano was undertaken at Indian Grassland and Fodder Research Institute, Southern Regional Research Station, Dharwad during 2012-13 and 2013-14. The freshly harvested seeds (February, 2012) were evaluated for the germinability. Since, the fresh seeds had exhibited large scale seed dormancy when evaluated under laboratory conditions, the seeds were imposed with following treatments to overcome this problem. The treatments were T_0: Control, T_1: Mechanical scarification by using sand paper, T_2: Soaking the seeds in hot water (80^0C initial temperature and then left for 12 hours), T_3: Scarifying the seeds in conc. H_2SO_4 for 2 min, T_4: Soaking the seeds in Sodium hydroxide solution @ 20% for 15 min., T_5: Soaking the seeds in PEG-6000 solution @ 200g/ l for 48 hrs, T_6: Soaking the seeds in Ethrel solution @ 1000 ppm for 24 hrs, T_7: Plant derived smoke treatment (exposing the seeds to the *Eupatorium odoratum* smoke for 30 min.) and T_8: Keeping the seeds to hot air oven at 80^0C for 10 min. Further, the treated seeds were also studied for their storage behaviour to document the performance of treated seeds under ambient conditions (RH: maximum 85 %, minimum 54 %; temperature: maximum 31° C minimum 16° C) of Dharwad for 12 months, along with untreated seed (T_0) in cloth bag. These seeds were evaluated under laboratory conditions in a completely randomized design with four replications.

Seed germination

Germination was tested by placing 400 seeds from each treatment, in four replications, on moist paper towels and kept at 25-35°C alternate temperature and relative humidity of 75% [3]. First count of germination was made at 4^{th} day and subsequent counts were made at 3 day interval till 15 days. Seeds were considered germinated when the radicle had emerged; germinated seeds were removed after each count. At the end of the test, seeds that had not germinated were categorized into hard and dead seed components by touching and piercing with a needle. Hard seeds could not be pierced with the needle.

Seed vigour

The vigour index of seedling was calculated by adopting the method suggested by Abdul-Baki and Anderson [6] and expressed as whole number by using the formula

Vigour index of seedling = Germination (%) × (root length + shoot length) in cm.

Statistical analysis:

The percentage of germinated, hard and dead seeds was transformed into arcsine values for conducting the statistical analysis adopting Completely Randomized Block Design as per Sundarraj *et al.* [7].

RESULTS AND DISCUSSION

Germination (%)

In fresh seeds significantly higher germination percentage (40.0 %) was achieved due to mechanical scarification (T_1) (Table 1). As storage period advanced, the germination of seeds increased sharply from 40 % to 85 % by fifth month and later declined to 73.50 %) at the end of 11^{th} months of storage. The increase in permeability of seed coat might have helped in germination enhancement initially, but the same might have made the seed prone to faster deterioration. Significantly lower germination was recorded in untreated control seeds (T_0) where germination increased to only 13 per cent even after 11 months of storage. The *Stylosanthes* seed with germination below 40% is not suitable for trading as per Indian Minimum Seed Certification Standards [8] and hence seed without any treatment cannot be marketed in the same year of production.

The previous studies showed that it was

Table 1. Effect of seed dormancy breaking treatments on germination (%) of *Stylosanthes hamata* seeds during storage

Treatments	Months of storage											
	0	1	2	3	4	5	6	7	8	9	10	11
T_0	0.00 (0.00)*	0.00 (0.00)	3.80 (11.1)	7.00 (15.3)	8.00 (16.4)	9.00 (17.5)	10.5 (18.9)	10.8 (10.8)	11.0 (19.4)	11.8 (20.0)	12.0 (20.3)	13.0 (21.1)
T_1	40.0 (39.2)	56.0 (48.4)	61.5 (51.6)	71.0 (57.4)	82.0 (64.9)	85.0 (67.2)	84.8 (67.0)	82.0 (64.9)	80.8 (64.0)	79.0 (62.7)	75.3 (60.1)	73.5 (59.0)
T_2	12.0 (22.3)	13.8 (21.8)	15.5 (23.2)	16.3 (23.8)	17.8 (24.9)	18.0 (25.1)	20.0 (26.6)	20.0 (26.6)	18.0 (25.1)	16.7 (24.1)	15.3 (23.0)	14.0 (22.0)
T_3	14.0 (20.3)	25.3 (21.8)	27.3 (23.2)	37.4 (37.7)	48.0 (43.8)	52.0 (46.1)	52.8 (50.2)	52.5 (41.5)	49.3 (44.6)	46.3 (42.8)	44.8 (42.0)	43.8 (41.4)
T_4	10.8 (19.1)	12.0 (20.3)	15.8 (23.4)	18.0 (25.1)	18.0 (25.1)	23.0 (28.6)	25.0 (30.0)	24.3 (29.6)	24.5 (29.7)	22.0 (28.0)	21.0 (27.3)	17.3 (24.5)
T_5	0.00 (0.00)	4.50 (12.2)	5.00 (12.9)	7.30 (15.6)	9.00 (17.4)	10.0 (18.4)	11.0 (19.4)	12.0 (12.0)	13.0 (21.1)	12.5 (20.7)	12.3 (20.5)	14.5 (22.4)
T_6	5.00 (12.9)	6.80 (15.0)	8.00 (16.4)	11.8 (20.0)	12.0 (20.3)	14.0 (22.0)	20.0 (26.6)	19.5 (19.5)	20.0 (26.6)	18.5 (25.5)	17.0 (24.3)	17.0 (24.3)
T_7	10.5 (18.9)	11.5 (19.8)	15.0 (22.8)	16.0 (23.6)	17.0 (24.3)	22.3 (28.1)	24.8 (29.8)	23.5 (23.5)	23.8 (29.2)	21.8 (27.8)	18.5 (25.5)	16.8 (24.1)
T_8	7.30 (15.6)	8.00 (16.4)	8.80 (17.2)	12.0 (20.3)	13.0 (21.1)	15.5 (23.2)	21.0 (27.3)	22.0 (22.0)	23.3 (28.8)	19.8 (26.4)	21.8 (27.8)	20.8 (27.1)
Mean	11.1	15.3	17.8	21.9	25.0	27.6	30.7	29.6	29.3	27.6	26.4	25.6
S.Em±	0.10	0.54	0.23	0.40	0.22	0.19	0.20	0.29	0.17	0.21	0.20	0.20
CD@1%	0.29	1.49	0.63	1.12	0.62	0.52	0.55	0.81	0.46	0.59	0.55	0.57

*Figures in the parentheses indicate arcsine root transformed values.

Seed treatments (T) : T_0 - Control (untreated) T_1 - Mechanical scarification for 5 min. T_2 - Hot water treatment @ 80°C for 5 min. T_3 - Conc.H_2SO_4 for 1 min. T_4 - Sodium hydroxide @ 20% for 15 min. T_5 - PEG-6000 @ 200g/lit for 48 hrs. T_6 - Ethrel @ 1000ppm for 24 hrs. T_7 - Smoke treatment with *Eupatorium odoratum* for 30 min. T_8 - Hot air oven @ 80°C for 10 min.

possible to overcome seed dormancy and increase germination in forage legumes by the application of mechanical and chemical treatments. Mechanical scarification was found to be the most effective seed dormancy breaking treatment in forage legume, *Pacheocoa venezuelensis*, where the germination percentage increased from 2.0 % in untreated seeds to 53.5 % after 28 days of treatment. In the present study, hand scarification of *Stylosanthes* seeds with sand paper improved germination, as shown in Table 1. These results are in close agreement with the results of Bhatt *et al.* [9] in different species of *Stylosanthes.* Similar results were recorded in other forage legumes *viz.*, *Medicago* and *Trifolium* species [10], *Desmanthus illinoensis* [11] and *Medicago scutelleta* [12].

Hard seed percentage

Initially (*i.e.* immediately after the treatment and before storage), the significantly lowest hard seed percentage (60.0%) was noticed in mechanically scarified seeds (T_1) preceeded by T_3 (acid scarified seeds with conc. H_2SO_4) (83.3 %). while, significantly highest value was recorded in T_0 (untreated control) (100%). As storage period advanced, these treatments recorded decline in hard seed content and recorded 9.5, 36.5 and 79.0%, respectively at the end of eleven months of storage period (Table 2). The seed coat of *Stylosanthes hamata* is resistant to the penetration of water and gases, resulting in poor germination. The hard seed coat dormancy can be overcome by enhancing the seed coat permeability through different treatments [13]. Even though initially, higher proportion of hard seeds were noticed in both treated and untreated seeds, as the storage period advanced the permeability of the seed increased. This resulted in enhancement of germination and reduction of hard seeds. The degree of hard seed reduction was more when seeds were subjected to mechanical scarification and sulphuric acid treatments (Table 2). Decrease in hard seed

Table 2. Effect of seed dormancy breaking treatments on hard seed (%) of *Stylosanthes hamata* during storage

Treatments	Months of storage											
	0	1	2	3	4	5	6	7	8	9	10	11
T_0	100 (90.0)*	100 (90.0)	95.3 (77.4)	91.0 (72.5)	89.8 (71.3)	88.5 (70.2)	86.3 (68.2)	85.3 (67.4)	83.8 (66.2)	82.3 (65.1)	81.5 (64.5)	79.0 (62.7)
T_1	60.0 (50.7)	44.0 (41.5)	37.0 (37.4)	27.0 (31.3)	15.5 (23.2)	12.5 (20.7)	11.0 (19.4)	12.0 (20.3)	11.5 (19.8)	11.0 (19.3)	10.0 (18.4)	9.50 (17.9)
T_2	86.0 (68.0)	83.3 (65.8)	81.8 (64.7)	80.5 (63.8)	78.3 (62.2)	78.0 (62.0)	75.3 (60.1)	69.0 (56.1)	67.3 (55.1)	65.3 (53.9)	63.7 (52.9)	63.0 (52.7)
T_3	83.3 (65.8)	71.7 (57.9)	70.0 (56.6)	59.3 (50.3)	48.0 (44.0)	44.0 (41.5)	43.0 (41.0)	40.8 (39.7)	39.8 (39.1)	38.7 (38.4)	38.2 (38.1)	36.5 (37.1)
T_4	87.2 (65.1)	84.5 (66.8)	81.3 (64.3)	79.3 (62.9)	79.0 (62.7)	74.0 (59.3)	72.0 (58.0)	70.8 (57.2)	68.0 (55.5)	65.0 (53.7)	61.0 (51.3)	61.5 (51.6)
T_5	99.0 (84.2)	93.5 (75.2)	92.0 (73.5)	89.8 (71.3)	87.8 (69.5)	86.8 (68.6)	85.3 (67.4)	83.0 (65.6)	80.0 (63.4)	78.5 (62.4)	76.3 (60.8)	73.5 (59.0)
T_6	94.0 (75.8)	91.0 (72.5)	88.8 (70.4)	85.5 (67.6)	84.8 (67.0)	82.8 (65.4)	76.8 (61.1)	75.8 (60.5)	74.0 (59.3)	73.5 (59.0)	71.0 (57.4)	69.3 (56.3)
T_7	89.5 (71.1)	88.5 (70.2)	85.0 (67.2)	82.0 (64.9)	82.5 (65.3)	74.8 (59.8)	72.3 (58.2)	71.0 (57.4)	66.8 (54.8)	66.3 (54.5)	65.5 (54.0)	66.3 (54.5)
T_8	92.8 (74.4)	92.0 (73.5)	91.3 (72.8)	86.0 (68.0)	84.8 (67.0)	82.0 (64.9)	76.0 (60.6)	75.0 (60.0)	73.5 (59.0)	73.5 (59.0)	70.5 (57.1)	64.0 (53.1)
Mean	88.0	83.2	80.2	82.7	72.3	69.4	66.4	64.7	62.7	61.6	59.7	58.1
S.Em±	0.16	0.55	0.28	0.40	0.50	0.22	0.21	0.17	0.14	0.19	0.23	0.21
CD@1%	0.43	1.51	0.77	1.10	1.39	0.60	0.58	0.47	0.38	0.55	0.65	0.58

*Figures in the parentheses indicate arcsine root transformed values.

Seed treatments (T) : T_0 - Control (untreated) T_1 - Mechanical scarification for 5 min. T_2 - Hot water treatment @ 80°C for 5 min. T_3 - Conc. H_2SO_4 for 1 min. T_4 - Sodium hydroxide @ 20% for 15 min. T_5 - PEG-6000 @ 200g/lit for 48 hrs. T_6 - Ethrel @ 1000ppm for 24 hrs. T_7 - Smoke treatment with *Eupatorium odoratum* for 30 min. T_8 - Hot air oven @ 80°C for 10 min.

percentage due to mechanical scarification was also reported in Persian clover (*Trifolium resupinatum*) by Ertan Ates [14].

Dead seed (%)

Irrespective of the seed treatments, as the storage period advanced, increase in the dead seed percentage of *Stylosanthes hamata* was noticed (Table 3). Initially, it was significantly highest (2.8 %) in hot water treatment (T_2) followed by sulphuric acid treatment (2.7 %). Likewise, at the end of storage period also, significantly higher dead seed percentage (23.0) was noticed in T_2 (seeds treated with hot water), followed by T_3 (seeds treated with sulphuric acid) (19.7%). Significantly lower dead seed percentage (8.00) was noticed in control, at the end of storage period.

The higher percentage of dead seeds in hot water treated seeds was mainly due to the attack of storage fungi which might have caused the death

Table 3. Effect of seed dormancy breaking treatments on dead seed (%) of *Stylosanthes hamata* during storage

Treatments	Months of storage											
	0	1	2	3	4	5	6	7	8	9	10	11
T_0	0.00 (0.00)*	0.00 (0.00)	1.00 (5.70)	2.00 (8.10)	2.30 (8.60)	2.50 (9.00)	3.30 (10.4)	4.00 (11.5)	5.30 (13.2)	6.00 (14.2)	6.50 (14.8)	8.00 (16.4)
T_1	0.00 (0.00)	0.00 (0.00)	1.50 (6.90)	2.00 (8.10)	2.50 (9.00)	2.50 (9.00)	4.30 (11.9)	6.00 (14.2)	7.80 (16.2)	10.0 (18.4)	14.8 (22.6)	17.0 (24.3)
T_2	2.80 (9.40)	3.00 (10.0)	3.00 (10.0)	3.30 (10.4)	3.80 (11.2)	3.30 (10.5)	4.50 (12.5)	11.0 (19.4)	14.6 (22.5)	18.0 (25.1)	21.0 (27.3)	23.0 (28.6)
T_3	2.70 (9.50)	3.00 (10.0)	2.70 (9.50)	3.30 (10.4)	4.00 (11.5)	4.00 (11.5)	4.20 (11.8)	6.70 (15.0)	10.9 (19.3)	15.0 (22.8)	17.0 (24.3)	19.7 (26.3)
T_4	2.00 (8.10)	3.50 (10.8)	3.00 (10.0)	2.80 (9.50)	3.00 (10.0)	3.00 (10.0)	3.00 (10.0)	5.00 (12.9)	7.50 (15.9)	13.0 (21.1)	18.0 (25.1)	21.3 (27.4)
T_5	1.00 (5.70)	2.00 (8.10)	3.00 (10.0)	3.00 (10.0)	3.30 (10.4)	3.30 (10.4)	3.80 (11.1)	5.00 (12.9)	7.00 (15.3)	9.00 (17.5)	11.5 (19.8)	12.0 (20.3)
T_6	1.00 (5.70)	2.30 (8.60)	3.30 (10.4)	2.80 (9.50)	3.30 (10.4)	3.30 (10.4)	3.30 (10.4)	4.80 (12.6)	6.00 (14.2)	8.00 (16.4)	12.0 (20.3)	13.8 (21.8)
T_7	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	2.00 (8.10)	2.50 (9.00)	3.00 (10.0)	3.00 (10.0)	5.50 (13.5)	9.50 (17.9)	12.0 (20.3)	16.0 (23.6)	17.0 (24.3)
T_8	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	2.00 (8.10)	2.30 (8.60)	2.50 (9.00)	3.00 (10.0)	3.00 (10.0)	3.30 (10.4)	6.80 (15.0)	7.80 (16.2)	15.3 (23.0)
Mean	1.10	1.50	1.90	2.60	3.00	2.90	3.60	5.70	8.00	10.9	13.8	16.3
S.Em±	0.11	0.09	0.11	0.12	0.16	0.16	0.15	0.09	0.15	0.06	0.16	0.12
CD@1%	0.33	0.25	0.30	0.33	0.45	0.43	0.41	0.25	0.42	0.16	0.44	0.33

*Figures in the parentheses indicate arcsine root transformed values.

Seed treatments (T) : T_0 - Control (untreated) T_1 - Mechanical scarification for 5 min. T_2 - Hot water treatment @ 80°C for 5 min. T_3 - Conc.H_2SO_4 for 1 min. T_4 - Sodium hydroxide @ 20% for 15 min. T_5 - PEG-6000 @ 200g/lit for 48 hrs. T_6 - Ethrel @ 1000ppm for 24 hrs. T_7 - Smoke treatment with *Eupatorium odoratum* for 30 min. T_8 - Hot air oven @ 80°C for 10 min.

of the cells in the softened tissue. Mc Ivor and Gardener [15] stated that immersion of seeds in hot water for more than one minute resulted in death of the seeds of *Stylosanthes* species. The increase in dead seed percentage in treated seeds was also observed in other related crops like seca stylo by Hopkinson and Paton [5] and in *Centrosema pubescens* by Win Pe *et al.* [16].

Seedling vigour index

Seed germination percentage under laboratory conditions is the standard measure of seed quality. However, seed lots with the same germination percentage may germinate at different rates. Therefore, seed vigour is becoming an increasingly important measure of seed quality especially in forage legumes [17]. As evident from the data (Table 4), the mechanical scarification in fresh seeds (T_1) recorded significantly highest vigour index (335) over rest of the seed treatments. During the storage, the vigour index of the seedlings increased (787) till 5th month of storage. However, the seedling vigour index decreased with further increase in storage period. Among the seed treatments, the second best treatment was the use of Conc. H_2SO_4 (T_3-116) in fresh seeds where, vigour index increased up to 536 at the end of six months of storage and later decreased to 376 by the end of storage period (11 months). Significantly lower vigour index was observed in T_0 (untreated control), initially (0) as well as at the end of eleven month storage (100). Similar findings have been reported by Paramathma *et al.* [18] in butterfly pea and siratro seeds, Bhatt *et al.* [9] in *Stylosanthes* species and Reshma *et al.* [19] in *Desmanthus virgatus*.

Since the seeds of *Stylosanthes hamata* are encased in a pod with leathery texture, as evidenced by experimental data, the mechanical scarification through coarse sand paper is the best method to make the leathery structure permeable to water, and reduce the impermeability of the seed coat which in turn promotes the rapid germination of seed. Hence, for getting better germination and seedling vigour, this method can be adopted compared to other scarification treatments *viz.*, hot water or Conc. H_2SO_4.

Table 4. Effect of seed dormancy breaking treatments on seedling vigour index of *Stylosanthes hamata* during storage

Treatments	Months of storage											
	0	1	2	3	4	5	6	7	8	9	10	11
T_0	0	0	28	53	62	70	84	87	91	97	93	100
T_1	335	478	535	628	736	787	780	749	735	714	674	656
T_2	91	106	123	130	143	147	165	168	150	140	126	119
T_3	116	211	232	328	425	463	536	472	437	407	389	376
T_4	98	110	133	153	157	203	222	214	215	192	180	147
T_5	0	34	39	58	72	80	89	98	108	103	100	117
T_6	39	54	65	97	100	118	170	169	174	157	143	140
T_7	84	93	124	134	144	194	219	207	207	186	157	142
T_8	57	65	72	99	109	133	183	192	203	166	184	172
Mean	91	128	150	187	217	244	272	262	258	240	227	219
S.Em±	0.93	4.68	2.14	3.84	1.56	2.94	1.77	2.49	1.49	2.12	1.79	1.73
CD@1%	2.58	12.9	5.94	10.64	4.33	8.13	4.91	6.89	4.14	5.88	4.99	4.79

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