Seed Res., Vol. 41(2): 143-156 December 2013

Standardization of seed testing protocols and TZ test in muskdana (*Abelmoschus moschatus*)

UDAYA BHASKAR K.*, S.S. PARIHAR, M. DADLANI AND SHIV K. YADAV
Division of Seed Science & Technology, IARI, New Delhi-110012
udaya9252@gmail.com

ABSTRACT Muskdana is cultivated mainly for the aromatic oil extracted from seeds that emits an aroma resembling natural musk. The present study conducted on thirty samples of muskdana revealed that working sample size for physical purity and other crop seed determination was 40 g and 400 g, respectively. Seeds can be tested using rolled paper towel method at 25°C with first and final count (days) 6 and 12. Muskdana exhibited physical dormancy as considerable number of seeds did not imbibe water at the end of germination test and sulphuric acid treatment for 15 minutes was found to be an effective treatment in counteracting coat-imposed dormancy. Germination studies for three years revealed normal seedling percentage in muskdana varied from 11 to 64 in control and 17 to 86 in H_2SO_4 treatment for 15 minutes. Topographical tetrazolium chloride (TZ) test was also standardized for muskdana. 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.Malvaceae*.

Keywords: *Abelmoschus moschatus*, medicinal and aromatic plants, muskdana, seed testing protocols and Tz test

Fundamental knowledge about mechanism underlying seed development, germinability, dormancy and storability is required to improve seed performance. 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, have been 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 and aromatic 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) on the basis of 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 muskdana is neither available in ISTA Rules nor any systematic study has been reported. Whereas regarding standardization of topographical tetrazolium chloride test in muskdana, 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 and aromatic plants. Because of growing importance of medicinal and aromatic plants in international market especially seed trade; the following study was taken up to standardize tetrazolium testing protocols in

*Corresponding author

Received: December 2012 Revised: November 2013 Accepted: December 2013

muskdana. Keeping in view the above, the present research work was undertaken in muskdana.

Muskdana or ambrette (*Abelmoschus moschatus*) is a native annual tall herb (Plate 1), where the seed coat contains an essential oil, which essentially is a mixture of farnesol and ambrettolide besides a few other minor aroma compounds. The crop is cultivated in small pockets all over the subtropical tracts in India. Its aromatic oil is used in perfumery. Different parts of the plant have uses in traditional and complementary medicine. Muskdana seeds as well as roots are also used in Indian system of medicine and are considered antispasmodic, aphrodisiac, carminative, cooling, cardio tonic, diuretic and [5].

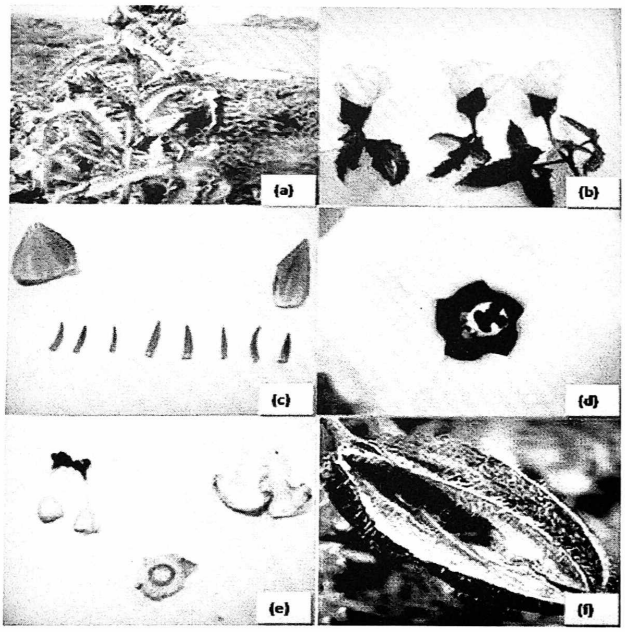

Plate 1. Muskdana botanical description and floral morphology

Note: (a): Muskdana plant; (b and c): Flower, epicalyx, 6 to 12; sepals 5, gamosepalous; (d and e): Staminal column and pentacarpellary ovary, stigma is capitate and crimson coloured, androecium (stamens indefinite, monodelphous); (f): capsule with seeds

MATERIALS 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 muskdana (10 seed lots or collections per year for three years) of recent 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 procedure of ISTA Rules for Seed Testing, 2012 [4]. The seed weight was determined in eight replicates of 1000 seeds each. For this, eight replicates of 1000 pure seeds each from the working sample were counted. Each replicate was weighed in grams to the same number of decimal place as in physical purity analysis. Seeds were counted from the entire pure seed fraction. Variance, standard deviation and coefficient of variation were calculated using the formulae of [6]. 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 standard [7] 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 [8] were taken. Due to hard seeded nature of the crop, imbibitional studies were conducted in muskdana. Imbibitional study is one of the principal measures to estimate the presence coat imposed (physical) dormancy. Hard seed coat prevents the imbibition of water, there by inhibits germination. In muskdana, the existence of coat-imposed dormancy was confirmed by this test. 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 15 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 of muskdana, which were procured during first year *i.e.* 2006, were used in this study. Treatments such as boiling water (100°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°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 muskdana (Plate 3 & 4), for determining the viability of seed lots. Muskdana belongs to the family *Malvaceae* and contains hard seed coat. Freshly harvested seed lots were also having physiological dormancy up to 30 days after physiological maturity in muskdana. Since the seed lots taken for study were more than a month old, there was no problem of physiological dormancy and to remove physical dormancy preconditioning was done. Preconditioning was performed 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 a thin slice was cut off transversely from the reverse side of seeds which were kept for staining with 1% tetrazolium chloride solution. This process of slicing was done for better penetration of stain into embryo and other seed structures. Thereafter, seeds were incubated at 30°C for 16 h for proper staining. Then seed coat was removed and embryo extracted for evaluation purpose. Later seeds were evaluated for viability depending on staining pattern. The data obtained during this entire study was statistically analyzed, Analysis of variance (ANOVA) was calculated following Completely Randomized Design (CRD). To stabilize the variance in germination percentages arc sin transformations were used [9].

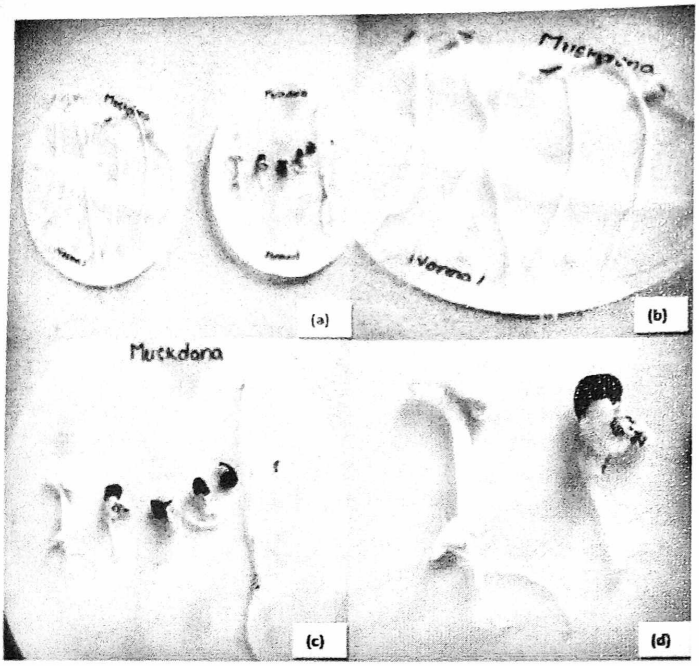

Plate 2. Normal seedlings, abnormal seedlings, hard seeds and dead seeds of germination test in muskdana

Note: (a and b): Normal seedlings, (c and d): Abnormal seedlings

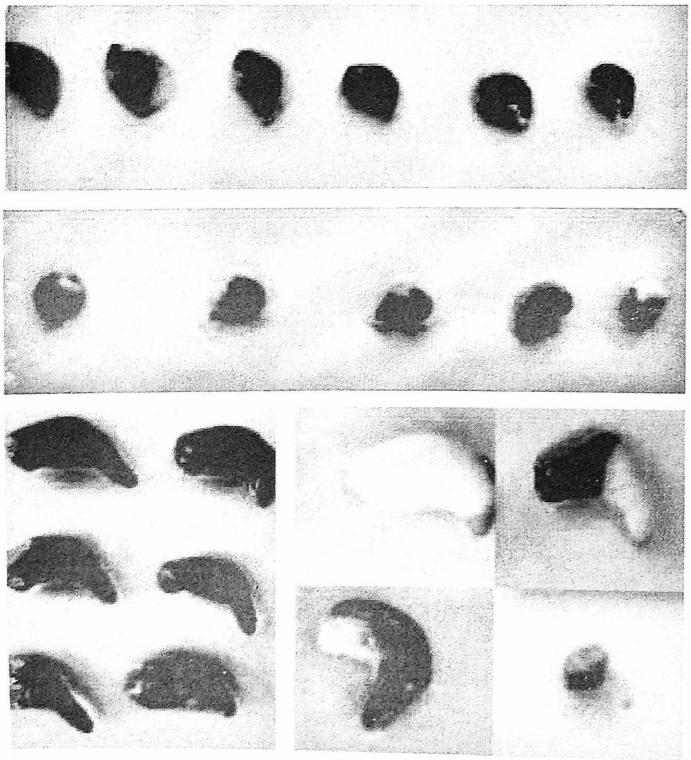

Plate 3. Tetrazolium staining test in muskdana

Note: (a and c): Viable seeds, (b and d): Non-viable seeds

RESULTS AND DISCUSSION

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

Muskdana, which belongs to family *Malvaceae*, the pure seed definition is in consonance with other members of this family, *i.e.* seed with or without testa, piece of seed larger than one-half the original size with or without testa was considered as pure seed.

Thousand seed weight

In muskdana, 1000 seed weight ranged from 15.68 g in lot 1 (from JNKVV 2005, Jabalpur) to 14.47 g in lot 12 (from JNKVV 2006, Jabalpur) with a mean of 15.17 g and standard deviation of 0.230 (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 gives a valuable input in determining working sample size (2500 seeds) which is 40g in muskdana and the sample size for other crop determination is 400g.

Optimization of temperature and substratum for germination testing

Effect of different temperature regimes (20^0, 25^0 and $20\sim30^0$C) on muskdana 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, revealed that mean germination of 27.97% and 27.37% were recorded at 25^0C and 20-30^0C, respectively, which were on par with each other. Lowest germination of 22.83% was recorded at 20^0C without any dormancy breaking treatments. A comparison of different treatments reveal that germination vary significantly in different lots and it ranged from

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

Lots	1000Seed wt	Lots	1000 Seed wt
LI	15.68	L16	15.05
L2	15.11	L17	15.02
L3	15.33	L18	15.31
L4	15.24	L19	15.14
L5	15.57	L20	14.91
6	15.03	L21	15.19
L7	15.07	L22	14.82
L8	15.01	L23	15.19
L9	15.09	L24	15.25
L10	15.11	L25	15.21
L11	15.39	L26	15.06
L12	14.47	L27	15.11
L13	15.42	L28	15.30
L14	15.44	L29	15.50
L15	15.18	L30	15.02
		Mean	15.17
		CD (p=0.05)	N.S.
		sd	0.230
		max	15.68
		min	14.47

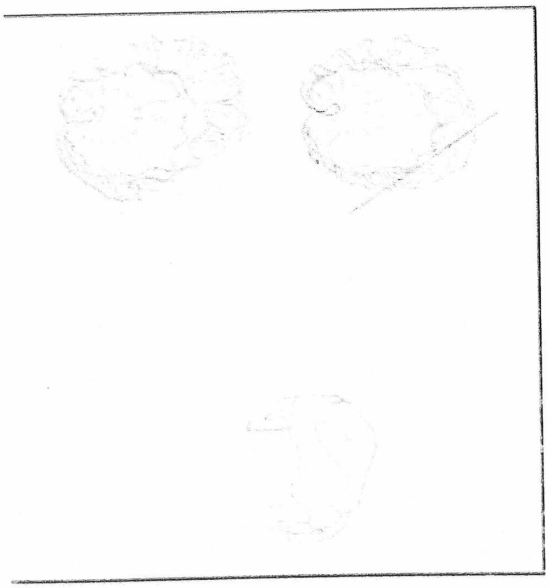

Plate 4. Cutting procedure for preparation to tetrazolium staining in muskdana

Note: A thin slice was cut off transversely from the reverse side of seed for proper entry of stain

64 % (in lot 8 at 25°C) to 9% (in lot 4 at 20°C). The mean hard seed was 28.33%, 28.90% and 32.00% at 25°C, 20-30°C and 20°C respectively. The results clearly indicated that the percent hard seed were more at 20°C and there was no significant difference among 25°C and 20-30°C without any dormancy breaking treatment.

Perusal of data revealed that the ideal temperature for germination is 25°C and there was no significant difference in mean germination percent among two temperature regimes 25°C (27.97) and 20-30°C (27.37). The other fact that muskdana being a kharif (June sown) crop and its habitat (sub-tropical) also suggest that it requires slightly higher temperature for quick and uniform germination.

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

Lots	25°C N	20-30°C N	20°C N	Mean	25°C H	20-30°C H	20°C H	Mean
L1	18 (25.1)	17 (24.5)	14 (22.1)	16.56 (23.89)	19 (26.1)	20 (26.5)	22 (27.7)	20.33 (26.78)
L2	13 (21.4)	11 (19.4)	10 (18.7)	11.56 (19.81)	21 (27.3)	22 (28.2)	22 (28.2)	21.89 (27.86)
L3	18 (25.0)	20 (26.8)	12 (19.9)	16.67 (23.91)	24 (29.3)	25 (29.7)	29 (32.8)	26.00 (30.60)
L4	11 (19.0)	11 (19.0)	9.0 (17.1)	10.00 (18.39)	12 (20.5)	16 (23.3)	17 (24.1)	14.89 (22.63)
L5	38 (37.8)	37 (37.2)	28 (32.1)	34.22 (35.73)	28 (32.1)	27 (31.5)	30 (33.4)	28.67 (32.34)
L6	18 (25.1)	19 (26.1)	17 (24.1)	18.00 (25.06)	22 (27.7)	22 (27.9)	25 (30.2)	23.00 (28.59)
L7	31 (34.0)	32 (34.2)	28 (32.1)	30.44 (33.42)	58 (49.4)	58 (49.6)	61 (51.5)	59.00 (50.17)
L8	64 (53.1)	61 (51.5)	55 (47.7)	60.00 (50.78)	22 (27.7)	23 (28.4)	26 (30.8)	23.56 (28.98)
L9	35 (36.2)	34 (35.6)	30 (33.4)	33.11 (35.09)	38 (38.0)	38 (37.8)	44 (41.3)	39.78 (39.07)
L10	34 (35.4)	31 (34.0)	25 (29.9)	30.00 (33.12)	39 (38.8)	39 (38.4)	43 (41.0)	40.33 (39.40)
Mean	27.97 (31.22)	27.37 (30.84)	22.83 (27.71)		28.33 (31.69)	28.90 (32.14)	32.00 (34.10)	
CD (p=0.05)		T	1.645 (1.107)	N.S. (N.S.)	CD (p=0.05)	1.34 (0.86)	N.S. (N.S.)	(n=4)
		L	3.004 (2.020)			2.44(1.57)		(n=4)
		T*L						

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

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 species under study. Hence, TP was not used as a method of germination testing. In muskdana, there was no significant difference in mean germination percent among germination media (BP method and Vermiculite). The mean germination of 27.97% and 28.73% were recorded with BP and Vermiculite, respectively (Table 3). Significant variation was observed among different lots, with germination as high as 64% in lot 8 to as low as 11% in lot 4 in muskdana without any dormancy breaking treatment. There was no significant difference between the two substrates (Germination paper and Vermiculite) as germination media but due to convenience in handling and ease in taking observations BP method (Between Paper) was followed for conducting germination test.

Days to first and final count of germination

In muskdana, days to first count were six and for final count were 12 and 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 muskdana

The results of imbibitional studies clearly revealed the presence of coat imposed (physical) dormancy in muskdana. In muskdana (Fig. 1), seeds reached to maximum mean fresh weight (3.35 g) at 48 hours after initiation of imbibitional study under control conditions, where as the maximum

Table 3. Effect of different substrates on germination percentage in muskdana

Lots	BP method	Vermiculite	Mean
L1	18 (25.1)	20 (26.5)	19.00 (25.80)
L2	13 (21.4)	12 (20.5)	12.83 (20.91)
L3	18 (25.0)	18 (25.1)	18.00 (25.05)
L4	11 (19.0)	12 (19.9)	11.17 (19.48)
L5	38 (37.8)	38 (38.2)	38.00 (38.04)
L6	18 (25.1)	18 (25.3)	18.17 (25.20)
L7	31 (34.0)	31 (33.8)	31.17 (33.91)
L8	64 (53.1)	64 (52.9)	63.83 (53.03)
L9	35 (36.2)	34 (35.6)	34.50 (35.94)
L10	34 (35.4)	40 (39.2)	36.83 (37.31)
Mean	27.97 (31.22)	28.73 (31.72)	
CD (p=0.05)	S	N.S. (N.S.)	
(n=4)	L	3.36 (2.25)	
	S*L	N.S. (N.S.)	

mean fresh weight were 3.37 g and 3.10 g with boiling water and H_2SO_4 treatments after 24 hours and 28 hours respectively.

Dormancy and its breakdown in muskdana

Imbibitional studies established the presence of coat imposed (physical) dormancy in muskdana beyond doubt. The results of

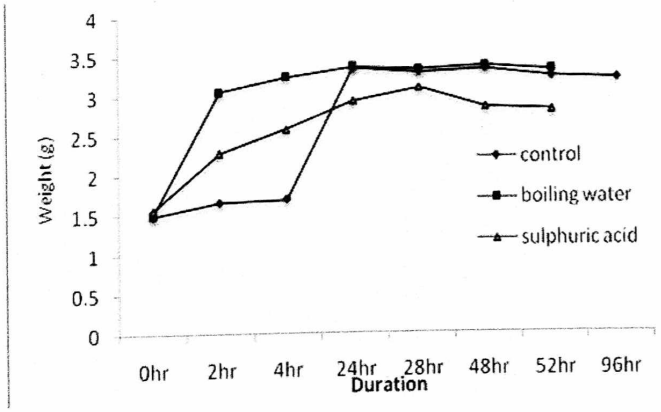

Fig 1. Imbibitional pattern in Muskdana (n=4)

dormancy and its breakdown clearly indicated that acid scarification and manual scarification are most effective treatments. Even though there was existence of slight physiological dormancy during first month in freshly harvested seeds, there was no sign of physiological dormancy afterwards and it

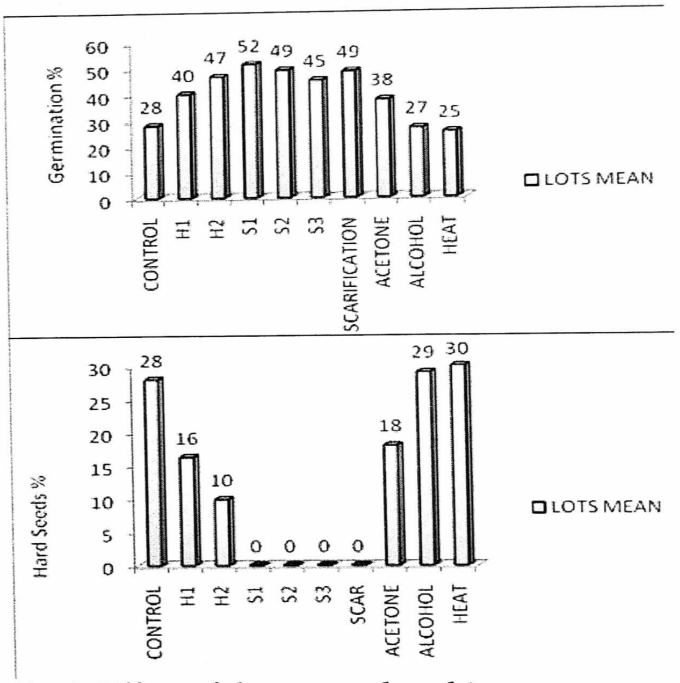

Fig 2. Effect of dormancy breaking treatments on germination and hard seeds percentage in muskdana. (n=4)

Note: H1= boiling water (equal volume); H2=boiling water (100ml); S1= H_2SO_4 (15min); S2=H_2SO_4 (30min); S3=H_2SO_4 (60min); SCARI=manual scarification with sand paper; ACETONE=over night soaking (equal volume); ALCOHOL= over night soaking (equal volume); HEAT= heat treatment for 6hr.

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

Lots	Control	H1	H2	S1	S2	S3	SCARI	ACETONE	ALCOHOL	HEAT	Mean
L1	18	28	34	36	36	29	34	29	16	15	27.40
	(25.1)	(31.7)	(35.4)	(36.8)	(36.6)	(32.4)	(35.6)	(32.4)	(23.8)	(23.0)	(31.28)
L2	13	23	29	32	31	31	29	21	11	11	23.13
	(21.4)	(28.6)	(32.5)	(34.1)	(34.0)	(33.6)	(32.8)	(27.2)	(19.6)	(19.0)	(28.29)
L3	18	25	31	37	32	29	33	27	19	13	26.43
	(25.0)	(30.0)	(33.6)	(37.6)	(34.6)	(32.6)	(34.8)	(31.3)	(25.8)	(21.4)	(30.67)
L4	11	15	16	17	18	17	17	14	8.0	8.0	14.10
	(19.0)	(22.8)	(23.5)	(24.5)	(24.7)	(24.0)	(24.6)	(22.2)	(16.0)	(16.8)	(21.82)
L5	38	45	50	61	56	49	57	44	33	33	46.73
	(37.8)	(42.3)	(45.2)	(51.3)	(48.4)	(44.4)	(49.2)	(41.7)	(35.2)	(35.0)	(43.07)
L6	18	23	29	33	31	28	29	21	20	16	24.57
	(25.1)	(28.4)	(32.4)	(34.8)	(33.6)	(32.1)	(32.4)	(27.0)	(26.3)	(23.3)	(29.54)
L7	31	70	79	86	85	79	85	63	32	29	63.87
	(34.0)	(57.0)	(62.5)	(67.9)	(67.0)	(62.7)	(66.9)	(52.7)	(34.6)	(32.3)	(53.77)
L8	64	72	78	80	79	72	78	66	61	60	71.00
	(53.1)	(58.0)	(62.3)	(63.4)	(62.9)	(57.8)	(61.8)	(54.5)	(51.1)	(50.8)	(57.58)
L9	35	51	62	68	65	63	62	49	32	34	51.97
	(36.2)	(45.4)	(51.7)	(55.3)	(53.7)	(52.5)	(52.1)	(44.2)	(34.2)	(35.6)	(46.11)
L10	34	50	61	65	60	56	62	41	34	32	49.47
	(35.4)	(45.0)	(51.3)	(53.9)	(50.5)	(48.6)	(51.7)	(40.0)	(35.4)	(34.4)	(44.64)
Mean	28.0	40.1	46.8	51.5	49.2	45.2	48.6	37.6	26.6	25.1	
	(31.2)	(38.9)	(43.0)	(46.0)	(44.6)	(42.1)	(44.2)	(37.3)	(30.2)	(29.2)	
CD (p=0.05)	T	1.32(0.88)									
(n=4)	L	1.32(0.88)									
	T*L	4.18(2.78)									

Note: H1= boiling water (equal volume); H2=boiling water (100ml); S1= H_2SO_4 (15min); S2=H_2SO_4 (30min); S3=H_2SO_4 (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

requires an after ripening period of at least one month (Fig. 2).

With respect to dormancy alleviation, sulphuric acid treatment for 15 minutes was proved effective which was having an advantage of 23.5% when compared with control (Table 4). With the increase in acid scarification duration beyond 15 minutes, there was decrease in germination percent and increase in abnormal seedling percent. Hence, acid scarification for 15 minutes was ideal in breaking coat-imposed dormancy in muskdana. Earlier reports also reflected similar results where acid scarification and manual scarification were ideal in alleviation of hard seededness in ambrette. Gupta [10] reported that 100% seed germination was obtained with acid scarification and sand paper scarification resulted in 85-90% seed germination.

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

Germination percentages, seedling length, seedling dry weight and vigour indices were tabulated over a period of three years (2006 to 2008) and

Table 5. Germination percentage and vigour of 10 seed lots during 1st year of testing in muskdana

Lots	N	H	G	SL	SD	VIG 1	VIG 2
L1	18 (25.1)	19 (26.1)	36 (36.8)	10.2	73	368	2626
L2	13 (21.4)	21 (27.3)	32 (34.2)	9.4	70	298	2230
L3	18 (25.0)	24 (29.3)	37 (37.6)	10.3	73	383	2737
L4	11 (19.0)	12 (20.5)	17 (24.5)	9.8	67	169	1170
L5	38 (37.8)	28 (32.1)	61 (51.3)	11.2	81	682	4959
L6	18 (25.1)	22 (27.7)	33 (34.8)	10.6	74	346	2426
L7	31 (34.0)	58 (49.4)	86 (67.9)	11.8	90	1012	7720
L8	64 (53.1)	22 (27.7)	80 (63.4)	12.0	101	962	8087
L9	35 (36.2)	38 (38.0)	68 (55.3)	11.9	92	801	6217
L10	34 (35.4)	39 (38.8)	65 (53.9)	11.0	90	721	5878
Mean	27.97 (31.22)	28.33 (31.69)	51.46 (46.00)	10.82	81.23	574.3	4405
CD (p=0.05)	5.73 (3.76)	3.92 (2.57)	5.38 (3.73)	0.717	6.17	61.27	625.4

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

Lots	N	H	G	SL	SD	VIG 1	VIG 2
L1	23 (28.9)	20 (26.3)	36 (36.6)	10.2	73	363	2626
L2	12 (19.9)	26 (30.8)	32 (34.6)	9.2	68	297	2205
L3	20 (26.3)	44 (41.3)	53 (46.7)	9.7	70	512	3694
L4	55 (48.0)	36 (36.9)	84 (66.1)	11.1	97	930	8151
L5	65 (53.5)	19 (25.6)	76 (60.8)	11.7	96	893	7321
L6	35 (36.4)	36 (37.0)	65 (53.7)	11.0	89	715	5803
L7	42 (40.4)	25 (30.2)	61 (51.5)	10.8	86	661	5293
L8	22 (28.1)	25 (29.8)	40 (39.2)	9.5	73	379	2929
L9	42 (40.6)	31 (33.8)	68 (55.7)	11.7	81	797	5519
L10	31 (33.6)	39 (38.4)	65 (53.7)	12.0	82	778	5303
Mean	34.73 (35.58)	30.03 (33.01)	58.07 (49.89)	10.67	81.53	632.7	4884
CD (p=0.05)	5.00 (3.19)	4.15 (2.67)	4.63 (2.86)	0.63	7.08	71	616

Note: N= normal seedlings; H =hard seeds; G =germination % after H_2SO_4 treatment; SL=seedling length (cm), SD= seedling dry weight (mg); VIG 1= vigour index 1; VIG 2= vigour index 2; (n=4).

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 muskdana the germination percent range was in between 17 to 86. The 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 might have variation in germination percentages. Since no seed standards were 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.

Correlation of tetrazolium staining data with germination percentage in muskdana

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, non-viable seeds are categorized into seeds with major portion of cotyledon unstained, radicle unstained, totally unstained and rotten. In lot 1, the total viable seed percent was 42, non viable seed percent was 58 and the corresponding germination percent was 36 after sulphuric acid treatment. In lot 2, the total viable seed percent was 34, non viable seed percent was 66 and the corresponding germination percent was 28. In lot 3, the total viable seed percent was 86, non viable seed percent was 14 and the corresponding germination percent was 80 and finally in lot 4, the total viable seed percent was 73, non viable seed percent was 27 and the corresponding germination percent was 68. The results (correlation coefficient, r) clearly established the correlation (highly significant) between viable seeds of tetrazolium chloride test and normal seedlings of germination test (Table 8).

Since there was no information available regarding tetrazolium staining procedures of muskdana [12], the present study has attempted to fill teh gap by standardizing the procedures such as method of pre conditioning, excision technique, concentration of staining solution, incubation time & 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 in each crop. Based on 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 muskdana 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 muskdana (Table 11).

Seed testing protocols for Abelmoschus moschatus

Muskdana seeds can be tested using BP (roll paper towel) method at 25^{0}C with first and final count being 6 and 12 days, respectively. Muskdana seeds exhibit combinational dormancy *i.e.* physical and physiological dormancy. Therefore, treatment of seeds with boiling water (equal volume) or scarification with concentrated H_2SO_4 for 15 minutes (depending on degree of hard seededness) is recommended to make the seed coat permeable to water and gas for inducing germination. Physiological dormancy lasts for a period of one month in muskdana, as the seeds after ripen due to increased growth potential of embryo [12] that is also due to increase in GA_3 levels and reduction in ABA.

Physical dormancy is reported to occur in 15 families of *Angiosperms*. *Malvaceae* appears to be the second family of *Angiosperms* after *Fabaceae* to exhibit physical dormancy [12-14]. As per Indian Seed Act, hard seeds are reported under normal seedlings category [1]. A variety of seed pre-treatments have been reported to break the physical dormancy in members of *Fabaceae* and *Malvaceae* reviewed from time to time [12, 15]. Acid scarification was found effective in breaking hard seededness in *Desmodium, Vicia and Sesbania* [16-19]. Boiling water [20-21] and heat treatment [22-23] have also been found effective in breaking dormancy.

Standardization of topographical tetrazolium chloride test in muskdana

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 (ISTA, 2012). Preparation

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

Lots	N	H	G	SL	SD	VIG 1	VIG 2
L1	32 (34.6)	34 (35.4)	61 (51.5)	10.8	76	664.6	4644
L2	31 (33.6)	33 (35.0)	59 (50.3)	11.0	77	654.9	4566
L3	21 (27.0)	25 (29.8)	40 (39.2)	10.3	72	410.1	2898
L4	13 (21.1)	62 (51.9)	69 (56.1)	10.0	79	692.2	5477
L5	41 (39.8)	32 (34.2)	67 (54.9)	11.9	81	797.4	5446
L6	29 (32.3)	22 (28.2)	44 (41.7)	10.5	76	468.1	3364
L7	47 (43.1)	32 (34.2)	72 (58.2)	10.9	95	789.1	6868
L8	53 (46.9)	25 (30.0)	73 (58.4)	11.8	91	854.5	6638
L9	65 (53.5)	27 (31.1)	83 (65.8)	12.1	98	1006	8167
L10	36 (37.0)	26 (30.8)	58 (49.5)	11.6	79	670.3	4605
Mean	36.73 (36.90)	31.70 (34.06)	62.73 (52.62)	11.09	82.50	700.7	5267
CD (p=0.05)	3.97 (2.56)	4.14 (2.64)	4.97 (3.03)	0.53	7.20	74.7	663.9

Note: N= normal seedlings; H =hard seeds; G =germination % after H_2SO_4 treatment; SL=seedling length (cm), SD= seedling dry weight (mg); VIG 1= vigour index 1; VIG 2= vigour index 2; (n=4).

Table 8. Correlation of tetrazolium staining data with germination test data in muskdana

Lots	Viable seeds				Non viable seeds				Germination %		
	TS	SE	TOTAL	MEU	RU	TU	ROT	TOTA	N	H	G
L1	35.0	7.0	42	36.5	1.3	3.3	17.3	58	18	19	36
	(36.2)	(15.3)	(40.4)	(37.1)	(5.5)	(10.2)	(24.5)	(49.6)	(25.3)	(25.6)	(36.7)
L2	27.8	6.3	34	43.3	5.3	2.5	15.0	66	13	21	28
	(31.8)	(14.4)	(35.6)	(41.1)	(13.1)	(9.0)	(22.8)	(54.3)	(21.5)	(27.3)	(32.1)
L3	75.3	10.8	86	9.8	1.3	0.8	2.3	14	64	20	80
	(60.2)	(19.1)	(68.0)	(18.2)	(6.3)	(3.5)	(8.38)	(21.9)	(53.3)	(26.3)	(63.4)
L4	62.8	10.3	73	19.5	0.5	1.0	6.0	27	35	38	68
	(52.4)	(18.6)	(58.7)	(26.2)	(2.0)	(4.9)	(14.1)	(31.3)	(36.2)	(37.7)	(55.5)
MEAN	50.2	8.56	58.8	27.3	2.06	1.9	10.1	41.3	32.8	24.3	53
	(45.1)	(16.9)	(50.7)	(30.6)	(6.8)	(6.9)	(17.4)	(39.3)	(34.1)	(29.2)	(46.9)
CD (p=0.05)	4.22	2.21	3.54	3.96	1.64	1.71	2.27	3.54	4.28	3.07	3.69
	(2.56)	(2.23)	(2.29)	(2.52)	(4.7)	(4.78)	(2.69)	(2.29)	(2.81)	(2.08)	(2.31)

Note: TS= totally stained; SE=some minor damages on embryo; MEU= major portion of embryo unstained; RU= radical unstained; TU= totally unstained; ROT= rotten; N= normal seedlings; H= hard seeds; G= germination % after H_2SO_4 treatment; (n=4).

of seeds for TZ test require knowledge of seed anatomy [24] so as to identify the embryo type, level of embryo differentiation, embryo-seed ratio etc. In muskdana, embryo is folded and for preparing the seed for TZ test, embryo removal was required as indicated in plate 4.

Muskdana 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 muskdana and the results were correlated (Table 12) with the

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

Species	Lot size (kg)	Working sample size for physical purity (g)	Sample size for other crop determination (g)
Abelmoschus moschatus	20000	40	400

Table 10. Seed testing protocols for muskdana

Species	Substrate	Temperature (°C)	First count (days)	Final count (days)	Remarks
Abelmoschus moschatus	Rolled paper towel (BP method)	25	6	12	H_2SO_4 treatment for 15 minutes

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

Species	Pre- conditioning and temperature	Hydration time of seed	Preparation	Incubation time required for staining in 1 % TZ solution
Abelmoschus moschatus	Boiling water for half an hour	20 °C for 17 hrs	Excision of embryo	30 °C for 16 hours

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

	Viable seeds (%)	Germination (%)
Viable seeds (%)	1	
Germination (%)	0.998	1

Table 13. Seed quality parameters formulated or recommendations

Factor	Standard for each class of seed Foundation	Certified
Pure seed (minimum)	99.0 %	99.0 %
Inert matter (maximum)	1.0 %	1.0 %
Other crop seed (maximum)	None	5 / kg
Weed seed (maximum)	None	None
Germination (minimum) including hard seeds	65 %	65 %
Moisture (maximum)	10 %	10 %
Moisture for vapour proof containers (maximum)	8.0 %	8.0 %

germination test results [11] and thus the standards were 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 and aromatic crop species grown by Indian farmers' *viz.* muskdana.

The above standards were formulated based on our results of experimentation and perusal of standards in IMSCS [1] of concerned family *i.e. Malvaceae*. As per Seeds Act, the hard seeds are to be considered as part of normal seedlings, as this is a standard practice for seeds of family *Malvaceae*. 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 muskdana and perusal of standards of IMSCS.

Suggested standards for pure seed (99%), other crop seed, weed seed and objectionable weed seed (Nil) and germination including hard seeds (65%) were suggested by careful observation and comparison with IMSCS of other *Malvaceae* members. The number of other crop seed and weed seed per kg are suggested on the basis of broad conclusions, and seed standards in other crop species of same family. However, no weed seed or other crop seed were found in the samples obtained, 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. However, when the seed certification becomes 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' for one of the most important medicinal and aromatic crop (muskdana), which will help in better handling of seeds of this species 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. PARIHAR SS, DADLANI MALAVIKA 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, *Abs* pp: 58.
3. PARIHAR SS, 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, *Abs* pp: 180-1.
4. ISTA (2012). International rules for seed testing. ISTA edition, Bassersdorf, CH. Switzerland.
5. SRIVASTAVA UC (1995). Ambretty seed. *In: Advances in Horti*, Vol. 11, Medicinal and Aromatic Plants (Eds. CHADHA KL AND GUPTA R). Malhotra pub. House, N. Delhi, pp: 887-97.
6. AGRAWAL PK AND SINGH DP (1993). In: *Handbook of Seed Testing*, Ministry of Agriculture, GOI, New Delhi, pp: 116-20.
7. ELLIS RH AND ROBERTS EH (1981). The quantification of ageing and survival in orthodox seeds. *Seed Sci Technol* **9:** 373-409.

8. ABDUL-BAKI AA AND ANDERSON JD (1973). Relationship between decarboxylation of glutamic acid and vigour in soybean seed. *Crop Sci* **13**: 227-32.
9. HOGG RV AND CRAIG AT (1995). *Introduction to Mathematical Statistics* (5th edn), Prentice Hall, New Jersey, USA.
10. GUPTA V (2003). Seed germination and dormancy breaking techniques for indigenous medicinal and aromatic plants. *J Medicinal Aromatic Pl Sci* **25**(2): 402-7.
11. ANONYMOUS (2009). 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.
12. BASKIN CC AND BASKIN JM (1998). *Seeds Ecology, Biogeography and Evolution of Dormancy and Germination*, Acad. Press, San Diego. pp: 324.
13. BASKIN JM, BASKIN CC AND LI X (2000). Taxonomy, ecology, and evolution of physical dormancy in seeds. *Pl Species Bio* **15**: 139–52.
14. BASKIN JM AND BASKIN CC (2004). A classification system for seed dormancy. *Seed Sci Res* **14**: 1-16.
15. PARIHAR SS (2003). Exogenous dormancy in forage seeds and its ecological significance. *Range Management Agroforestry* **24**: 1-8.
16. ASWATHAIAH B AND DELOUCHE JC (1987). The effectiveness of sulphuric acid scarification in reducing hard seed content in vetch seeds. *Seed Res* **15**: 20-3.
17. AKINOLA JO, AFOLAYAN RA AND OLORUNJU SAS (1991). Effect of storage, testa colour and scarification method on seed germination of *Desmodium velutinum* (Wild.). *Seed Sci Technol* **19**: 159-66.
18. ZODAPE ST (1991). The improvement of germination of some forest species by acid scarification. *Ind Forestry* **117**: 65-7.
19. SHEELAVANTAR MN, BHAT RS AND HALEPYATI AS (1993). Breaking dormancy in *Sesbania rostrata* by acid and boiling water treatment. *Seed Res (Spl. Vol.)* **I**: 484-9.
20. MCIVOR JG AND GARDENER CJ (1987). Effect of boiling water treatment on hardseededness and germination in some *Stylosanthes* species. *Australian J of Exptl Agril* **27**: 857-62.
21. VAISH CP, KATIYAR RP AND KANAUJIA VP (1992). Effect of hot water treatment on breaking hardseededness in subabul (*Leucaena leucocephala*). *Seed Res* **20**: 158-9.
22. MOTT JJ (1979). High temperature contact treatment of hard seeds of *Stylosanthes*. *Aus J Agril Res* **30**: 847-54.
23. GILBERT MA AND SHAW KA (1979). The effect of heat treatment on hard seededness of *Stylosanthes scabra*, S. *hamata cv. Verano* and *S. viscosa*. *Trop Grasslands* **13**: 171-5.
24. CHOWDHURY KA AND BUTH GM (1970). Seed coat structure and anatomy of Indian pulses. *J Linn Soc Bot* **63**: 169-79.