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Standardization of quick viability and test weight protocol for *Moringa oleifera* L. Var. PKM 1

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Abstract
Moringa oleifera is a woody tree belongs to moringaceae family and popularly known as drum stick in India and it is considered as important nutritive vegetable in southern parts of Indian and also has high medicinal value. It is mainly propagated through seeds. Seed is the basic agricultural input and its quality is important. If the quality of the seed is poor at the time of sowing, then expenditure of the farmers on the other inputs may become ineffective. It is therefore imperative to have certainty about the quality and viability of the seed before commencing sowing and planting work. Testing the seed viability is therefore required to estimate the number of viable seeds in a seed lot. There is no standard protocol for viability testing and test weight for moringa. Hence, a study were undertaken in Moringa var. PKM 1 to determine the quick test of seed viability and test weight. To conduct Tetrazolium test, removal of perisperm of the seed kernel after soaking in water for 12 h and later soaking in 3, 6, 12 and 24 h in 0.5 and 1.00% concentrations and incubated at 40 ^{O}C temperature. The results revealed that 1% concentration for 12 h soaking showed better staining pattern to distinguish viable and non viable seeds. For the test weight, the optimum test weight (100 seeds) was 30 g.

Keywords: Moringa, seed viability, test weight, concentration and duration

Introduction
Moringa olifera L. is a tropical flowering plant belonging to the family Moringaceae. It is commonly known as the "Tree of life" due to its medicinal and nutritional values such as Fe, Ca, Cu, Mg, and vitamins such as A, C and E. It alleviates the problem of undernourishment among women and children (Anwar *et al*., 2007, Beaulah and Mariappan, 2016) [1, 2]. It is especially adapted to dryness and can withstand several months of drought, owing to its swollen roots that store water. It is a back yard tree commonly seen in villages and is popularly cultivated for its young seed pods, leaves and used as vegetable. The tree is mainly propagated through seeds. Seeds are brown in colour and the seed coat has three conspicuous ridges from which leathery wings are extended for air dispersion. The seed coat acts like a shell and protects a single white colour kernel. Seed kernel has two protective seed coat layers; first layer on kernel is the perisperm and outer hard seed coat with wings. Size of seeds are light in weight and weigh approximately 30 gm for 100 seeds. Understanding the quality of seed is important which has great impact on the planting stock. Seed viability testing is important for separating all the non-viable seeds from viable seeds. Several methods have been used to estimate the viability of seeds. One of the most reliable techniques is the Tetrazolium test, often referred to as the quick viability test. The change in colour of the cotyledon from colourless to red colour is an indication of viable seeds. The concentration of the tetrazolium solution and the duration of treatment varies from species to species based on the seed morphology.
However, there is no defined standard protocol for test weight and viability of moringa. With this background study was conducted to determine the protocol for test weight and viability in moringa.

Materials and Methods
The variety PKM 1 seeds were obtained from Horticultural College & Research Institute, TNAU, Periyakulam used for this study. Standardization of the viability test experiment was conducted to optimize the concentration of Tetrazolium solution and soaking duration. Outer seed coat was removed and seed kernel was soaked for 12 h for pre-conditioning (Sivasubramaniam, 1996) in distilled water.

At the end of the soaking period, the perisperm on the seeds kernel was removed and soaked in two different concentrations of Tetrazolium solution *viz.,* 0.5 and 1.0%, with different durations *viz.,* 3, 6, 12 and 24 h. The treatment was imposed at 40 °C for the activation of enzymes and biochemical reactions. At the end of the treatment, the seeds were washed with water and evaluated for its viability based on the colour intensity and pattern. The seeds with red cotyledons and radicle were classified as viable and the colourless cotyledons with radicle as non-viable seeds (ISTA, 1999) [4].

Statistical Analysis

The data observed were analysed statistically using AGRES software, by the methods described by Panse and Sukhatme (1985) [12]. Whenever necessary value in the percent data was transformed to arcsine transformation and 5% level critical difference was computed.

Standardization of test weight (ISTA, 2015) [3].

One hundred seeds of 11 replications and its mean, standard deviation, and coefficient of variation were computed. According to ISTA the recommended replication group with a coefficient of variation of less than 4.0 percent for non-chaffy seeds and less than 6.0 percent for chaffy seeds for determining the test weight was calculated. Data was analyzed by using the method given by Agrawal and Singh as follows

$$\text{Variance} = \frac{n(\sum x^2) - (\sum x)^2}{n(n-1)}$$

Where

$\sum$ = sum of

x = weight of each replicate in gms

n = number of replicate

$$\text{Standard Deviation (S.D)} = \sqrt{\text{variance}}$$

$$\text{Coefficient of variation} = \frac{\text{S.D}}{\bar{x}} \times 100$$

Where,

$\bar{x}$ = mean weight of 100 seeds

Results and Discussion

Viability Test: The Tetrazolium test is referred as the quick viability test. The advantage of this test is a rapid evaluation of viability, detection of seed weaknesses and timely guidance in quality control programme. Tetrazolium solution is a colourless one and when imbibed by the seed tissues intereferes in the reduction process of living cells and accept hydrogen from dehydrogenase resulting in the production of red stable and non diffusible substance. Triphenyl formazon in the living cells which makes it possible to distinguish the red coloured living parts of the seeds from the colourless dead ones. Water soaked seed kernels exposed to 0.5 and 1.0% tetrazolium solution showed significant differences in staining patterns. Decortication and removal of perisperm allows easier penetration of tetrazolium solution into the seed (Mukta Shrivastava, 2013) [10] kernels soaked in higher concentration showed faster staining pattern than the lower concentration of tetrazolium solution. Staining is a gradual process which required the activation of dehydrogenase enzymes and conversion of tetrazolium to formazon (Fig 1). Staining after 3 h of soaking in 1% showed higher percentage of 32 but it was lower in 0.5% solution (20%). The staining pattern was from pheripheral region to centre. Kernel soaked in 1% took 12 h for complete staining and it was able to distinguish viable and non viable seeds. whereas, kernels soaked in 0.5% solution took 24 h for complete staining (Table 1). The results of the present study are also in line with Jerlin 2004 [6] in pungam; Carvalho *et al.,* 2013 [13] in wheat; Pallavi *et al.,* 2015 [7] in moringa and Mohd. Aslam *et al.,* 2010 in pinus Spp. Bruna *et al.,* 2011 advocated that the tetrazolium test proved to a definite test for viability than germination test which takes days to complete and it is efficient for evaluating the physiological potential.

Table 1: Effect of seed staining on different concentration of TZ solution and soaking duration

Duration of Soaking (h) (D)	Concentration of TZ solution (%) (C)		
	0.50	1.00	Mean
3	20 (26.25)	32 (34.45)	26 (30.65)
6	60 (50.76)	76 (60.66)	68 (55.55)
12	96 (78.46)	100 (89.71)	98 (81.87)
24	100 (89.71)	100 (89.71)	100 (89.71)
Mean	69 (56.16)	77 (61.34)	
	Concentration(C)	Duration(D)	Interaction(CxD)
SEd	0.926	1.309	1.852
CD (0.05)	1.926**	2.776**	3.926**

(Figure in parentheses are arcsine transformation value)

A) Non-viable B) Viable seed

Fig 1: Staining pattern of unviable and viable seeds

Test Weight

Test weight is a varietal character. Finding the test weight of seeds is one-way of determining the optimum weight of submitted and working samples. Navamaniraj (2005) [5] in *Bixa orellana* reported the submitted sample size as 640 g, and the working sample size as 64 g, with a replication requirement for test weight determination of 8 (ISTA).

The study revealed that the coefficient of variations for the replication was within the acceptable limit of 6.0% for moringa (Fig 2). As there was no variation in CV% beyond eight replications, the number of replications for determination of test weight can be fixed as eight as suggested by ISTA. Therefore, the study suggests that the test weight for moringa is 30 g.

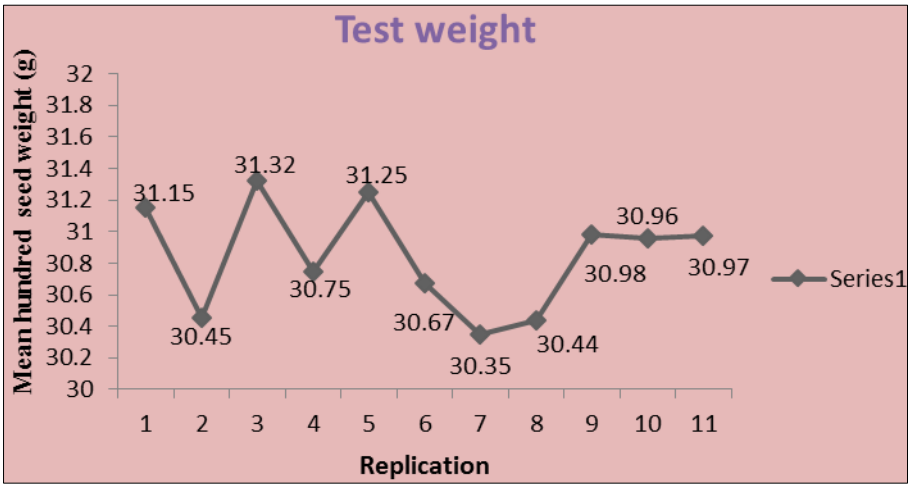

Fig 2: Standardization of Test weight.

Conclusion

It could be concluded that, seed viability can be tested after preconditioning the seeds by removing outer seed coat and inner perisperm layer for easy visualization of staining pattern of seeds. It is recommended that preconditioned seeds should be soaked in 1.0% TZ solution for 12 h at 40 ^{0}C for efficient evaluation of seeds. The number of replications taken for determination of test weight in moringa is 8 and the test weight is 30 g, this helps the seed analyst to determine the test weight and viability.

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