



Overcoming the hard seededness in *Centrosema pubescens* seeds

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Abstract

In *Centrosema pubescens*, hard seed coat is the main problem impeding the germination by preventing water and gaseous exchange in the seed. Therefore, to improve the germination and other seed quality parameters, three different types of dormancy breaking treatments were employed. Accordingly physical dormancy breaking was done with hot water and hot air, mechanical scarification with sandpaper and chemical treatment with GA_3 , KNO_3 , H_2SO_4 and Thiourea. Results obtained during the study revealed that control seeds showed very low germination (10.0%) and hot water treatment increased germination (66.67%). The complete and sharp increase in germination achieved by physical scarification with hot water shows that the dormancy originated by seed coat (hard seededness) whereas, acid scarification resulted in lowest germination (2.67%) due to charring. The hot water treated seeds performed better over other treatments under storage conditions with regards to higher and increased seed germination, reduced hard seeds percentage seedling vigour index. Whereas the control treatment exhibited large scale hard seed coat dormancy even after 12 months of storage (58.0%).

Keywords: *Centrosema pubescens*, Forage legume, Hard seed, Scarification, Seed dormancy, Seed germination, Storage, Vigour index

Abbreviations: GA_3 : Gibberellic acid; H_2SO_4 : Sulphuric acid; KNO_3 : Potassium nitrate; **RH**: Relative humidity

Introduction

Centrosema pubescens is a climbing forage legume with unique floral structure, pod like fruits and nodule formation systems in the roots. The cultivation of *Centrosema pubescens* has increased many folds in recent past mainly due to production of high quality forage and ability to improve soil quality by fixing atmospheric nitrogen. One of the major constraints in successful stand establishment of *C. pubescens* 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. Therefore, breaking the physical dormancy due to hard seed coat is important before planting. Irrespective of the seed lots evaluated at Indian Grassland and Fodder Research Institute, Southern Regional Research Station, Dharwad, poor germination was experienced in freshly harvested seeds of *Centrosema pubescens*. The major cause was dormancy associated with hard seed. Although different pre-planting treatments are reported to be effective for breaking hard seed dormancy in different forage legume species (Ramamoorthy and Rai, 1990), little has been documented in case of *Centrosema* species. Hence, a laboratory experiment was carried out with the aim to identify the suitable pre-planting seed treatment practice to break seed dormancy to enhance seed germination of *Centrosema pubescens*. Further, the storage behaviour of treated seeds was also studied to document the performance of treated seeds over a period of twelve months.

Materials and Methods

An experiment on overcoming seed dormancy in *Centrosema pubescens* was undertaken at Indian Grassland and Fodder Research Institute, Southern Regional Research Station, Dharwad during 2012-13 and 2013-14. The freshly harvested seeds were evaluated for the germinability and only meagre germination was recorded. Therefore, the fresh seeds were imposed with following treatments. T_0 : Control, T_1 : Soaking of seeds in GA_3 solution @ 400 ppm, T_2 : Soaking of seed in KNO_3 solution @ 4 g/l, T_3 : Soaking of seed in hot water (80°C) for 5 min, T_4 : Keeping the seeds in hot air oven @ 80°C for 10 min, T_5 : Mechanical scarification by sand paper, T_6 : Soaking of seeds in Conc. H_2SO_4 solution for 2 minute and washing in running tap water and T_7 : Soaking the seeds in Thiourea solution @ 4 g/l for 30 minutes. The treated seeds were also studied for storage behaviour to document the performance of treated seeds under

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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.

By drawing the required number of seeds every month the germination test was conducted in the laboratory by following the procedures outlined by International Seed Testing Association (ISTA, 1999). This was done upto 12 months. On the day of final count, the number of normal seedlings obtained was taken as germination percentage. Simultaneously, the observations on abnormal seedlings, hard seeds and dead seeds were made. The vigour index of seedling was calculated by adopting the method suggested by Abdul-Baki and Anderson (1973) and expressed as whole number by using the formula;

Vigour index of seedling = Germination (%) $\times$ (root length + shoot length) in cm

The percentages of germinated, hard and dead seeds were transformed into arcsine values for carrying out the statistical analysis adopting completely randomized block design as per Sundarraj *et al.* (1972).

Results and Discussion

Effect of seed treatments on seed quality components

Germination (%): The preliminary germination tests showed that the fresh seeds cannot germinate easily in *Centrosema pubescens* (Table 1). The results showed that the seeds can germinate after subjecting them into various dormancy releasing treatments. Soaking of seeds in hot water (T_3) significantly helped to cause some metabolic changes within the dormant seeds and significantly enhanced the germination percentage (66.67) over control treatment (10.0), while only 2.67 per cent germination was observed in acid scarification with H_2SO_4 (Table 1). Seeds of fodder legumes undergo seed dormancy of many kinds causing delay in germination of variable duration. Similar results were also obtained by Omokanye and Onifade (1993) in *Centrosema pubescens* and Agboola (2006) in *Tithonia diversifolia*. They discovered that heat treatment of seeds helped to cause some metabolic changes within the dormant seeds. The ability of the embryo to germinate appears only when seeds have undergone warm stratification. There have been various instances where hot water treatments have been used to terminate dormancy in many range legume species (Ajiboye, 2006). High temperature might have caused the changes in the structure of the seed coat thereby causing permeability of seeds to water and gases and enhance germination (Fasidi *et al.*, 2000).

Hard seed (%): After treatment, significantly higher proportions of hard seeds were observed in control (88.44 %) than in physically or chemically treated seeds (Table 2). The integument of the seed of many leguminous species is resistant to the penetration of water and gases. This results in poor germination caused by hard seed coat, which can be overcome by treating seed to reduce the impermeability of the integuments (Elberse and Berman, 1989). Seeds treated with conc. H_2SO_4 for 2 min (T_6) broke hard seed coat dormancy (97.34%) to a significantly greater extent than untreated control, followed by hot water treatment (T_3) (74.0%). However, it is interesting to note that, majority of the acid scarified seeds with H_2SO_4 has charred and burnt, hence, recorded the minimum percentage of hard seed component and maximum component of dead seed percentage. 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 targeted levels of rapid establishment. Previous studies with *Leucaena leucocephala* report that manipulation of hot water temperature is more effective than immersion time in breaking hard seededness (Oakes, 1984).

Dead seed (%): Significantly higher dead seed percentage (94.67) was recorded in seeds treated with conc. H_2SO_4 for 2 min (T_6), whereas, significantly lower (1.56%) dead seed percentage was noticed in T_0 (control) treatment (Table 3). This is probably due to acid scarification with H_2SO_4 for two minutes burnt the seed testa and damaged the embryo, while the effect of other treatments was mainly through rupturing of the seed coat by ejecting the strophilolar plug and cracking the testa (Argel and Paton, 1999). This leads to water imbibition over a relatively longer period of time, which might have helped in better germinability of the seeds. This agrees with the results of Hopkinson and Paton (1993), who had reported increased laboratory germination of *Stylosanthes scabra* cv. Seca seed following scarification, with a slightly increased risk of causing seed death.

Seedling vigour index: Seed germinating percentage under laboratory conditions is the standard measure of seed quality. However, seed lots with the same germination percentage may germinate at different rates

(Duorado, 1989). Therefore, seed vigour is becoming an increasing important measure of seed quality especially in forage legumes (Chin and Wong, 1993). Because seedling vigour is difficult to measure quantitatively, germination and seedling growth rates have been used as reference indices for vigour tests (Wang and Hampton, 1993). In our experiment also, significantly highest vigour index (705.3) was observed in seeds treated with hot water (T_3), which was followed by mechanical scarification (T_5) (371.7). Significantly lowest vigour index (28.30) was observed in acid scarified treatment (T_6) (Table 4). The acid and mechanical scarified seeds reduced the impermeability of the seed coat by softening the outer layer, which might have promoted entry of water and exchange of gases into the seed. Probably during imbibition the embryo became metabolically active which resulted in the emergence of root and shoot and finally increased vigour index of the seedling. Similar results have been achieved by Paramathma *et al.* (1991) in butterfly pea (*Clitoria ternatea*) and Siratro (*Macroptilium atropurpureum*) seeds.

Effect of seed treatments on seed storability

Germination (%): Irrespective of different seed treatments, germination percentage increased till seventh month of storage then it declined gradually with the advancement in storage period. The germination percentage differed significantly due to seed treatments in all months of storage period (Table 1). At initial month of storage, significantly higher germination (66.67 %) was recorded in hot water treatment (T_3) and lower germination was recorded in control (T_0) (10.0%). Similarly, at the end of twelve months of storage higher germination was recorded in hot water treatment (65.0%) whereas, sulphuric acid treated seeds recorded nil germination, while mechanical scarification treatment (T_5) recorded the second best germination (56.0%). 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).

Hard seed (%): Hard seed coat impermeability is of ecological importance, since it lengthens the lifespan of viable seed and allows for the progressive germination of small proportions of a given seed lot over time, thus increasing opportunities for species survival. However, it also poses practical problems in agriculture, where high hard seed levels reduce the crop establishment. The efficiency of dormancy breaking treatments on hard seed

percentage of *Centrosema pubescens* during storage is shown in Table 2. In general, it was observed that the hard seed content decreased gradually with the advancement in the storage period. The percentage of hard seed remaining at the end of the germination test was significantly higher in the control than in those either physically or chemically treated seeds. On an average the hard seed percentage recorded at the beginning and end of storage period in control treatment was 88.44 and 58.0 per cent, respectively.

At initial stage of storage, significantly lower hard seed percent (2.66) was recorded in sulphuric acid treatment (T_6), followed by hot water treatment (T_3) (26.0). Similarly, at the end of 12th month of storage treatment T_6 (H_2SO_4) recorded nil hard seed percentage and the second best treatment was T_3 (hot water treatment) (18.0 %), while the control treatment recorded the maximum hard seed percentage (58.0). The hard seed character is heritable, but its expression is strongly related to prevailing climatic factors during plant growth and seed maturation as well as the degree of seed dehydration. Different degree of hard seededness is achieved as seed matures and loses moisture to reach equilibrium in accordance with the prevailing atmospheric humidity. At the end of storage, the high proportion of hard seeds was observed in the control compared to other treatments. Similar results were also obtained by Ertan Ates (2011) in Persian clover.

Dead seed (%): The dead seed percentage differed significantly due to different seed treatment during twelve months storage period. With advancement in the storage period, the dead seed percentage increased gradually irrespective of treatments (Table 3). However, higher dead seed percentage (94.67) at initial stage was recorded in T_6 (seeds soaked in H_2SO_4 solution for 2 min) followed by T_5 (mechanical scarification with sand paper) 8.67%. Significantly lower (1.56%) dead seed percentage was noticed in T_0 (control) treatment. The acid treated seeds were almost charred and burnt hence, maximum death of seeds were recorded. At the end of twelve month of storage, significantly lower dead seed percentage was recorded in seeds exposed to hot air (T_4) (1.33) which was followed by T_2 (2%) and T_7 (2%) treatments and higher dead seed was recorded in sulphuric acid treatment T_6 (100.0 %). The results are in conformity with the findings of Win Pe *et al.* (2012) in *Centrosema pubescens* who reported that acid treated seeds produced more abnormal seedlings than untreated seeds after a month of storage as increase in storage period caused death of the seeds.

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

Treatments	Duration of storage (Months)												
	Initial	1	2	3	4	5	6	7	8	9	10	11	12
T ₀	10.00	13.33	16.67	23.33	24.00	25.33	26.67	28.50	29.67	31.33	36.50	37.12	39.33
T ₁	18.00	16.00	18.00	18.58	20.00	19.33	19.83	20.23	16.00	22.00	22.67	26.00	31.33
T ₂	19.33	20.17	22.00	19.90	19.50	20.65	20.00	26.00	22.67	19.33	20.67	21.33	32.00
T ₃	66.67	76.67	75.33	64.00	69.33	68.67	62.67	68.00	66.67	64.00	69.50	69.33	65.00
T ₄	21.33	18.00	16.67	16.00	20.67	23.50	36.00	29.47	28.17	34.00	34.00	28.00	44.00
T ₅	30.67	31.17	34.00	42.67	40.67	46.67	35.33	46.67	37.33	44.00	53.03	54.00	56.00
T ₆	2.67	3.33	4.67	3.33	4.67	2.00	3.33	6.00	2.67	4.00	0.67	0.00	0.00
T ₇	23.33	20.67	18.67	16.17	13.33	18.00	19.33	23.33	20.00	18.40	21.33	22.00	27.33
Mean	24.00	24.92	25.75	25.50	26.52	28.02	27.90	31.03	27.90	29.63	32.30	32.22	36.88
S.Em±	1.76	1.58	1.91	1.59	1.89	1.71	4.10	2.15	1.94	3.81	1.38	3.50	1.80
CD (0.01)	5.15	4.62	5.56	4.64	5.53	4.99	11.99	6.28	5.68	11.12	4.02	10.22	5.24

Table 2. Effect of seed dormancy breaking treatments on hard seed (%) during storage of *Centrosema pubescens* seeds

Treatments	Duration of storage (Months)												
	Initial	1	2	3	4	5	6	7	8	9	10	11	12
T ₀	88.44	84.00	80.33	73.67	72.55	70.67	68.66	67.50	65.83	62.87	58.83	58.88	58.00
T ₁	74.00	80.67	80.00	76.75	75.33	74.00	76.84	77.77	82.00	67.33	72.00	69.30	63.34
T ₂	76.67	75.16	72.00	72.10	74.50	75.35	76.67	73.33	72.00	77.34	75.00	74.00	66.00
T ₃	26.00	20.66	19.33	28.00	26.00	25.33	33.33	25.33	23.33	24.67	19.50	20.17	18.00
T ₄	76.00	80.00	81.33	80.67	74.00	73.17	60.67	65.20	70.50	64.00	63.33	68.00	54.67
T ₅	60.66	63.50	64.00	47.33	55.33	51.33	59.33	46.00	54.00	42.00	41.97	40.00	35.00
T ₆	2.66	2.00	0.66	0.00	0.33	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
T ₇	74.67	76.66	76.00	77.83	84.00	81.33	76.67	72.00	77.33	78.60	75.34	74.67	70.67
Mean	59.89	60.33	59.21	57.04	57.76	56.40	56.52	53.39	55.63	52.10	50.75	50.63	45.71
S.Em±	2.30	1.43	2.24	1.14	1.12	1.29	4.20	2.94	1.92	2.50	1.02	1.78	1.42
CD (0.01)	6.71	4.18	6.55	3.33	3.28	3.78	12.26	8.59	5.60	7.30	2.98	5.19	4.16

Table 3. Effect of seed dormancy breaking treatments on dead seed (%) during storage of *Centrosema pubescens* seeds

Treatments	Duration of storage (Months)												
	Initial	1	2	3	4	5	6	7	8	9	10	11	12
T ₀	1.56	2.67	3.00	3.00	3.45	4.00	4.67	4.00	4.50	5.80	4.67	4.00	2.67
T ₁	8.00	3.33	2.00	4.67	4.67	6.67	3.33	2.00	2.00	10.67	5.33	4.70	5.33
T ₂	4.00	4.67	6.00	8.00	6.00	4.00	3.33	0.67	5.33	3.33	4.33	4.67	2.00
T ₃	7.33	2.67	5.34	8.00	4.67	6.00	4.00	6.67	10.00	11.33	11.00	10.50	17.0
T ₄	2.67	2.00	2.00	3.33	5.33	3.33	3.33	5.33	1.33	2.00	2.67	4.00	1.33
T ₅	8.67	5.33	2.00	10.00	4.00	2.00	5.34	7.33	8.67	14.00	5.00	6.00	9.00
T ₆	94.67	94.67	94.67	96.67	95.00	98.00	96.67	94.00	97.33	96.00	99.33	100.0	100.0
T ₇	2.00	2.67	5.33	6.00	2.67	0.67	4.00	4.67	2.67	3.00	3.33	3.33	2.00
Mean	16.11	14.75	15.04	17.46	15.72	15.58	15.58	15.58	16.48	18.27	16.96	17.15	17.75
S.Em±	1.22	0.84	1.54	1.63	1.23	1.22	0.73	1.19	1.29	0.90	0.47	0.56	0.89
CD (0.01)	3.56	2.45	4.50	4.77	3.60	3.56	2.13	3.47	3.77	2.64	1.38	1.64	2.61

Table 4. Effect of seed dormancy breaking treatments on seedling vigour index during storage of *Centrosema pubescens* seeds

Treatments	Duration of storage (Months)												
	Initial	1	2	3	4	5	6	7	8	9	10	11	12
T ₀	82.60	168.7	188.3	299.3	285.6	423.9	286.6	261.3	329.1	531.6	641.5	672.3	506.9
T ₁	194.6	157.1	219.5	269.0	230.7	271.6	210.6	287.3	252.9	345.1	377.1	450.8	382.1
T ₂	240.4	231.6	263.1	345.3	271.9	267.1	220.3	391.1	368.3	321.7	355.5	374.3	471.2
T ₃	705.3	993.5	995.9	1077.6	1053.9	1233.7	913.2	1152.0	917.1	1171.0	1302.45	1328.9	1000.18
T ₄	274.9	212.1	201.9	271.6	299.4	388.1	511.8	438.9	382.2	613.7	624.3	520.7	640.8
T ₅	371.7	516.3	442.8	736.5	699.5	779.6	557.0	744.3	555.9	862.3	1076.67	1120.5	845.8
T ₆	28.30	40.80	42.10	35.5	67.30	23.90	43.30	72.90	43.10	59.30	11.10	0.00	0.00
T ₇	248.7	234.3	223.1	278.1	162.7	290.4	258.6	327.8	247.6	304.6	371.9	397.1	406.9
Mean	268.3	319.3	322.1	414.1	384.0	459.8	375.2	459.5	387.0	526.2	595.05	608.5	531.7
S.Em±	24.99	19.18	25.20	35.30	24.81	33.22	57.73	36.63	22.88	70.54	25.57	68.9	39.4
CD (0.01)	73.00	56.01	73.59	103.1	72.45	97.04	168.6	107.0	66.81	206.0	74.68	201.3	115.1

Seed treatments (T) : T₀ - Control (untreated), T₁ - Soaking of seeds in GA₃ @ 400 ppm, T₂ - Soaking of seeds in KNO₃ @ 4 g/l, T₃ - Hot water treatment (80°C) for 5 min., T₄ - Keeping the seeds in hot air oven @ 80°C for 10 min., T₅ - Mechanical scarification, T₆ - Soaking of seeds in H₂SO₄ for 2 min., T₇ - Soaking of seeds in Thiourea @ 4 g/l

Seedling vigour index: The results on seedling vigour index as influenced by seed treatments during twelve months of storage period are presented in Table 4. Irrespective of seed treatments, the seedling vigour index increased gradually with advancement in the storage period. On an average the seedling vigour index recorded at the beginning and end of storage period was 268.3 and 531.7, respectively. Among the seed treatments, significantly highest vigour index at initial stage up to the end of storage period (12 month) was observed in T₃ (hot water treatment) which recorded 705.3 at initial and increased up to 1328.9 at the end of the 11 month of storage, later decreased to 1000.2 at the end of 12 month of storage. Significantly lowest vigour index (28.30) was observed in T₆ at initial stage and nil vigour index was recorded on 11 and 12 month of storage, respectively. It was followed by control treatment which recorded 82.60 and 506.9 seedling vigour index at initial and 12 month of storage, respectively. These results are in conformity with the findings of Win Pe *et al.* (2012) in *Centrosoma pubesecens*. They observed that acid treated seeds produced more abnormal seedlings than untreated seeds as increase in storage period caused increased death of seeds. However, in general the acid and mechanical scarified seeds reduced the impermeability of the seed coat that promoted entry of water and exchange of gases into the seed. Upon imbibition, the embryo becomes metabolically active and resulted in the emergence of root and shoots and contributed for the increased vigour index of the seedling. Similar results have been reported by Paramathma *et al.* (1991) in butterfly pea (*Clitoria ternatea*) and Siratro (*Macroptilium atropurpureum*).

Conclusion

In *Centrosema pubesecens* seeds, an effective treatment method to improve germination rate of the seed lots without causing mortality of potentially viable seeds was hot water treatment. Further, the hot water treated seeds can be stored for a minimum period of seven months with better seed germinability and without losing its viability and vigour. It was also concluded that under natural course, the hard seed coat dormancy in *C. pubescens* seeds maintained till 12 month of storage.

References

- Abdul-Baki, A. A. and J. D. Anderson. 1973. Vigour determination of soybean seeds by multiple criteria. *Crop Sci.* 13: 630-633.
- Agboola, D. A. 2006. Seed germination and seedling growth of the Mexican Sunflower, *Tithonia diversifolia* composition in Nigeria, Africa. *Rev. Biol. Trop.* 54(2): 385-402.
- Ajiboye, A. A. 2006. *Seed germination and seedling physiology of to multi-Purpose Savanna Tree Species*. M.Sc. Thesis. University of Agriculture, Abeokuta, Ogun State of Nigeria.
- Argel, P. J. and C. J. Paton. 1999. Overcoming legume hard seededness. In: Loch, D.S. and Ferguson, J.R. (eds.) *Forage Seed Production*. Volume 2: *Tropical and Sub-Tropical Species*. pp. 247-266. CABI Publishing: New York, USA.
- Chin, H. F and C. C. Wong. 1993. The importance of tropical pasture seed quality and factors affecting it in smallholder farming systems. *International Grassland Congress* 2: 1863-1866.

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- Duorado, A. M. 1989. The effect of seed size, method of harvest and accelerated ageing on germination of Grasslands Matuaö prairie grass (*Bromus catharticus*). *Seed Sci. & Technol.* 17: 283-288.
- Elberse, W. T. and H. Breman. 1989. Germination and establishment of Sahelian rangeland species. I. Seed properties. *Oecologia*. 80: 477ñ 484.
- Ertan Ates, 2011. Influence of some hard seededness breaking treatments on germination in Persian clover *Trifolium resupinatum* ssp. *Typicum* seeds. *Romania Agril. Res.* 28: 229-239.
- Fasidi, I. O., Karidi, Tsamani and Agboola. 2000. Dormancy Types and Water Uptake in Seeds of *Parkia biglobossa* (Jaca). *R. Br. Don J. of Natural and Applied Sciences*. 1(1):14-20.
- Hopkinson, J. M. and C. J. Paton. 1993. Treatment of Seca Stylo seed to reduce hard seed content. *Tropical Grasslands*. 27: 327ñ334.
- ISTA, 1999. International rules for seed testing. *Seed Sci. & Technol.* 27(Suppl.): 1-333.
- Oakes, A. J. 1984. Scarification and germination of seeds of *Leucaena Leucacephala* (Lam.) de Wit. *Tropical Agriculture*. 61: 125ñ132.
- Omokanye, A. T. and O. S. Onifade. 1993. Effect of storage and scarification time on seed germination of Centro (*Centrosema pubescens*). *Seed Res.* 20:11-15.
- Paramathma, M., C. Surendran, R. S. Vinaya Raj and P. Srimathi. 1991. Studies on maximizing germination and vigour in Forage legumes. *Range Mgmt. & Agroforestry*. 12(2): 125-128.
- Phipps, R. H. 1973. Methods of increasing the germination percentage of some tropical legumes. *Tropical Agriculture*. 50: 291ñ296.
- Ramamoorthy, K. and R.S.V. Rai. 1990. Scarification treatments to overcome hard seededness in hedge Lucerne. *East African Agricultural and Forestry Journal*. 55: 179ñ182.
- Sundarraj, N., S. Nagaraju, M. N. Venkataram and K. Jagannath. 1972. *Design and Analysis of Field Experiments*, Univ. Agric. Sci., Bangalore.
- Wang, Y. R. and J. G. Hampton. 1993. Seed vigour in lucerne and white clover. *International Grassland Congress 2*: 1868-1869.
- Win Pe., M. J. Hill and E. H. Margot. 2012. Storage conditions and acid treatment. *New Zealand J. Exp. Agric.* 3(1): 85-89.