Introduction to Seed Testing

(Methodology and Terminology)

The main purposes of seed testing are to:

- Study the physiological quality of seed
- Study seed procurement of the species

Seed tests are based on the standards recommended by International Seed Testing Association (ISTA) and include:

- Purity
- Seed weight
- Moisture content
- Viability

■ **Purity** is the composition by weight of pure seed in a sample. **Seed samples** must be carefully prepared in order to **represent the whole seed lot**. Sub-samples should be drawn from 2-3 different places within the seed lot, mixed thoroughly, and then divided into test samples of about 2,500 seeds.

Methodology:

-Weigh the sample

-Separate the pure from impure seed, using the following criteria listed in Table 1.

Table 1 – Seed Purity Criteria

Pure Seed	Impure Seed
Mature seed	Seed of other species
Undamaged seed	Stones, leaves, twigs, etc
Undersized seed	Seed wings
Pieces of seed more than half original size	Pieces of seed less than half original size
Shriveled seed	Legume seed without seed coat
Immature seed	
Germinated seed	

-Weigh the pure fraction and calculate to a percentage using the formula below.

$$\textbf{Purity\%} = \frac{\textbf{Weight of pure seed}}{\textbf{Total weight of sample}} \times 100$$

Purity affects the number of pure seeds per kilogram, and therefore, the price and quantity requirements for sowing

Purity is affected by the level of seed extraction.

Purity is needed before seed sales; before seed storage; during seed processing

■ **Seed weight** refers the **weight of 1,000 seeds** according to ISTA. However, in the market, seed weight refers to the **amount seed per kilogram.**

Methodology:

-Weigh 100 seeds for 8 replicates, separately

-Calculate the average weight of 100 seeds, and multiply by 10

1000 seeds weight= 100 seeds weight x 10

- Determine the calculation accuracy through statistic analysis using the formula below

$$\textbf{Coefficient of variation (must be less than 4)} = \frac{\textbf{100 x largest different between replicates}}{\textbf{2.85 x average of 100 seeds}}$$

-The seed weight can be used to calculate the amount of seed per kilogram, as follows:

$$\textbf{Amount of seed per kilogram} = \frac{\textbf{1,000,000}}{\textbf{Average weight of 1,000 seeds}}$$

Seed weight affects	seed quality (big seed produces healthy seedling)
Seed weight is affected by	seed size; moisture content; degree of processing.
Seed weight is needed	before sale; before storage

■ **Moisture content** represents the percentage of water in seed, and can be determined through two methods.

1) Direct method

Using this method, water is removed from seed by heating in an oven, and the lost weight measured. This method is also known as the 'oven method'.

Methodology

-Weigh 2 empty containers.

-Drop seed into containers and weigh (at least 5g for small seed, and at least 10g for large seed). The weight of the container is not included

-Cut seeds into small pieces

-Dry seed in the oven at ± 103°C for ± 17 hours

-Allow the samples to cool in an incubator for 15 minutes

-Weigh dry sample and calculate the moisture content as follows:

$$\textbf{Moisture content (\%)} = \frac{\textbf{Weight of fresh sample – Weight of dry sample}}{\textbf{Weight of fresh sample}} \textbf{ x 100}$$

-To ensure accuracy, statistical analysis has determined that the largest difference between replicates is as illustrated below.

1000 seed weight	**Average moisture content**		
	< 12%	**12%-25%**	**>25%**
<200g	0.3%	0.5%	0.5
>200g	0.4%	0.8%	2.5%

Moisture content affects	seed weight; seed storage
Moisture content is affected by	the degree of maturity; the degree of drying; the degree of processing; storage conditions
Moisture content is needed	before collection (to determine degree of maturity); before processing (to determine the need for processing or further drying) after processing (to determine need for further drying) before testing; before storage; before sales

2) Indirect method

This method mostly relies on the use of a moisture meter that reflects moisture content. However, calibration curves for each species are needed, and accuracy is not always satisfactory. This method is not used in the CTSP seed lab.

■**Viability** is tested through a 3 step process.

1) **Cutting test**

-Prepare a sample of 100 randomly collected seeds

-Cut along the side of each seed, and if the embryo part is fresh, the seed is regarded as viable

-Calculate the percentage of viable seed as follows:

$$\textbf{Viability} = \frac{\textbf{Fresh seed}}{\textbf{Total cut seed}}$$

Cutting test affects	germination rate evaluation; seed collection; maturity assessment.
Cutting test is effected by	insect infection; yield of fruit and seed, weather conditions and pollination status.
Cutting test is needed	before maturity degree evaluation; before collection; before testing; before storage

2) **Pre treatment tests** need to be applied before sowing to gain maximum germination

Pre treatment affects	germination degree; speed; and uniformity (seeds germinate at the same time)
Pre treatment is affected by	seed viability; method of pretreatment; medium (moisture, status of infection); conditions (light, temperature)
Pre treatment is needed	when germination for a species is unknown; For species difficult to germinate in normal conditions

3) **Germination tests** determine viability under optimum conditions, where optimal conditions refers to best pre-treatment; sufficient moisture; optimum temperature (about 25°C), and most suitable medium (without pest and fungi infection)

Methodology

-Sow 4 replicates, each with 100 seeds (for big and medium seed), or 0.1-0.5g for very small seed

-Count the germinated seed (seed with root growth about twice the diameter of the seed), and stop when

$$\textbf{Germination (\%)} = \frac{\textbf{Total germination in all replicates}}{\textbf{Number of replicates}}$$

Germination affects	assessments of seed viability
Germination is affected by	genetics; degree of maturity at the time of seed collection; processing; insect and fungi infection; age of the mother tree; pre treatment; storage; germination conditions (medium, light, temperature water supply)
Germination tests are needed	during storage; before sale;

The **medium** can be sand (the preferred medium within the CTSP seed lab); top of paper; or between paper.

The seed of some species is structured in such a way that prevents germination under normal conditions, a state known as **dormancy.** Dormancy can be broken before seed is sown through methods appropriate to the type of dormancy:

-*Embryo dormancy*	After the fruit and seed are mature and collected, the embryo is still not fully developed for germination
-*Mechanical dormancy*	The fruit or seed obstructs the embryo from taking up water for development and germination
-*Physical dormancy*	The fruit or seed obstructs the embryo from absorbing water from outside, even if the seed itself is soaking in the water
-*Chemical dormancy*	Normally this affects fleshy fruit where the seed is surrounded by a chemical element such as sugar, which obstructs contact between the seed and water and sometimes blocks germination
-*Photo dormancy*	Seed requires a suitable light regime for germination
-*Thermo dormancy*	Seed requires a specific temperature for germination

■**Desiccation** is a method for removing moisture from seed, specifically as a possible method to allow storage of seed of recalcitrant species. Usually, silica gel is used to absorb the moisture to avoid the impact of temperature on seed viability. The target moisture content is determined by seed weight according to the following formula:

$$TW = \frac{100\text{-}IMC}{100\text{-}TMC} \times IW$$

Where:

TW	Target weight of seed at identified moisture content
IMC	Initial moisture content
TMC	Target moisture content
IW	Initial weight of seed

Normally, target moisture contents are identified according to the initial moisture of seed. Target moisture contents for desiccation trials are calculated as follows:

Initial moisture content (IMC)	**Target moisture content (TMC)**
≤10%	9%. 6%. 3%
11%-15%	12%, 9%. 6%. 3%
16%-20%	15%, 12%, 9%. 6%. 3%
21%-25%	20%, 15%, 12%, 9%. 6%.
26%-30%	25%, 20%, 15%, 12%, 9%. 6%
31%-35%	30%, 25%, 20%, 15%, 10%, 5%
36%-40%	35%, 30%, 25%, 20%,10%, 5%
41%-45%	40%,35%, 30%, 20%,10%,5%
45%-50%	45%, 40%, 35%, 25%, 15%, 8%
51%-55%	50%, 45%, 40%, 35%, 25%, 10%
56%-60%	55%, 50%, 45%, 35%, 25%, 10%
≥60%	60%, 50%, 40%, 30%, 20%, 10%