

## Standardization of seed testing protocols and TZ test in senna (*Cassia angustifolia*)

UDAYA BHASKAR K.\*, S.S. PARIHAR, M. DADLANI AND SHIV K. YADAV  
Division of Seed Science and Technology, IARI, New Delhi,  
udaya9252@gmail.com

**ABSTRACT** Senna is a cultivated annual herb valued in medicine for its cathartic properties. Based on studies conducted on thirty samples of senna revealed that working sample size for physical purity and other crop seed determination was found to be 65 g and 650 g respectively. Seeds can be tested using rolled paper towel method at 25°C with first and final count (days) 6 and 11. Senna exhibited physical dormancy as considerable number of seeds did not imbibe water at the end of germination test and sulphuric acid treatment for 30 minutes was found to be effective for alleviation of coat-imposed dormancy. Germination studies for three years revealed normal seedlings percentage in senna varied from 10 to 65 in control and 28 to 90 in H<sub>2</sub>SO<sub>4</sub> treatment for 30 minutes. Topographical tetrazolium chloride (TZ) test was also standardized for senna and finally seed standards were suggested based on our results of experimentation and perusal of standards in IMSCS (Indian Minimum Seed Certification Standards) of concerned family *i.e. fabaceae*.

**Key words:** Senna, *cassia angustifolia*, medicinal and aromatic plants, seed testing protocols, Tz test

Seeds are fundamental to agriculture and natural ecosystem. Since the success of crop production relies heavily on high quality planting material, the Government of India enacted Seed Act in 1966, so that all seed sold should conform to the minimum standards of physical and genetic purity, germination, moisture content and seed health. These seed quality parameters, known as Indian Minimum Seed Certification Standards, were notified for more than 95 crops *viz.* cereals, pulses, vegetables etc. and compiled in "Indian Minimum Seed Certification Standards" [1]. However, no such standards of seed quality parameters are available for medicinal crops, which have high commercial value. Seed testing protocols are also not available, which is a pre-requisite for testing the seeds and for recommending minimum limits of germination [2-3]. Seed testing protocols are regularly updated by ISTA (International Seed Testing Association) based on research work done globally through publication of research papers. The latest

International Rules for Seed Testing [4] contain seed testing protocols for a large number of plant species cultivated all over the world and it forms the basic reference book for all kinds of seed testing activities and also for the international seed trade.

The information on requirement of temperature and substrate for seed germination in senna is neither available in ISTA Rules nor any systematic study has been reported. Whereas regarding standardization of topographical tetrazolium chloride test in senna, the main significance was it is the most common test used all over the world for determining seed viability together with germination test. ISTA is providing guide lines and this test is standardized in almost all of the crop plants but it has not been standardized in majority of medicinal plants. Because of growing importance of medicinal plants in international market especially seed trade; the following study was taken up to standardize tetrazolium testing protocols in senna. Some fragmented information on seed germination in senna and muskdana is available in literature

---

\*Corresponding author

Received: November 2012

Revised: May 2013

Accepted: June 2013

[5-7] and needs validation. Keeping above in view, the present research work was undertaken on senna.

Senna is a cultivated annual herb (Plate 1), valued in medicine for its cathartic properties. The active ingredients of leaves and pods are sennosides A, B, C, D rhein, aloe-amine, kaempferin and iso-rhein in free and compound/glycoside forms [8]. Both the dried leaves and pods were used as laxative, cathartic and purgative [9].

### MATERIAL AND METHODS

The present investigation was undertaken during the years 2006 to 2008 at Indian Agricultural Research Institute, New Delhi, India. Experimental material *i.e.*, seeds of senna (10 seed lots or collections per year for three years) of fresh harvest from different places and agencies *viz.*, Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur; Directorate of Medicinal and Aromatic Plants Research, Anand; commercial sources (Neemuch, M.P) were procured and used for the study. For physical purity analysis, the working sample size for purity determination and other crop seeds (2500 seeds) was calculated by taking into account the thousand seed weight. Inert matter, other crop seed and weed seed were separated and seeds with less than half of the original size were considered as inert matter. Physical pure fraction of seed was used for subsequent germination studies. Thousand seed weight was calculated following the ISTA procedure [4]. Where, seed weight was determined in eight replicates of 100 seeds each. For this, eight replicates of 100 pure seeds each from the working sample were counted. Each replicate is weighed in grams to the same number of decimal place as in physical purity analysis. Seeds are counted from the entire pure seed fraction. Variance, standard deviation and coefficient of variation were calculated using the formulae of Agrawal and Singh [10]. Seed germination studies based

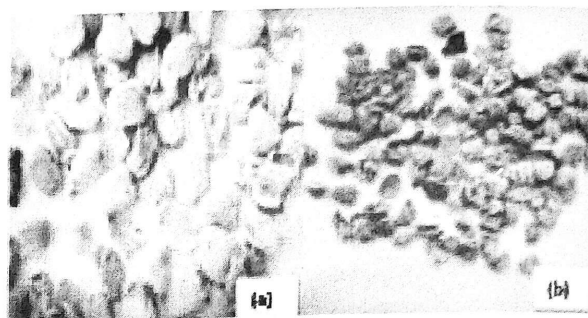
on procedures of ISTA rules [4], were followed using different substrata *i.e.* roll towel paper (BP), and vermiculite, under different temperature regimes *i.e.* constant temperatures of 20°C, 25°C and alternate temperatures of 20~30°C. From each seed lot 400 seeds (*i.e.* 100 seeds each in 4 replicates) of pure seed fraction were drawn at random and placed equidistantly in rolled towel or on a leveled layer of moist vermiculite medium (Covered with 10-20 mm of vermiculite medium substrate). The germination media were incubated at constant temperatures of 20°C, 25°C and alternate temperatures of 20~30°C in walk-in-room germinator (maintaining requisite temperature) and having 95±5% RH. Only morphologically normal seedlings were scored and the average percentage germination for the four replicates was calculated. The un-germinated seeds, which did not appear rotten or released any foetid odour, were gently pressed with thumb to judge their internal state. The seedlings and seeds were categorized into normal seedlings, abnormal seedlings, hard seeds and dead seeds. The germination was observed regularly, and first count was taken when more than 50 percent of seeds produced seedlings with all the essential structures visible. Final count was taken for the date from which no further germination occurred. The Optimum germination time was estimated using methodology of Ellis and Roberts [11] with a modification, that full seedling with all essential structures was taken as criteria for normal seed germination. Under germination studies, observations such as germination percentage, seedling length (cm), vigour indexes I and II [12] were taken. Due to hard seeded nature of senna seed, imbibitional studies were conducted. Imbibitional study is one of the principal measures to estimate the presence of coat imposed (physical) dormancy. Hard seed coat prevents the imbibition of water, there by inhibits germination. In senna, this test

confirmed existence of coat-imposed dormancy. In this study 3 seed lots (100 seeds in 4 replicates) were taken and the imbibitional pattern was studied at hourly interval by comparing control (seeds without scarification) with boiling water (seeds scarified by boiling water) and  $H_2SO_4$  (seeds scarified by conc. sulphuric acid for 30 min) treatments by taking fresh weights at periodic interval. Initial germination studies revealed presence of hard seeds as considerable number of seeds did not imbibe water at the end of experiment. Therefore dormancy (physical) breaking treatments were given. Seeds from freshly harvested 10 seed lots, which were procured during first year *i.e.* 2006, were used in this study. Treatments such as boiling water ( $100^\circ C$  @ equal volume and 100 ml), Conc. Sulphuric acid (for 15min, 30min and 60 min), manual scarification (sand paper), Acetone (equal volume), ethyl alcohol and dry heat treatment ( $50^\circ C$  for 6 h) were used for dormancy release.

The methodology (preconditioning, cutting procedure, concentration of Tz solution, incubation time & temperature and finally evaluation) for topographical tetrazolium chloride test was standardized in senna (Plate 4), for determining the viability of seed lots. Senna belongs to the family *fabaceae* and contains hard seed coat (physical dormancy). So preconditioning is an essential requirement to soften the hard seed coat. Preconditioning was done with boiling water for half an hour after that seeds which were still having hard seed coats were manually scarified with sand paper. Then the seeds were soaked overnight in water and prepared for removal of seed coat.

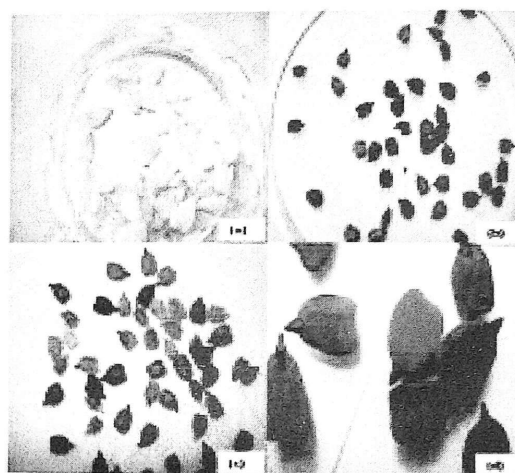
After that seed coat was removed and seeds were kept for staining with 1% tetrazolium chloride solution and seeds were incubated at  $30^\circ C$  for 16 h for proper staining. Later the embryos were evaluated for viability depending on staining pattern. The data obtained was statistically analyzed, following Completely Randomized Design (CRD). To stabilize the variance in germination percentages arc sin transformations were used [13].

**Plate 4a. Seeds without radicle tip are not viable and considered as inert matter as shown below:**



**Note:** (a and b): Seeds without radicle tip (Inert matter)

**Plate 4b. Tetrazolium staining test in senna**



**Note:** (a): Presence of hard seeds after seed soaking, (c): Non-viable seeds, (b and d): Viable seeds

## RESULTS AND DISCUSSIONS:

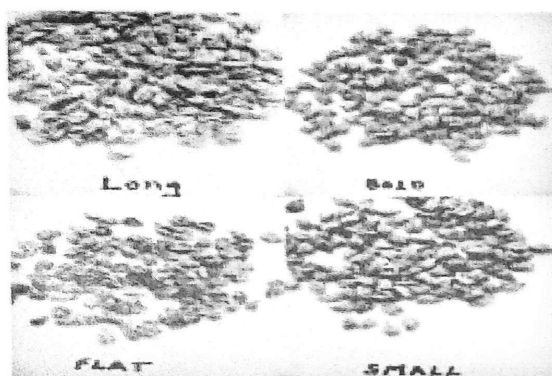
Seed testing protocols were developed to determine the maximum germination potential of a given seed lot, which were used to compare the quality of different seed lots and to estimate the field planting value.

### *Physical purity*

For senna, which belongs to family *fabaceae*, pure seed definition is common to other members of this family *i.e.* seed provided a portion of the testa attached, piece of seed larger than one-half the original sizes, provided a portion of testa attached. Seeds and pieces of seed entirely without test are regarded as inert matter, for *fabaceae*, separated cotyledons are regarded as inert

matter irrespective of whether or not the radicle-plumule axis and/or more than half of the testa may be attached. Whereas the only exception through our experimentation was seeds without radicle tip, not counted as viable and considered as inert matter (Plate 3).

**Plate 3. Pure seed fractions (with varying size, shape and colour) of different seed lots in senna**



*Thousand seed weight*

In senna the thousand seed weight ranged from 21.84 g in lot 21 (from JNKVV 2007, Jabalpur) to 28.35 g in lot 4 (from JNKVV 2005,

Jabalpur) with a mean of 25.47 g and standard deviation of 2.012 (Table 1). The results of thousand seed weight clearly indicated that there was lot of variation in seed weight among various seed lots across different agro climatic zones of India. The mean 1000 seed weight of 30 seed lots in each crop gives a valuable input in determining working sample size (2500 seeds), which is 65 g and the sample size for other crop determination 650 g in senna.

*Optimization of temperature and substratum for germination testing*

Effect of different temperature regimes (20<sup>o</sup>, 25<sup>o</sup> and 20~30<sup>o</sup>C) on senna seed germination (Plate 2) following BP method are given in Table 2. While comparing the mean germination across the different temperature regimes without any dormancy breaking treatments, maximum germination of 40.57% was observed at 25<sup>o</sup>C, which reduced to 38.00 and 33.47% at 20-30<sup>o</sup>C and 20<sup>o</sup>C, respectively. A comparison of different treatments revealed that germination percent vary significantly in different lots and ranged from 65 (in lot 3 at 25<sup>o</sup>C) to 8 (in lot 6 at 20-30<sup>o</sup>C).

**Table 1. 1000 seed weight (g) of seed lots of senna collected from different agro- climatic zones in India. (n=8)**

Lots	1000 Seed wt	Lots	1000 Seed wt
L1	22.98	L16	28.00
L2	22.55	L17	24.96
L3	27.18	L18	24.11
L4	28.35	L19	25.27
L5	24.73	L20	26.58
L6	22.88	L21	21.84
L7	24.63	L22	27.58
L8	23.72	L23	25.13
L9	27.20	L24	27.41
L10	25.60	L25	27.09
L11	23.80	L26	28.07
L12	28.02	L27	28.21
L13	25.85	L28	24.58
L14	22.33	L29	24.48
L15	24.41	L30	26.46
Mean	25.47		
CD (p=0.05)	0.091		
sd	2.012		
max	28.35		
min	21.84		

It was also evident from Table 2, that mean hard seed percent was low (27.57) at 25°C and gradually increased to 28.23 and 31.97 at 20-30°C and 20°C, respectively. Perusal of data in Table 2 revealed that in senna, ideal temperature for germination is 25°C. There was significant difference in germination percent among three temperature regimes (20°, 25° and 20~30°C) and 25°C was proved better with high mean germination percent (40.57) and low mean hard seed percent (27.57) without any dormancy breaking treatment. The other fact is that senna is a *kharif* (June sown) crop and its habitat (sub-tropical) also suggest that it requires slightly higher temperature for quick and uniform germination.

#### Substratum

The seed size and the resultant seedling dimensions make it difficult to conduct the germination studies on top of the paper (TP) in the crop under study. Hence, TP was not used as a method of germination testing. The mean germination of 40.57% and 39.87% were recorded with BP method and Vermiculite as substrate (Table 3), which were at par. However, significant variance was observed among diverse seed lots. There was no significant difference between the two substrates (Germination paper and Vermiculite) as germination media. Nevertheless, due to convenience in handling and ease in taking observations BP method (Between papers) was followed for conducting germination test.

#### Days to first and final count of germination

In senna, first count was taken on 6<sup>th</sup> day while final count was recorded on 11<sup>th</sup> day. Similar pattern was observed in all most all seed lots. For obtaining the above-mentioned results, germination was observed regularly, and first count was taken when more than 50 percent of seeds produced seedlings with all the essential structures visible. Final count was taken for the date from which no further germination occurred.

#### Imbibitional studies in senna

The results of imbibitional studies clearly revealed the presence of coat imposed (physical) dormancy in senna. In senna (Fig. 1), seeds reached to maximum mean fresh weight (7.13 g) at 96 hours after initiation of imbibitional study under control conditions where as the maximum mean fresh weight were 8.10 g and 8.32 g with boiling water and H<sub>2</sub>SO<sub>4</sub> treatments respectively after 48 hours. The reduction in time of imbibition to reach maximum mean seed fresh weight in two treatments compared to control was a clear-cut indication for the presence of coat imposed (physical) dormancy.

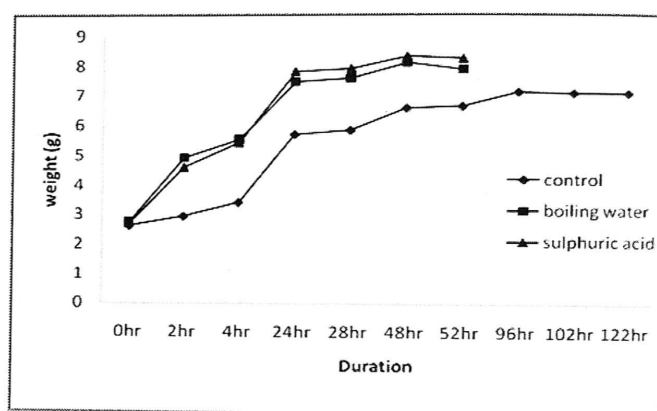


Fig. 1. Imbibitional pattern in Senna. (n=4)

#### Dormancy and its breakdown in senna

Imbibitional studies established the presence of coat imposed (physical) dormancy in senna. The results of dormancy and its breakdown (Fig. 2) clearly indicated that acid scarification and manual scarification are most effective treatments. This also reiterated the fact that seeds are having coat imposed dormancy.

In senna the most effective dormancy breaking treatment recommended as per our experimentation was sulphuric acid treatment for 30 minutes because of which we got an advantage of 24% in mean germination when compared with control (Table 4). The germination percent of three most effective dormancy breaking treatments *i.e.* sulphuric acid treatment for 30 minutes,

Table 2. Effect of different incubation temperatures on germination percentage and hard seed percentage in senna

Lots	25°C	20-30°C	20°C	Mean	25°C	20-30°C	20°C	Mean
	N	N	N		H	H	H	
L1	30 (33.0)	28 (32.1)	23 (28.6)	27.00 (31.25)	39 (38.8)	36 (37.0)	40 (39.2)	38.56 (38.36)
L2	26 (30.4)	21 (27.5)	19 (25.6)	21.89 (27.81)	32 (34.4)	35 (36.0)	34 (35.6)	33.56 (35.37)
L3	65 (53.9)	61 (51.5)	57 (48.8)	61.11 (51.42)	12 (19.9)	19 (25.6)	22 (28.2)	17.56 (24.56)
L4	58 (49.6)	55 (48.1)	48 (43.6)	53.67 (47.10)	27 (31.5)	29 (32.6)	36 (36.6)	30.67 (33.56)
L5	54 (47.3)	51 (45.7)	47 (43.3)	50.78 (45.43)	9.0 (17.7)	10.0 (18.7)	10.0 (18.4)	9.89 (18.27)
L6	10 (18.1)	8.0 (15.9)	9.0 (17.8)	8.89 (17.26)	22.0 (28.2)	22.0 (27.7)	22.0 (27.7)	21.89 (27.86)
L7	33 (35.0)	31 (34.0)	27 (31.3)	30.44 (33.45)	32 (34.4)	29 (32.6)	35 (36.4)	32.11 (34.47)
L8	45 (42.3)	41 (39.8)	35 (36.0)	40.33 (39.38)	49 (44.2)	49 (44.6)	57 (49.2)	51.78 (46.01)
L9	48 (43.8)	46 (42.9)	39 (38.4)	44.33 (41.71)	13.0 (21.1)	14.0 (21.9)	20.0 (26.5)	15.67 (23.18)
L10	37 (37.4)	36 (36.8)	32 (34.4)	35.00 (36.24)	40 (39.2)	39 (38.8)	43 (41.2)	40.89 (39.73)
Mean	40.57 (39.09)	38.00 (37.44)	33.47 (34.79)		27.57 (30.95)	28.23 (31.55)	31.97 (33.91)	
CD (p=0.05) (n=4)	T	1.61 (0.98)			27.57 (30.95)	28.23 (31.55)	31.97 (33.91)	
	L	2.93 (1.80)			1.35 (0.89)			
	T*L	N.S. (N.S.)			2.47 (1.62)	4.28 (2.80)		

Note: N= Normal seedlings; H= hard seeds; L =lots; T= temperature regimes; 20-30°C=20°C for 16hr and 30°C for 8 hr

**Table 3. Effect of different substrates on germination percentage in senna**

Lots	BP method	Vermiculite	Mean
L1	30(33.0)	29(32.8)	29.50(32.88)
L2	26(30.4)	27(31.1)	26.17(30.73)
L3	65(53.9)	63(52.3)	64.00(53.12)
L4	58(49.6)	56(48.4)	57.00(49.01)
L5	54(47.3)	55(47.7)	54.33(47.47)
L6	10(18.1)	9(17.7)	9.50(17.90)
L7	33(35.0)	33(35.2)	33.17(35.14)
L8	45(42.3)	45(42.3)	45.33(42.30)
L9	48(43.8)	44(41.5)	46.00(42.68)
L10	37(37.4)	37(37.6)	37.17(37.54)
Mean	40.57(39.09)	39.87(38.67)	
CD (p=0.05)	S	N.S.(N.S.)	
(n=4)	L	3.32(2.04)	
	S*L	N.S.(N.S.)	

Note: S = substrate; L = lots

sulphuric acid treatment for 15 minutes and manual scarification were 64.20, 62.17 and 62.13 respectively. Even though first treatment was significantly different from other two treatments, there was lot-to-lot variation in response. For some lots sulphuric acid treatment even for 15 minutes was enough but sulphuric acid treatment for 30 minutes was proved better in majority of seed lots and there no detrimental effects were reported up to that duration. The results obtained were in conformity with the results obtained by other scientists. Ramamoorthy *et al.* [14] reported that acid scarification for 5 minutes was effective for alleviation of hard seededness in senna. Bhuse *et al.* [5] identified that H<sub>2</sub>SO<sub>4</sub> scarification for 12 min was effective for enhancing seed germination and seedling vigour in senna. Whereas, other study indicated that the mechanical scarification with sand gave highest seed germination and lowest percentage of abnormal seedlings [6]. Although there was slight disagreement with respect to duration of sulphuric acid treatment in our experimentation with observations of previous scientists, it was mainly due to

handling of more number and diversified seed lots from various agro climatic zones.

*Germination percentage and vigour of 30 seed lots tested over a period of 3 years in senna*

Germination percentages, seedling length, seedling dry weight and vigour indices were tabulated over a period of three years (2006 to 2008) and the data was given in tables 5 to 7. There was lot of variation in germination percent and vigour of seed lots procured from various agro climatic zones. In senna germination percent ranged from as low as 28 to as high as 90. This much wide variation was due to varied climatic conditions, genotype variations and environmental parameters at the time of seed development and maturation and post harvest seed handling. The results clearly indicated that commercial seed lots obtained would have various germination percentages. Since no seed standards prescribed, there was need to establish seed quality before taking up commercial cultivation. Proper processing and grading may help to solve this problem to some extent.

Table 4. Effect of dormancy breaking treatments on normal seedlings percentage in senna

Lots	CONTROL	H1	H2	S1	S2	S3	SCARI	ACETONE	ALCOHOL	HEAT	Mean
L1	30(33.0)	45(41.9)	57(49.0)	59(50.4)	62(52.1)	46(42.9)	61(51.6)	29(32.4)	21(27.0)	20(26.80)	43.03 (40.70)
L2	26(30.4)	29(32.4)	51(45.4)	49(44.2)	54(47.1)	36(36.6)	51(45.7)	29(32.6)	24(29.5)	16(23.8)	36.40 (36.77)
L3	65(53.9)	68(55.3)	69(56.2)	73(58.7)	74(59.5)	66(54.5)	72(58.0)	66(54.5)	60(50.6)	49(44.2)	66.23 (54.55)
L4	58(49.6)	66(54.4)	77(61.6)	79(63.0)	80(63.3)	63(52.7)	76(60.9)	57(49.0)	50(45.2)	53(46.5)	66.00 (54.61)
L5	54(47.3)	56(48.4)	57(49.2)	61(51.3)	62(51.9)	57(49.2)	61(51.5)	51(45.4)	48(43.6)	45(42.3)	55.27 (48.02)
L6	10(18.1)	21(27.0)	24(29.1)	26(30.6)	28(32.1)	21(27.0)	26(30.4)	18(25.1)	15(22.5)	7(15.7)	19.47 (25.75)
L7	33(35.0)	44(41.5)	53(46.9)	59(50.4)	60(50.8)	45(42.1)	58(49.4)	30(33.2)	27(31.5)	30(33.2)	43.97 (41.40)
L8	45(42.3)	69(55.9)	80(63.4)	89(70.4)	90(71.6)	73(58.5)	89(71.0)	51(45.4)	44(41.5)	41(39.8)	67.03 (55.98)
L9	48(43.8)	50(45.0)	53(46.7)	55(48.0)	57(49.2)	48(43.8)	55(48.0)	39(38.6)	37(37.6)	44(41.3)	48.70 (44.22)
L10	37(37.4)	53(46.9)	57(49.2)	71(57.4)	74(59.5)	60(50.9)	71(57.4)	39(38.8)	34(35.8)	33(35.2)	53.13 (46.87)
Mean	40.57 (39.09)	49.97 (44.87)	57.87 (49.67)	62.17 (52.44)	64.20 (53.72)	51.57 (45.83)	62.13 (52.40)	40.87 (39.49)	36.03 (36.49)	33.87 (34.89)	
CD(p=0.05) (n=4)	T L T*L	1.59(0.98) 1.59(0.98) 5.02(3.09)									

Note: H1= boiling water (equal volume); H2=boiling water (100ml); S1= H<sub>2</sub>SO<sub>4</sub> (15min); S2=H<sub>2</sub>SO<sub>4</sub> (30min); S3=H<sub>2</sub>SO<sub>4</sub> (60min); SCARI=manual scarification with sand paper; ACETONE=over night soaking (equal volume); ALCOHOL= over night soaking (equal volume); HEAT= heat treatment for 6hr; T=treatments; L=lots.

#### Correlation of tetrazolium staining data with germination percentage in senna

In this experiment, four seed lots were taken for ascertaining the relation between viable seeds of quick viability test and normal seedlings of germination test. As per ISTA regulations the viable seeds are categorized into totally stained and seeds with some minor damages on cotyledons. Whereas, the non-viable seeds were categorized into seeds with major portion of cotyledon unstained, radical unstained, totally unstained and rotten. The data relating to tetrazolium chloride test of four seed lots and corresponding germination percent are given in table 8. In Lot-1, the total viable seed percent was 58, non viable seed percent was 42 and the corresponding germination percent was 53 after sulphuric acid treatment. In Lot-2, the total viable seed percent was 80, non viable seed percent was 20 and the corresponding germination percent was 73. In Lot-3, the total viable seed percent was 85, non viable seed percent was 15 and the corresponding germination percent was 79 and finally in Lot 4 the total viable seed percent was 35, non viable seed percent was 65 and the corresponding germination percent was 28. The results (correlation coefficient, r) clearly established the correlation between viable seeds of tetrazolium chloride test and normal seedlings of germination test.

Since there was no information available regarding

**Table 5. Germination percentage and vigour of 10 seed lots during 1<sup>st</sup> year of testing in senna**

Lots	N	H	G	SL	SD	VIG 1	VIG 2
LI	30(33.0)	39(38.8)	62(52.1)	10.5	152	656	9484
L2	26(30.4)	32(34.4)	54(47.1)	10.4	152	561	8182
L3	65(53.9)	12(19.9)	74(59.5)	13.1	159	972	11845
L4	55(47.7)	31(33.5)	80(63.3)	11.9	159	951	12672
L5	54(47.3)	9(17.7)	62(51.9)	12.5	164	773	10168
L6	10(18.1)	22(28.2)	28(32.1)	10.0	157	283	4432
L7	33(35.0)	32(34.4)	60(50.8)	13.0	161	782	9642
L8	45(42.3)	49(44.2)	90(71.6)	13.1	158	1174	14246
L9	48(43.8)	13(21.1)	57(49.2)	13.4	154	767	8832
L10	37(37.4)	40(39.2)	74(59.5)	11.3	154	839	11428
Mean	40.23 (38.89)	27.90 (31.15)	64.20 (53.72)	11.91	157	776	10093
CD (p=0.05)	5.94(3.55)	6.42(4.12)	5.35(3.40)	1.07	6.60	99	1041

**Table 6. Germination percentage and vigour of 10 seed lots during second year of testing in senna**

Lots	N	H	G	SL	SD	VIG 1	VIG 2
LI	61(51.1)	22(28.2)	78(61.8)	12.1	158	936	12265
L2	37(37.2)	46(42.5)	78(61.8)	13.0	152	1008	11819
L3	69(56.4)	14(21.9)	79(63.0)	12.2	160	972	12721
L4	76(60.9)	9(17.7)	82(64.7)	12.3	159	1009	12963
L5	36(37.0)	42(40.2)	64(53.3)	11.9	154	769	9935
L6	44(41.3)	19(25.6)	60(50.8)	13.1	157	785	9393
L7	57(48.8)	14(21.8)	64(53.3)	13.0	159	839	10233
L8	21(27.5)	39(38.4)	42(40.2)	12.0	151	498	6295
L9	29(32.6)	39(38.4)	51(45.4)	11.6	154	586	7786
L10	36(37.0)	36(37.0)	61(51.3)	12.4	153	754	9336
Mean	46.63(42.99)	27.93(31.18)	65.83(54.55)	12.36	155.7	815	10275
CD(p=0.05)	4.79(2.89)	4.75(3.32)	4.62(3.00)	N.S.	N.S.	114	836

Note: N= normal seedlings; H =hard seeds; G =germination % after H<sub>2</sub>SO<sub>4</sub> treatment; SL=seedling length (cm), SD= seedling dry weight (mg); VIG 1= vigour index 1; VIG 2= vigour index 2; (n=4).

tetrazolium staining procedures of senna in (updated 2011) 'Tetrazolium Testing Handbook' [15], the present study filled that gap by standardizing the procedures such as method of pre conditioning, excision technique, concentration of staining solution, incubation time and temperature and finally method of evaluation. To ascertain the fact that tetrazolium test is a very good indication of viability of a seed lot the results were compared with the germination data in four seed lots. On the basis of thousand seed

weight of 30 seed lots, lot size, working sample size for physical purity and for other crop determination is given in table 9 and seed testing protocols for senna is mentioned in table 10. Keeping in view the seed morphology and anatomy the test for seed viability *i.e.* topographical tetrazolium chloride test was standardized in senna (Table 11).

#### *Seed testing protocols for senna*

Seeds of senna can be tested using BP (roll paper towel) method at 25°C with first and final counts being 6 and 11 days respectively.

Table 7. Germination percentage and vigour of 10 seed lots during third year of testing in senna

Lots	N	H	G	SL	SD	VIG 1	VIG 2
L1	29(32.7)	49(44.4)	70(56.6)	12.0	161	838	11240
L2	43(41.0)	44(41.7)	76(60.9)	12.0	155	914	11808
L3	28(32.1)	39(38.6)	58(49.6)	11.7	148	678	8607
L4	52(46.3)	37(37.6)	81(64.4)	12.4	163	1009	13229
L5	42(40.2)	42(40.6)	77(61.2)	11.4	160	874	12279
L6	65(53.9)	19(26.0)	82(64.9)	11.9	159	978	13007
L7	59(50.2)	29(32.3)	81(63.9)	11.6	168	934	13523
L8	48(43.8)	41(39.6)	79(62.5)	12.1	154	951	12145
L9	57(48.8)	34(35.6)	84(66.2)	12.2	169	1024	14147
L10	43(41.0)	43(41.1)	75(60.0)	11.9	153	895	11494
Mean	46.67(43.00)	37.80(37.78)	76.20(61.01)	11.93	159	909	12148
CD	5.58(3.30)	3.81(2.32)	4.48(3.15)	N.S.	6.44	84	812

Note: N= normal seedlings; H =hard seeds; G =germination % after H<sub>2</sub>SO<sub>4</sub> treatment; SL=seedling length (cm), SD= seedling dry weight (mg); VIG 1= vigour index 1; VIG 2= vigour index 2; (n=4).

However, seeds require pre-treatment for germination *viz.* acid scarification or boiling water treatment indicates presence of physical dormancy. Variation was observed in the intensity of dormancy in different seed lots. Depending on level of dormancy, hard seeds require concentrated H<sub>2</sub>SO<sub>4</sub> treatment up to 30 minutes.

Physical dormancy is the second most widely distributed seed dormancy in *Angiosperms* after physiological dormancy [16-18]. A large number of pulses *viz.* mung, urd, lentil etc. have been reported to exhibit physical dormancy or hard seededness [19-22]. Fodder legumes *viz.* *Acacia*, *Cassia*, *Trifolium*, *Prosopis*, *Alylosa*, *Tephrosia*, *Leucaena*, *Medicago*, *Desmanthus*, *Stylosanthes* and *Sesbania* species also exhibit hard seededness [23-36]. Senna, being the member of leguminosae family also falls in the above category.

#### Standardization of topographical tetrazolium chloride test in senna

Topographical tetrazolium test (Tz test) is a measure of seed viability, as a dormant seed may not germinate in germination tests even after dormancy breaking treatments. Therefore, Tz test is necessary to identify the un-germinated seeds [4]. Preparation of seeds for TZ test require knowledge of seed

anatomy [37], so as to identify the embryo type, level of embryo differentiation, embryo-seed ratio etc. In senna embryo is bent axile with high embryo-seed ratio. Therefore, it required removal of seed coat after treating the seeds with boiling water (Plate 4). Senna exhibited high level of embryo differentiation and differentiation of viable and non-viable seeds was easy by the staining pattern. As the germination test is incomplete without viability test (ISTA, 2012), Tz test was standardized in senna and the results were correlated (Table 12) with the germination test results [15] and based upon it standard formulated for each class of seed (Table 13). Seed standards are essential for the notification of a variety under Seed Act and for the production of certified seeds. Because of its utmost importance, an attempt was made to formulate Seed Standards in one of the most important medicinal crop species grown by Indian farmers' *viz.* senna.

The above standards were formulated based on our results of experimentation and perusal of standards in IMSCS [1] of concerned family *i.e.* *Fabaceae*. In senna standard of physical purity is being reduced to 96%, in view of the fact that during seed processing or threshing, a sizeable portion

Table 8. Correlation of tetrazolium staining results with germination test results in senna

Lots	Viable seeds				Non viable seeds				Germination %		
	TS	SC	TOTAL	TOTAL	MCU	RU	TU	ROT	TOTAL	N	H
L1	50(44.7)	8(16.6)	58(49.4)	23(28.3)	1(5.5)	5(13.2)	13(21.2)	42(40.5)	25(30.1)	32(34.4)	53(46.8)
L2	70(56.9)	10(17.9)	80(63.2)	6(14.4)	6(13.8)	1(4.9)	7(15.6)	20(26.7)	66(54.0)	11(19.6)	73(58.8)
L3	71(57.4)	14(21.5)	85(66.8)	5(12.5)	4(11.7)	2(8.0)	5(12.2)	16(23.1)	59(50.0)	28(31.6)	79(62.9)
L4	28(31.8)	7(15.3)	35(36.1)	22(27.8)	4(11.1)	10(18.4)	30(33.0)	65(53.9)	10(18.6)	24(29.0)	28(31.9)
Mean	54.6(47.7)	9.56(17.8)	64.1(53.9)	13.8(20.7)	3.75(10.5)	4.56(11.1)	13.6(20.5)	35.8(36.0)	39.9(38.2)	23.5(28.6)	
CD(p=0.05)	2.48(1.52)	2.94(2.89)	3.58(2.39)	2.96(2.39)	1.85(3.88)	1.61(3.35)	3.22(2.73)	3.58(2.39)	4.00(2.58)	3.39(2.30)	4.27(2.65)

Note: TS= totally stained; SC=some minor damages on cotyledons; MCU= major portion of cotyledon unstained; RU= radical unstained; TU= totally unstained; ROT= rotten; N= normal seedlings; h= hard seeds; G= germination % after H<sub>2</sub>SO<sub>4</sub> treatment; (n=4).

Table 9. Sample sizes for physical purity and other crop determination in senna

Species	Lot	size (kg)	Working sample size for physical purity (g)	Sample size for other crop determination (g)
<i>Cassia angustifolia</i>		10,000	65	650

Table 10. Seed testing protocols for senna

Species	Substrate	Temperature (°C)	First count (days)	Final count (days)	Remarks
<i>Cassia angustifolia</i>	Rolled paper towel (BP method)	25	6	11	pre- treatment of seeds with H <sub>2</sub> SO <sub>4</sub> for 30 minutes for dormancy breaking

Table 11. Standardized procedure of topographical tetrazolium chloride test in senna

Species	Pre-conditioning	Hydration time and temperature	Preparation of seed	Incubation time required for staining in 1 % Tz solution
<i>Cassia angustifolia</i>	Boiling water for half an hour and manual scarification for the remaining hard seeds	20°C for 17 hrs	Seed coat removal	30°C for 16 hrs

Table 12. Correlation value (r) between total viable seeds (%) and total germination (%) among 4 seed lots in Senna

	Viable seeds (%)	Germination (%)
Viable seeds (%)	1	
Germination (%)	0.999	1

of seeds are likely to be broken (especially the radicle - the pointed portion of the seed). As it has been observed in the seed lots (plate 3), suggesting that the broken seeds may be considered as a part of inert matter. As per Seeds Act, the hard seeds are counted towards normal seedlings, as this is a standard practice for seeds of *Fabaceae*. Standards for other crop seed, weed seed and germination, including hard seeds are suggested by careful comparison with other members of family *Fabaceae*. Optimum moisture content for storage (10% for normal storage and 8 % for vapour proof container storage) was fixed because of the typical orthodox storage behaviour exhibited by senna and perusal of standards of IMSCS. However, no weed seed or other crop seed

Table 13. Seed quality parameters formulated or recommendations

Factor	Standard for each class of seed	
	Foundation	Certified
Pure seed (minimum)	96.0 %	96.0 %
Inert matter (maximum)	4.0 %	4.0 %
Other crop seed (maximum)	5 / kg	10 / kg
Weed seed (maximum)	5 / kg	10 / kg
Germination (minimum) including hard seeds	85 %	85 %
Moisture (maximum)	10 %	10 %
Moisture for vapour proof containers (maximum)	8.0 %	8.0 %

was found in the samples, because of low quantity of procured seed material.

It would be desirable that initially the seed quality control measures in medicinal and aromatic plants should not be very rigid. Otherwise, the seed programme will suffer. However, when the seed certification is becoming more popular and adequate infrastructural facilities have been developed, the quality control measures should be applied rigidly at later stages. The present study helped in formulation of 'Seed Standards' in one of the most important medicinal crop (senna), which will help in better handling of seeds during sowing, storage, labelling, seed certification, seed law enforcement and at national and international trade.

#### REFERENCES:

1. TUNWAR NS AND SINGH SV (1988). Indian Minimum Seed Certification Standards. Central Seed Certification Board, Department of Agriculture & Cooperation, Ministry of Agriculture, Government of India, pp :388
2. PARIHARSS, DADLANIM AND JAIN SK (2006). Status of seed science studies in medicinal crops and future needs. National Seminar on Medicinal and Aromatic Plants: Perspectives and Potentials, IGAU&TCB College and Research Station, Bilaspur, December 18-19, Abstracts, pp: 58
3. PARIHARSS, RAJENDRA KUMAR AND JAIN SK (2005). A need for the formulation of seed testing protocols in medicinal and aromatic plants. National Seminar on Medicinal and Aromatic Plants-Biodiversity, Conservation, Cultivation and Processing: IGAU, Raipur, February 26-27, Abstracts, pp: 180-1.
4. ISTA (2012). International rules for seed testing. ISTA edition, Bassersdorf, CH. Switzerland.
5. BHUSE VH, LAD BL AND GHULE ST (2001). Effect of various seed treatments for enhancement of seed germination and vigour in senna (*Cassia angustifolia* Vahl.). *Res Crops*, 2(3): 359-63
6. RATHNAVALLI P, SUNDHARAIYA K, RADHA NS AND PONNUSWAMY V (2002). Seed treatment to overcome hard seededness in Senna (*Cassia angustifolia* L.). *South Ind Hort*, 50(416): 671-5.
7. PONNUSWAMY V, SUNDHARAIYA K, JASMINE AJ AND RATHNAVALLI P (2002). Studies on seed germination and leaf defoliation on leaf and seed yield of senna (*Cassia angustifolia* Vahl.) under red soil tract. *South Ind Hort*, 50(4/6): 667-70.
8. KHARE CP (2004). *Encyclopedia of Indian Medicinal Plants*. Springer - Verlag, Berlin, Heidelberg.

9. CHEVALLIER A (1996). Phytochemistry of plants used in traditional medicine. *The Encyclopedia of Medicinal Plants*, Dorling Kindersley. London ISBN 9-780751-303148.
10. AGRAWAL PK AND SINGH DP (1993). In: *Handbook of Seed Testing*, Ministry of Agriculture, GOI, New Delhi, pp: 116-20.
11. ELLIS RH AND ROBERTS EH (1981). The quantification of ageing and survival in orthodox seeds. *Seed Sci Tech*, **9**: 373-409.
12. ABDUL-BAKI BS AND ANDERSON JD (1973). Vigour determination in soybean seed by multiple criteria. *Crop Sci*, **13**: 630-3.
13. HOGG RV AND CRAIG AT (1995). *Introduction to Mathematical Statistics* (5<sup>th</sup>edn), Prentice Hall, New Jersey, USA.
14. RAMAMOORTHY K, RAJENDRAN C AND SIVASUBRAMANIAN S (2005). Seed treatment for alleviation of hard seededness in senna (*Cassia angustifolia* L.). *Adv Pl Sci*, **18**(1): 429-30.
15. ANONYMOUS (2011). Tetrazolium Testing Handbook (2011 update). Contribution no. 29 to the *Handbook on Seed Testing*. The Tetrazolium Subcommittee of the Association of Official Seed Analysts: [www.aosaseed.com](http://www.aosaseed.com).
16. BASKIN CC AND BASKIN JM (1998). *Seeds Ecology, Biogeography and Evolution of Dormancy and Germination*, Acad. Press, San Diego. pp: 324.
17. BASKIN JM, BASKIN CC AND LIX (2000). Taxonomy, ecology, and evolution of physical dormancy in seeds. *Pl Species Biol*, **15**: 139-52.
18. BASKIN JM AND BASKIN CC (2004). A classification system for seed dormancy. *Seed Sci Res*, **14**: 1-16.
19. SUBBURAMU K AND SRIDHAR K (1977). Pre-treatment studies on black gram (*Phaseolus mungo*) cv. T-9 to improve germination. *Seed Res*, **5**: 177-9.
20. BORIKAR ST, SINGH AR AND KATKADE JL (1985). Effect of pre-treatment of green gram (*Vigna radiata*) seeds with certain chemicals on its germination. *Seed Res*, **13**: 192-4.
21. SINGH JN, JHA BN, SINHA SK AND SINGH RS (1985). Effect of seed treatment on dormancy of lentil seeds. *Seed Res*, **13**: 28-32.
22. VERMA K AND KHANNA PP (1988). Hard seeds in legumes. *Seed Res*, **16**: 235-7.
23. SUNDARARAJ DANIL D, BALASUBRAMANIAM G AND PANDIAN GS (1966). Pre-treatment of seeds of *Prosopis juliflora* for improving germination. *Madras Agri J*, **53**(6): 259-61.
24. SUNDARARAJ DANIL D, BALASUBRAMANIAM G AND PANDIAN GS (1971). Effect of pre-treatment on germination of Kolingi seeds (*Tephrosia purpurea*). *Madras Agri J*, **58**(1): 1-4.
25. RAMDEO KD (1971). Studies on seed coat dormancy in *Leucaena glauca* Benth. *Japanese J Ecol*, **21**: 14-7.
26. YADAV RBR, SINGH A AND TRIPATHI M (1976). Methods to increase germination in Dashrath grass (*Desmanthus virgatus*). *Seed Res*, **4**: 120-3.
27. TAYLER GB (1981). Effect of constant temperature followed by fluctuating temperature on the softening of hard seed of *Trifolium subterraneum* L. *Australian. J Pl Physiol*, **8**: 547-58.
28. RAO NK, MAESAN LJGV AND PREMANANDAN P (1985). Breaking seed

- dormancy in *Atylosia* species. *Seed Res*, **13**: 47-50.
29. BIRADAR BB, MAHADEVAPPA M AND MUNEGOWDA MK (1988). Seed scarification studies in subabul. *Seed Res*, **16**: 238-40.
30. RANA U AND NAUTIYAL AR (1989). Coat imposed dormancy in *Acacia farnesiana*. *Seed Res*, **17**: 122-7.
31. RADHAKRISHNAN J, MAHADAVAPPA M, JOSHI B AND PRASAD TG (1989). Dormancy studies in *Cassia sericea* seeds. *Seed Res*, **17**(2): 118-21.
32. RAMAMOORTHY K AND RAI RSV (1990). Scarification treatments to overcome hardseededness in hedge lucerne. *East Af Agri Forestry J*, **55**: 179-82.
33. HOPKINSON JM AND PATON CJ (1993). Treatment of Seca-stylo seed to reduce hard seed content. *Trop Grasslands*, **27**: 327-34.
34. PADMA V, SATYANARAYANAG AND REDDY BM (1994). Effect of scarification treatments on the germination of *Leucaena leucocephala*, *Albizia lebbeck* and *Samanea saman*. *Seed Res*, **22**: 54-7.
35. KUMAR D AND LAL P (1999). Improving germination of *Sesbania rostrata*. *Seed Res*, **27**: 20-4.
36. RESHMA C, SRIMATHI P AND RAJASEKARAN R (2002). Effect of scarification treatments and containers on storability of hedge lucerne (*Desmanthus virgatus*). *Seed Tech News*, **32**(1): 176.
37. CHOWDHURY KA AND BUTH GM (1970). Seed coat structure and anatomy of Indian pulses. *J Linn Soc Bot*, **63**: 169-79.