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Standardization of seed testing procedure techniques in Sunnhemp (*Crotalaria juncea*) var ADT 1

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Abstract

This abstract presents a comprehensive overview of seed testing procedure techniques designed explicitly for sunnhemp (*Crotalaria juncea* L.) var ADT 1, an essential leguminous plant exploited for its versatile uses in agriculture and various industries. The seed testing procedure comprises a systematic approach to evaluate the quality, viability and germination potential of sunnhemp seeds, which is crucial for ensuring successful crop establishment and yield optimization. The seed testing procedure was highlighted, viz., test weight determination, sample size estimation, purity assessment, standardization of germination media, temperature requirement for germination, and determination of first and final counts. The experiment results revealed that the submitted sample size could be 700 g. In comparison, the working sample size was 70 g and the minimum standards for the pure seed and the inert matter percentage were 98 and 1.5 %, respectively. In the seed germination test, paper medium with a roll towel registered a maximum germination percentage (96 %) and vigour index (2146). Similarly, 30 °C temperature was registered as the maximum germination percentage. Based on this result, the first and final count was fixed as 3rd and 10th days in sunnhemp var ADT 1. Thus, seed testing procedures contribute to advancement in evaluating sunnhemp seed quality, promoting sustainable agriculture and economic prosperity.

Keywords

grading; germination; physical purity; seed testing; seed quality; viability

Introduction

In recent years, land degradation has been one of the significant problems in agriculture. Land degradation in agriculture leads to a reduction in crop productivity and usability of land. Overuse of synthetic fertilizers and pesticides are the foremost causes of agricultural land degradation (1). To overcome this problem, sustainable farming is the only solution. The main objective of sustainable agriculture is to enrich soil fertility and productivity using green manure crops (2). Green manuring incorporates the crop biomass into the soil and ploughs it, which acts as mulch and serves as a soil amendment. It helps improve soil structure, soil fertility enhancement, amelioration of soil problems, crop yield and quality improvement, pest control, increased population of soil microflora (bacteria, fungi and actinomycetes) and weed control (3). The total area of green manuring in India is

around 1.23 M ha. About 80 % of this land is covered by Uttar Pradesh Andhra Pradesh, Madhya Pradesh, Karnataka, Orissa, Rajasthan, Punjab and West Bengal. Sunnhemp plays an essential role as a multipurpose crop among green manure crops.

Sunnhemp (*Crotalaria juncea* L.) is an annual herbaceous plant in the Fabaceae family. Its origin is India and it thrives well in tropical and subtropical conditions (5) and well-drained soils. Growing up to 3 meters tall, it features bright yellow flowers and narrow leaves. Sunnhemp is known for its rapid growth (6), nitrogen-fixing ability, and soil health improvement (7). It serves as a multipurpose crop (8) used as a green manure, cover crop, fodder and source of fibre. It also plays a vital role in weed suppression (9) and erosion control. Its versatility and ecological benefits make it a valuable asset in agricultural systems.

In sunnhemp, the availability of quality seeds during the growing season is the major problem (10). Seed quality refers to the seeds with essential physical and genetic purity, good physiological soundness and freedom from pests and disease. It involves factors such as germination rate, seedling vigour and genetic integrity, which are crucial for achieving desired crop yields and performance (11). After seed processing, the quality of the seed lot is primarily evaluated through a seed testing procedure. There is no proper seed testing procedure for sunnhemp var ADT 1. Seed quality testing ensures the supply of quality seeds. Therefore, this study was carried out to standardize sunnhemp var ADT 1 seed testing standards.

Materials and Methods

Sunnhemp var ADT 1 (*Crotalaria juncea* L) seeds were obtained from the Department of Seed Science and Technology, Agricultural College and Research Institute, Madurai. The preliminary laboratory analysis was conducted at the Department of Seed Science and Technology, Agricultural College and Research Institute, Madurai. The seeds were used for experiments after proper sterilization.

After a preliminary study, seed testing procedures like test weight standardization, sample size determination, purity analysis, standardization germination media, germination temperature requirement and first count final count standardization were carried out in the sunnhemp var ADT 1 seeds (12-14).

For test weight determination, the mean, standard deviation and coefficient of variation were estimated by categorizing the hundred seed replications into groups of 2, 3, 4 and up to 11. As per ISTA recommendation, for the determination of the test weight of green manure seeds, the replication group which had a coefficient of variation less than 4.0 % was recommended

For sample size estimation, sunnhemp seeds were subjected to mechanical divider to confirm that the entire seed lot's sample composition was homogeneous, with no bias introduced during dividing. From the samples, 2500 seeds were counted in four replicates for determining the working sample size based on standard error (15). The submitted sample size was calculated by multiplying the working sample sizes by 10. The mean of two sample size were given in grams.

In purity analysis, the seed were separated into pure seed, inert matter and other crop seed. The given formula calculated the percentage of pure seed, inert matter and other crop seeds in Equation 1-2.

$$\text{Pure seed (\%)} = \frac{\text{Weight of pure seed}}{\text{Total wt. of working sample}} \times 100 \quad \text{.....(Eqn. 1.)}$$

$$\text{Inert matter (\%)} = \frac{\text{Weight of inert matter}}{\text{Total wt. of working sample}} \times 100 \quad \text{.....(Eqn. 2.)}$$

Other crop seeds were expressed in number per kilogram

In the germination test, the seeds were sown in different media (roll towel, sand) and placed in a germination room maintained at 25 °C ± 1 °C and 95 ± 2 % relative humidity. The physiological parameters determined the best method.

Sixteen replicates of 25 seeds were sown in four distinct temperatures, *viz*., 15, 20, 25 and 30 °C and maintained for 7 days. Germination percentage was estimated in a given distinct temperature to standardize the germination temperature. Determination of the first count and the final count was carried out based on the first count and final count standardization (15).

Statistical Analysis

The interpreted data were analyzed statistically using AGRES software and standard methods (16). The value in the percentage data was transformed into arcsine value and a 5 % level critical difference was computed.

Results and Discussion

Seed testing is essential for ensuring the quality and performance of seeds. It validates the purity, viability and germination rates, improving crop yields and economic efficiency. ISTA is an international seed testing association responsible for prescribing seed testing procedures. Test weight is an essential varietal character that varies from crop to crop and variety. Based on the test weight, the submitted and working sample size was determined in seed testing. In this experiment, from the given data (Table 1), the mean hundred seed weight was statistically non-significant. It varied between 2.744 g to 2.809 g, with an overall mean value of 2.772 g. The coefficient of variation ranged from 0.73 to 2.96 %, with an overall mean value of 2.47 %, which does not exceed 4% (Table 1).

The overall mean coefficient variation was less than the acceptable limit of 4 %. Further, there was no much variation in CV % beyond eight replications (R_8). Therefore, the number of replications for test weight determination

Table 1. Standardization of the number of replications for determination of test weight in sunnhemp var ADT 1

S.No.	Replication	Mean hundred seed weight (g)	SEd	CV%
1	Replication-2	2.788	0.0167	0.74
2	Replication-3	2.744	0.0165	0.73
3	Replication-4	2.757	0.0579	2.56
4	Replication-5	2.777	0.0590	2.59
5	Replication-6	2.792	0.0483	2.13
6	Replication-7	2.764	0.0494	2.18
7	Replication-8	2.809	0.0477	2.10
8	Replication-9	2.803	0.0661	2.93
9	Replication-10	2.798	0.0670	2.96
10	Replication-11	2.800	0.0663	2.94
$\overline{x}$	Mean	2.772	0.0562	2.47

SEd- Standard Error; **CV**- Coefficient of variation; ******- Highly Significant at 5 % levels; **NS**- Non Significant.

can be fixed as eight replications per ISTA recommendation. Similar findings were reported in *Bixa orellana* and *Moringa oleifera,* respectively.

According to the Seed Act 1966, quality seed has to meet the minimum seed testing standards. The seed testing should be uniform, comparatively stable and economical with a shorter duration for result declaration (18). Seed testing starts with sample submission, which evaluates the various seed quality attributes like germination, viability, physical purity, vigor and termination with result declaration. For proper seed testing, the uniform size for submitted and working samples has to be standardized. These sizes differed according to crop size. As per ISTA recommendation, the weight of 2500 pure form of seed was taken as a working sample and 10 times the size of the working sample was taken as the submitted sample as the additional quantity of seed was required for moisture estimation and as a guard sample.

The parameters working and submitted samples were statistically non-significant. The working sample weight ranged from 68 g to 72 g (weight of 2500 seeds). Subsequently, the submitted sample weight ranged from 686 g to 724 g. The overall mean values for the working and submitted samples were 70 g and 704 g, respectively (Table 2). In sunnhemp, the working sample size ranges from 68 g to 72 g with a mean value of 70 g and the submitted sample size varies from 686 g to 724 g and its overall mean value 704. The same trend in working and submitted samples was observed in *Trichosanthes tricuspidata*, *Embelia Tsjeriam*-cottam and *Moringa oleifera* (12,19).

Purity analysis in seed testing involves assessing various factors to determine the composition and quality of a seed lot. Purity analysis is the initial test in seed testing. These analyzed seeds were used for further seed testing procedures. This test separated seeds into three components: pure seed, inert matter and other crop seeds (15). In this experiment, the physical purity analysis was statistically non-significant. Pure seed percent ranged from 99.4 % to 97.2 % with 98 % of the overall mean; subsequently, the inert matter percentage varied from 1.2 % to 1.8 % with 1.5 % overall mean. No other crop seeds were registered (Table 2). From the analysis, pure seed percent ranges from 97 to 99 % with 98 % overall mean value. Inert matter percentage ranges from 0.6 to 1 % with 1.5 %. Similar results were reported in *Bixa orellana* and cow pea (19–20).

In germination test, germination media is the basic requirement. Germination media were the substances in which seeds are placed and observed the germination. The choice of germination media depends on various factors such as the seed size being tested, their specific germination requirements and the test's purpose. The most commonly recommended germination media were paper, sand, vermiculite, etc. Among them, germination paper is the most popular media (15). Germination media significantly influenced the seed physiological parameters. Maximum germination percent (96 %), root length (8.89 cm), shoot length (13.46 cm), Dry matter production (0.10 g) and vigor index (2146) was recorded in roll towel medium followed by sand medium germination percent (92 %), root length (5.77 cm), shoot length (10.50 cm), Dry matter production (0.08 g) and vigor index (1497) (Table 3). The better performance of the roll towel method was due to the availability of enough space and easy seedling emergence. The poor germination in sand media was due to the depth and obstacles that restricted the seeds' germination. The following researchers reported roll towel (top of paper) media as superior in *Parkia timoriana* and in tobacco (21**–**22).

Table 2. Standardization of submitted sample size, working sample size and purity analysis for Sunnhemp Var ADT 1

Replication	Sample size (gram)		Standards for purity analysis (%)		
	Weight of working sample size (WS) 2500 seeds (gram)	Submitted sample size (g) (WS × 10)	Pure seed (%)	Inert matter (%)	Other crop seed
1	69	694	99.2	0.8	-
2	70	702	98.8	1.2	-
3	72	724	99.3	0.7	-
4	71	715	98.9	1.1	-
5	68	686	99.4	0.6	-
Mean	70	704	99.12	0.88	-
SEd	1.29	14.85	2.21	0.025	-
CD	NS	NS	NS	NS	-

SEd- Standard Error; **CD**- Critical Difference; ******- Highly Significant at 5 % levels; **NS**- Non Significant.

Table 3. Standardization of germination media for Sunnhemp Var ADT 1

Media	Germination (%)	Abnormal seedling (%)	Hard seed (%)	Dead (%)	Root Length (cm)	Shoot length (cm)	Dry matter Production (g/10 seedlings)	Vigour index
Roll towel (M_1)	96 (78.46)	1 (5.73)	1 (5.73)	(8.13)	8.89	13.46	0.10	2146
Sand (M_2)	92 (73.57)	2 (8.13)	2 (8.13)	4 (11.53)	5.77	10.50	0.08	1497
Pleated (M_3)	86 (68.02)	5 (12.92)	2 (8.13)	7 (15.34)	4.64	7.87	0.07	1076
Mean	91 (72.54)	3 (9.97)	2 (8.13)	5 (12.92)	6.43	10.61	0.08	2158
SEd	1.515	0.025	0.039	0.072	0.191	0.213	0.002	32.954
CD	3.708**	0.059**	0.095**	0.177**	0.467**	0.522**	0.005**	80.640**

SEd- Standard Error; **CD**- Critical Difference; ******- Highly Significant at 5 % levels; **NS**- Non Significant

Temperature is a dynamic factor. Low temperature inhibits the enzymatic activity and prevents germination, while high temperature can regulate the germination process (20). Germination temperature was a crucial factor that directly influenced the accuracy and reliability of the germination test. The seed testing laboratory conducts the germination test at a constant temperature (15). In this experiment, four different temperatures were used *viz.*, 15 °C, 20 °C, 25°C and 30°C. The germination temperature made a significant variation in seed germination percentage. Higher germination percentage (94 %) was registered at 30 °C (Fig. 1.), followed by 25 ˚C (73%) germination. No germination was found at 15°C (Fig. 2.). In this experiment, low temperature (15°C) inhibited the germination; subsequently, high temperature (30°C) promoted germination in sunnhemp var ADT 1. This was because the low temperature and critically higher temperature will affect the metabolic rate in the pathway, which was essential for onset of germination. Temperature may influence the initial phase of seed water uptake or the subsequent metabolic processes resulting in cell multiplication. The results were similar to the previous reports recorded in beans, maize and rapeseed (23–25).

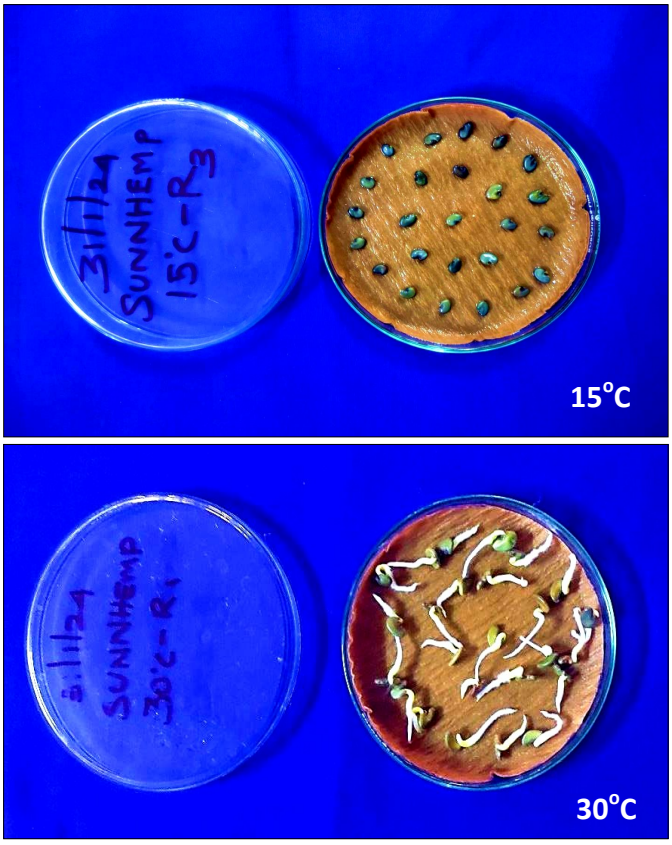

Fig. 1. Standardization of germination temperature in sunnhemp var ADT 1.

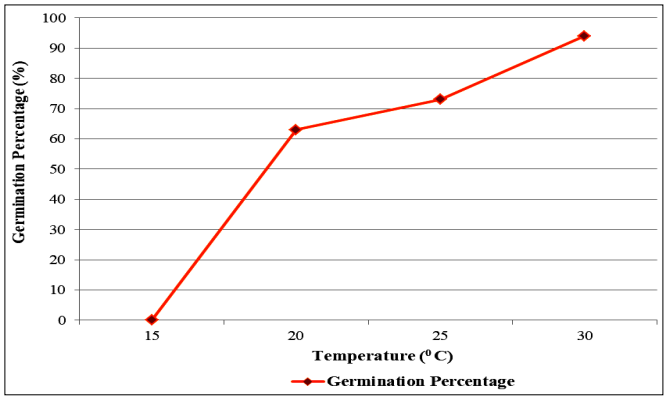

Fig. 2. Standardization of temperature requirement for seed germination test in Sunnhemp ADT 1.

In the germination test, the critical component is the seedling evaluation, first count and final count. Days taken by the seed for 50 % emergence is considered the first count and the sustainability of autotropic ability of the emerging seedling is fixed as the final count in Kalmegh (14). This experiment observed significant results on seedlings' physiological characteristics due to germination duration. The radicle emergence was noticed on the 2nd day after sowing and the seedlings produced all the essential structures on 3rd day and it reached its maximum growth with the fullest expression of germination on the 10th day for sunnhemp. Further, the seedling height or dry matter accumulation did not increase. Beyond the 10th day, mortality of seedlings occurred. Based on this result, the first and final count was fixed as the 3rd and 10th days in sunnhemp var ADT 1 (Fig. 3–4).

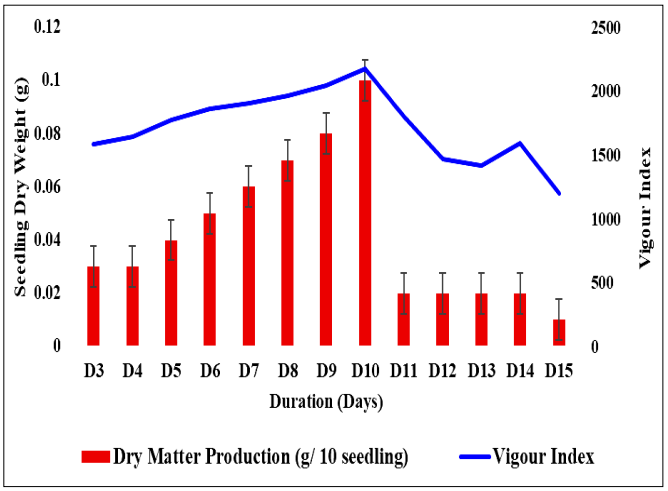

Fig. 3. Determination of days to final count on seedling characters for sunnhemp var ADT 1.

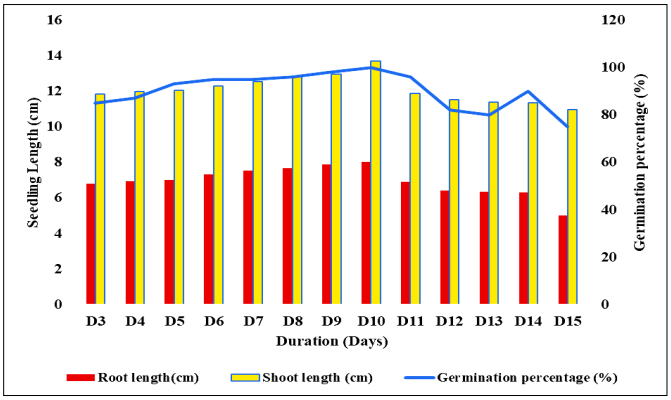

Fig. 4. Determination of days to final count on dry matter production (per 10 seedlings) and vigour index for sunnhemp var ADT 1.

Conclusion

It could be concluded that the seed testing procedure standardized for sunnhemp was a sample size of 70 g and a submitted sample size of 700 g. The pure seed was 98 % and inert matter was 2 %. Roll towel media for germination test and temperature for germination could be 30 °C. For seedling evaluation, the 3rd and 10th days were fixed as the first and final counts.

Authors' contributions

All the authors have contributed equally to data collection, analysis, writing the original manuscript draft, editing and reviewing.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None

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