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Short Communication

Standardization of seed germination testing protocol in *Moringa oleifera* Lam

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Moringa oleifera Lam. is a fast–growing, soft wood, drought–resistant and multipurpose tree of Moringaceae family (Quattrocchi 2000), popularly known as drumstick in India and horseradish tree in other parts of the world. This miracle tree is native to India and cultivated in the tropics and sub–tropics of the world for its nutritional, medicinal and therapeutic properties (Nsofor et al. 2012). Propagation of moringa through seed is easy (Radovich 2012) and can be achieved in a large area rapidly when compared to stem cutting methods. Moringa seeds, not only used as planting material but are also a rich source of oil (ben–oil) (Anwar and Rashid 2007), which is considered as good substitute for Olive oil. Seed cake after oil extraction is used for water purification. It has dimeric cationic proteins that neutralize the colloidal charge of turbid water and remove impurities by flocculation (Delelegn et al. 2018). Moringa seeds generally exhibit late, erratic, and poor germination (WAC 2002; Yerima 2016). Under the Indian seed laws, it is mandatory to label seed bags or containers and specify seed germination and other seed quality attributes (Seed Act 1966). These prescriptions are based on testing protocols developed in a national level reputed laboratory and subsequently got tested for its proficiency. Information on seed germination for moringa seeds was either fragmented and neither available in Indian Minimum Seed Certification Standards nor the International Seed Testing Association. These seed germination testing protocols are very useful and used to determine the maximum germination potential of a given seed lot, which will be used to compare the quality of different seed lots and to estimate the field planting value (Anon 2008). Unlabeled seeds available to the farmers are spurious in nature, which leads to poor crop stand. Mature moringa seed consists of the embryo and two cotyledons of 10–14 mm in diameter, covered by the epidermal layer of the inner integument and enclosed by the triangular seed coat (outer integument) from which three thin wings of 5–25 mm in length are attached. The effect of these wings in seed germination was also explored. Hence, the present investigation was undertaken with the objective of developing standard and tangible seed germination testing protocols for moringa.

The experiments were conducted at the ICAR–Indian Institute of Vegetable Research, Varanasi (82.50ÚN, 25.10ÚE) during 2017–18. Mature seeds of three genotypes (VRMO–10, VRMO–17 and PKM–1) were collected in the month of July 2017, at harvest maturity (brown pod stage). Seed germination studies were carried out based on procedures of ISTA rules (Anon 2008 and 2015), using different substrata i.e. rolled paper towel or between paper (BP), top of paper (TP) and sand (S), under different temperature regimes i.e. at constant temperatures of 10°C, 20°C, 25°C, 30°C, 35°C and an alternating temperature of 20°~30°C (16 h & 8 h, respectively). A combination of light and dark (petri plates were covered properly with double layer of aluminum foil) was also used to optimize the seed germination percentage. From each seed lot, 400 seeds (i.e. 100 seeds each in 4 replicates) from pure seed fraction were drawn at random and placed equidistantly either on a double layer of moist filter paper discs in plastic petri plates (11 cm diameter) or in the rolled paper towel or on a levelled layer of moist sand medium (covered with 10–20 mm of sand medium substrate). The germination media were incubated in dark or light at above mentioned temperatures in cabinet seed germinator maintaining 95±5% RH for 21 days. Only morphologically normal seedlings (intact seedlings with slight defects or with secondary infection, possessing all its essential structures) were scored for the purpose of seed

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germination. The un-germinated seeds were gently pressed with a thumb to judge their internal state. If internal content oozed out or appeared rotten or produces any foul odour, it was considered dead. Otherwise, its viability was adjudged using tetrazolium chloride test (TZ). The results of the germination test were calculated as the average of 4 × 100 seeds replicates. This is expressed as the percentage by number of normal seedlings. The percentage was calculated to the nearest whole number, 0.5 was taken to the higher side. The percentage of abnormal seedlings, hard, fresh un-germinated and dead seeds was calculated in a similar way.

Optimum germination time or duration of seed germination is an obvious attribute of seed quality performance and could serve as measure for seed vigour. The optimum germination time was estimated using methodology of Ellis and Roberts (1981) with slight modification, that complete seedling with all essential structure was taken as criteria for normal seed germination. 200 seeds from each genotype (i.e. 50 seeds each in 4 replicates) were placed in between paper and incubated at 25°C in dark in cabinet seed germinator. The seeds were monitored and recorded daily for germination up to 21 days, starting from day 1. Observations on time taken (days) for approximately 75 percent germination, of the total seed germinated as first count (day) and maximum germination as final count (day) were recorded.

The data recorded were subjected to factorial Completely Randomized Design analysis. The data taken as percent based on count value were transformed to the respective angular (arcsine values) before subjecting them to statistical analysis. The computer software used was AgRes version 3.01. Results of different substrates used for moringa seed germination with different treatments *viz.* whole seed (seed with an outer coat and wings, T1), seed without wings (T2), seed without wing and outer seed coat (T3) are presented in table 1. Significant differences were observed in germination due to different treatments and substrates. The treatment T2 (wingless seed) gave highest mean germination (55%) percentage over the other treatments. Treatment T3 showed the lowest germination (11%) mostly due to secondary fungal infection and seed rotting in all the substrates. Similarly, presence of wings in T1 also showed secondary fungal infection. The mean germination value among different seed lots was maximum on the between paper (BP) followed by sand (S) and top of paper (TP) (47%, 40% and 24%, respectively). Higher germination on BP can be attributed to proper moisture availability to seeds. Moringa, being large seeded, sand method for germination was expected to be best suitable. Germination (%) in sand method is

Table 1. Germination percentage of moringa seeds on different substrates.

Genotypes	Seed type	Top of Paper (M1)	Roll Towel Paper (M2)	Sand (M3)	Mean (VxT)
VRMO–10 (V1)	Whole seed (T1)	21 (27.27)	56 (48.06)	58 (49.41)	45 (41.58)
	Seed without wing (T2)	39 (38.84)	65 (53.73)	63 (52.73)	56 (48.44)
	Seed without wing and seed coat (T3)	11 (19.65)	21 (27.50)	1 (5.74)	11 (17.63)
	Mean (V×M)	24 (28.59)	47 (43.10)	41 (35.96)	
VRMO–17 (V2)	Whole seed (T1)	23 (28.62)	56 (48.64)	57 (49.03)	45 (42.11)
	Seed without wing (T2)	40 (39.43)	65 (53.54)	63 (52.34)	56 (48.44)
	Seed without wing and seed coat (T3)	11 (19.32)	23 (2842)	1 (5.74)	12 (17.83)
	Mean (V×M)	25 (29.13)	48 (43.53)	40 (35.70)	
PKM–1 (V3)	Whole seed (T1)	23 (28.65)	56 (48.26)	57 (48.83)	45 (41.91)
	Seed without wing (T2)	36 (36.67)	64 (53.14)	63 (52.54)	54 (47.45)
	Seed without wing and seed coat (T3)	11 (19.03)	19 (26.07)	1 (3.93)	10 (16.34)
	Mean (V×M)	23 (28.11)	46 (42.49)	40 (35.10)	

Mean table for T × M

	M1	M2	M3
T1	22 (28.19) d	56 (48.32) b	57 (49.09) b
T2	38 (38.31) c	65 (53.47) a	63 (52.54) a
T3	11 (19.33) e	21 (27.33) d	1 (5.14) f

Overall mean table

V mean		T mean		M mean	
V1	37 (35.88) ab	T1	45 (41.87) b	M1	24 (28.61) c
V2	38 (36.12) a	T2	55 (48.11) a	M2	47 (43.04) a
V3	37 (35.24) b	T3	11 (17.27) c	M3	40 (35.59) b

ANOVA

	V	T	M	VxT	TxM	VxM	VxTxM
	0.65*	0.65**	0.65**	NS	1.12**	NS	NS
CV (%)	3.34						

V–Variety, T–Treatment, M–Germination media

Table 2. Germination percentage of moringa seeds without wing (T2) on BP under different temperature regimes.

Genotypes	10°C (C1)	20°C (C2)	25°C (C3)	30°C (C4)	35°C (C5)	20°~30°C* (C6)	Mean (V)
VRMO–10 (V1)	3 (9.88)	34 (35.66)	65 (53.54)	43 (41.16)	6 (13.69)	64 (53.34)	36 (34.54)
VRMO–17 (V2)	4 (10.96)	35 (36.07)	67 (54.75)	42 (40.59)	9 (17.05)	65 (53.74)	37 (35.52)
PKM–1 (V3)	4 (11.90)	32 (34.44)	65 (53.75)	41 (40.00)	5 (12.28)	65 (53.54)	35 (34.38)
Mean (C)	4 (10.92)e	34 (35.39)c	65 (54.01)a	42 (40.59)b	6 (14.34)d	65 (53.54)a	
CD @ 5%	V	C	CxV				
	NS	1.74**	NS				
CD (%)	5.03						

*alternate temperature (20° C for 16 hrs. and 30° C for 8 hrs.)

numerically equal with BP for T1 and T2, but seed took more time to germinate in sand (more than 10 days). Sand method is space consuming and laborious than BP method, the latter provides an opportunity of measurement of speed of germination over former method.

Results of different temperature regimes (C1 to C6) were used for moringa seed germination on between paper were given in table 2. Significant differences were observed in seed germination due to different temperature treatments. Seed germination under 25°C (65%) and alternate temperature of 20°~30°C (65%) treatment recorded better than any temperature in the present study. Altering day and night temperature for some plant species gave better germination results than constant temperature (Hartmann and Kester, 2001).

Table 3. Germination percentage of moringa seeds without wing (T2) using TP method at 25°C with light and/or dark.

Genotypes	Light* (L1)	Dark** (L2)	Mean
VRMO–10 (V1)	37 (37.46)	39 (38.64)	38 (38.04)
VRMO–17 (V2)	39 (38.76)	40 (39.22)	40 (38.99)
PKM–1 (V3)	39 (38.76)	41 (40.04)	40 (39.40)
Mean	38 (38.32)	40 (39.30)	
	V	L	VxL
CD @ 5%	NS	NS	NS
CV (%)	4.83		

*Fluorescent tube light for 8 hrs alternate with 16 hrs dark
**Complete dark

Better germination on alternate temperature may be attributed to natural diurnal temperature in characterizing the season, the climate and the microclimate. Alternating temperatures have been reported to increase the rate of germination of different vegetables (Motsa et al. 2015). Results of light or complete darkness treatment used for moringa seed germination on TP under 25°C are shown in table 3. No significant differences were observed in germination under dark and light conditions. Germination was recorded to be 38% in light and 40% under complete darkness, the differences were non–significant, suggesting that moringa seeds are non–photoblastic.

Observations made on the number of days required for the first count and final count are given in table 4. Significant differences were found in number of days required for the first count and the final count in achieving seed germination. Moringa seed registered slow germination. Seed germination in all the genotypes started on the 7th day onwards, germination percentages steadily progressed up to 17 days. Maximum germination was obtained on 17th day in majority of the seed lots studied. ANOVA showed that seed germination values were at par within 12, 13, 14 and 15 days and 17, 18, 19, 20, 21 days in all the seed lots studied. Over 75 percent of the total achievable seed germination was observed on the 12th day, whereas the total achievable

Table 4. Germination percentage of moringa seeds without wing (T2) using BP method under 25°C for first and final count (days).

Genotypes	Days* from seed planting													
	09 (D1)	10 (D2)	11 (D3)	12 (D4)	13 (D5)	14 (D6)	15 (D7)	16 (D8)	17 (D9)	18 (D10)	19 (D11)	20 (D12)	21 (D13)	Mean
VRMO–10 (V1)	23 (28.64)	30 (33.20)	38 (38.05)	49 (44.42)	49 (44.14)	49 (44.43)	53 (46.73)	56 (48.45)	63 (52.54)	65 (53.43)	65 (53.75)	65 (53.74)	65 (53.44)	51 (45.76)a b
VRMO–17 (V2)	24 (28.97)	33 (35.05)	41 (39.52)	50 (45.00)	51 (45.58)	51 (45.57)	54 (47.30)	58 (49.61)	63 (52.54)	67 (54.95)	67 (54.65)	67 (54.64)	67 (54.94)	53 (46.80)a
PKM–1 (V3)	20 (26.50)	28 (31.62)	38 (38.04)	48 (43.85)	48 (43.57)	49 (44.14)	50 (45.00)	55 (47.88)	61 (51.36)	64 (53.14)	64 (53.15)	65 (53.44)	65 (53.45)	50 (45.01)b
Mean	23 (28.04)f	30 (33.29)e	39 (38.54)d	49 (44.43)c	49 (44.43)c	50 (44.71)c	53 (46.34)bc	57 (48.64)b	62 (52.15)a	65 (53.84)a	65 (53.85)a	65 (53.94)a	65 (53.95)a	
	V	D	VxD											
CD @ 5%	1.13**	2.35**	NS											
CV (%)	4.43													

*Seed germination (Normal seedling) were available from 09 day onwards from seed planting

seed germination was observed on the 17th day. After, the 17th day to the 20th day, there was no increase in seed germination percentage. Therefore, based on statistical analysis, it is suggested that the germination on 12th day is at par with 13th, 14th and 15th day and were most suitable for first count while, 17th day for final count from the seed planting. Various dormancy breaking and seed enhancement treatments *viz.* abrasion against sandpaper, soaking in sulphuric acid for 2 minutes, hot water for 10 minutes, cold water for 24 hr and 10% gibberellic acid (Eghobor et al. 2015, Materechera 2017) were used for germination studies in moringa. Abrasion of seed (physical scarification) and acid scarification is normally used for softening the seed coat to facilitate radicle emergence and to remove acid soluble germination inhibitors. These treatments enhanced germination percentage in moringa but, the presence of any physical and physiological dormancy was not reported. Similarly, in this study, we also found that the seeds abraded with sandpaper gave at par gemination with seed without wing (T2) placed for gemination (data not shown).

Standard germination test is a viability test used to determine the suitability of a seed lot for planting. These results are used for labelling seeds for sale and are printed on the seed tag to inform the buyer of the physiological quality of the seed lot. Seed testing protocols were developed to determine the maximum germination potential of a given seed lot, which is used to compare the quality of different seed lots; and to estimate the field planting value. Results suggest that use of between paper method (BP) at 25°C or alternate temperature of 20°~30°C with the first count on 12th day and final count on 17th day is the best suitable protocol for seed germination testing in moringa.

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