

Seed Germination, Purity and Tetrazolium Methods for Malabar Spinach (*Basella spp.* L).



Region: 3



STRF funded project

(Developed and Validated Test Method AOSA/SCST/ISTA for the *Basella spp.*-Malabar Spinach)

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Disclaimer

The Research views, material, content of this presentation is that of presenter based on the fact of the findings with evidence (*literature) to support.

Objective of the study:

Addition of seed quality test methods on Malabar spinach (*Basella alba* spp.) to the AOSA/SCST for testing seeds.

- I. Addition of Pure Seed Unit (PSU) for *Basella alba* L. spp.
- II. Standard germination method to *Basella alba* L. spp.
- III. Tetrazolium Test procedure methods to *Basella alba* L. spp.



Introduction

Basella or **vine spinach/summer spinach** is a popular versatile leafy-green vegetable

- ✓ Malabar spinach cultivars are (***Basella alba* L. and *Basella rubra* L**) are popular in some of the tropical & temperate climates of America, Australia, and Europe, and are known for their lush, nutritious greens, and tender stems in addition to abundant **vitamin C** compared to English spinach.
- ✓ Originated in the monsoon tropical regions of Malabar Coast of India and Sri Lanka.
- ✓ It is revered in some East Asian cultures for its wholesome phytonutrient profile.
- ✓ They impart a delicious crunch to a dish.



*<https://www.epicgardening.com/malabar-spinach>

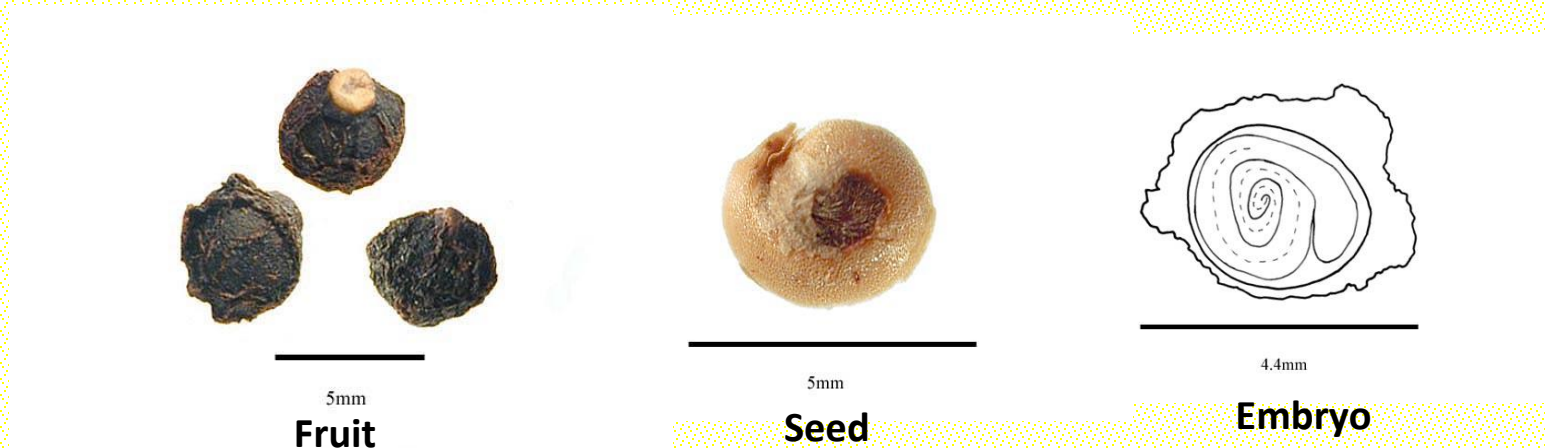
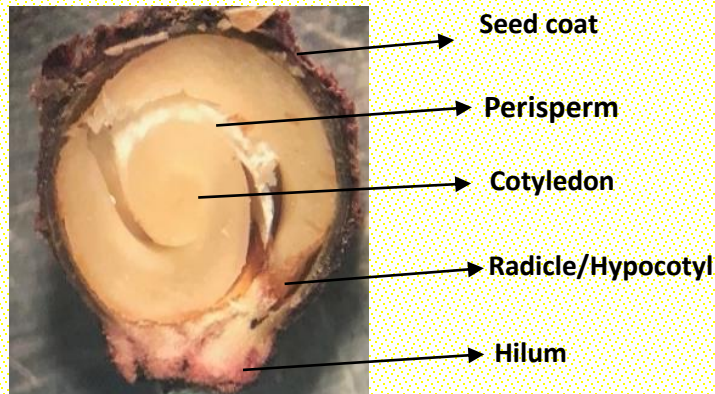
- ✓ In Thailand, they are added to green curries alongside other garnishes like shredded basil.
- ✓ There are **no prescribed standard methods for testing Malabar seed (*Basella spp.*)** in the **AOSA** and **ISTA** rules.

Seed anatomy of *Basella* spp. L.

Succulent and viney plants with small flowers and a single seeded fruit surrounded by the persistent perianth.

Seed unit: Single seeded / a dried fleshy one-seeded fruit including accessory structures (perianth) or the actual seed

PSU- The half seed rule is the operating principle



Seed globular; testa membranous; endosperm copious; embryo spirally twisted or semicircular to horseshoe-shaped.

Morphological differences between two varieties of *Basella alba* L. species

Green variety

Basella alba L - Green Spinach



potted
plants

shoot-tops at
edible stage

adaxial abaxial flower

Red variety

Basella rubra L. Red Spinach



potted
plants

shoot-tops at
edible stage



adaxial abaxial flower


Nutritional Value

- ✓ Flowers contain vitamin A, vitamin C, calcium and iron
- ✓ Anthocyanin, the natural pigment that has antioxidant properties.
- ✓ known to have wound-healing effects and is an antibacterial and anti-inflammatory.

Reference for classification of order Caryophyllales-Pure seed Unit/ TZ study

Classification | USDA PLANTS Page 1 of 1

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Classification for Kingdom **Plantae** Down to Order **Caryophyllales**

Click on names to expand them, and on P for PLANTS profiles.

Up to the Kingdom

Kingdom **Plantae** - Plants
Subkingdom **Tracheobionta** - Vascular plants
Superdivision **Spermatophyta** - Seed plants
Division **Magnoliophyta** - Flowering plants
Class **Magnoliopsida** - Dicotyledons
Subclass **Caryophyllidae**
Order **Caryophyllales**

Contains 11 Families and 1873 accepted taxa overall

Down one level

- Family **Achatocarpaceae** - Achatocarpus family
- Family **Alzooaceae** - Fig-marigold family
- Family **Amaranthaceae** - Amaranth family
- Family **Basellaceae** - Basella family
- Family **Cactaceae** - Cactus family
- Family **Caryophyllaceae** - Pink family
- Family **Chenopodiaceae** - Goosefoot family
- Family **Molluginaceae** - Carpet-weed family
- Family **Nyctaginaceae** - Four o'clock family
- Family **Phytolaccaceae** - Pokeweed family
- Family **Portulacaceae** - Purslane family

Enter a scientific or common name at any rank. The report will display the kingdom and all descendants leading down to the name you choose.

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Basella alba spp. Classification- as a variety -*Basella alba* L. *Basella rubra* L.

Five species: Family Basellaceae

Basella spp. has long been cultivated as a vegetable and an ornamental.

The juice is harmless and used as a food coloring.

The plants are used for medicinal purposes

1 Endemic to tropical Africa,

3-Endemic to Madagascar,

1-Pantropical



Report data for sql:					
Taxon	Taxon Name Authority	Taxonomy Species ID	Current Taxonomy Species ID	Current Taxon	Current Taxon Authority
Basella alba	L.	6531			
Basella cordifolia	Lam.	6532	6531	Basella alba	L.
Basella rubra	L.	6533	6531	Basella alba	L.
Basella tuberosa	(Caldas) Kunth	6534	40814	Ullucus tuberosus	Caldas
Basella vesicaria	Lam.	413898	413896	Anredera vesicaria	(Lam.) C. F. Gaertn.

Plant taxonomy or classification is the science of naming organisms and placing them in a hierarchical structure, each level being given a name (e.g., kingdom, division (phylum), class, order, family, genus, species). Taxonomic units at a given level are termed taxa (singular taxon). Names of higher order taxa (e.g., kingdom, phylum, class, order, family, genus) are uninominal (i.e., each name is a single word). Names of species are binomial (e.g., *Magnolia virginiana*), and names of taxa below the rank of species (e.g., subspecies, varieties) are comprised of three or more words (e.g., *Panicum virgatum* var. *cubense*). Any given organism can be classified throughout the hierarchy. For example, the species sweet bay magnolia (*Magnolia virginiana*) is in the genus *Magnolia*, the family Magnoliaceae, the order Magnoliales, the class Magnoliopsida, the division Magnoliophyta, and the kingdom

[Reference- *Images \(ars-grin.gov\)](#)

[* Reference -Plant Classification | NRCS Plant Materials Program \(usda.gov\)](#)

Biology and Ecology

Genetics

- There are no reports of hybrids in cultivation or in the wild for *B. alba* ([PIER, 2017](#)).
- The chromosome numbers reported for the species are **2n=44,48** ([PROTA, 2017](#)).
- Germplasm resources are available at The World Vegetable Center and at USDA-ARS facilities ([Hughes and Ebert, 2013](#); [USDA-ARS, 2017](#)).
- **DNA barcodes** are available for this species at Barcode of Life Data System ([BOLDS, 2017](#)).
- The species is reported by [Ganashan et al. \(1996\)](#) as showing high genetic diversity in Sri Lanka.

Reproductive Biology

- *Basella alba* reproduces by seeds and vegetatively by stem cuttings and rhizomes
- Pollination is cleistogamous although some pollination by short-tongued insects is reported
- No breeding programmes are known to exist, although several seed companies in India and the United States produce seeds to be sold locally and internationally ([PROTA, 2017](#)).

Physiology and Phenology

Basella alba will not flower if the length of daylight is more than 13 hours per day ([Useful Tropical Plants, 2017](#)).

It is reported as flowering from May to September and fruiting from July to August ([ZipcodeZoo, 2017](#)).

The seeds require temperatures of **18 - 21°C and 10-21 days to germinate**. Pre-soaking the seeds for 24 hours in warm water will shorten the germination time. The species can produce mature fruits within 70 days of planting in warm climates ([PIER, 2017](#)).

Longevity

- *Basella alba* is a perennial vine that is variously reported as being short and long lived .
- If not taken care of, it tends to die back after **two or more years**.

[*Ref:Basella alba \(malabar spinach\) \(cabi.org\)](#)

Distribution

Taxon: *Basella alba* L.

[Nomenclature](#)

[Common Names](#)

[Distribution](#)

[Economic Uses](#)

Distribution

[Exportable format](#)

Cultivated

Africa

EAST TROPICAL AFRICA: **Kenya, Tanzania, Uganda**
WEST TROPICAL AFRICA: **Benin, Côte D'Ivoire, Nigeria, Sierra Leone, Togo**
SOUTH TROPICAL AFRICA: **Malawi, Zambia**

Asia-Temperate

CHINA: **China**
EASTERN ASIA: **Japan**

Asia-Tropical

INDIAN SUBCONTINENT: **Sri Lanka, Nepal, Pakistan**
INDO-CHINA: **Cambodia, Laos, Thailand, Vietnam**
MALESIA: **Indonesia** [Jawa], **Malaysia, Philippines, Singapore**

Pacific

NORTH-CENTRAL PACIFIC: **United States** [Hawaii, United States Minor Outlying Islands]
SOUTH-CENTRAL PACIFIC: **French Polynesia**

Southern America

CARIBBEAN: **Barbados, St. Lucia**
CENTRAL AMERICA: **Guatemala, Panama**
NORTHERN SOUTH AMERICA: **French Guiana, Guyana, Suriname**
WESTERN SOUTH AMERICA: **Colombia, Ecuador, Peru**

Naturalized

Africa

NORTHEAST TROPICAL AFRICA: **Ethiopia, Sudan**
EAST TROPICAL AFRICA: **Kenya, Tanzania, Uganda**
WEST-CENTRAL TROPICAL AFRICA: **Burundi, Cameroon, Democratic Republic of the Congo, Congo, Rwanda**
SOUTH TROPICAL AFRICA: **Angola, Mozambique, Malawi, Zambia**

Asia-Temperate

CHINA: **China**
EASTERN ASIA: **Taiwan**

Asia-Tropical

INDIAN SUBCONTINENT: **Sri Lanka**
PAPUASIA: **New Guinea**
INDO-CHINA: **Cambodia, Laos, Myanmar, Thailand, Vietnam**
MALESIA: **Borneo, Indonesia** [Jawa, Sumatera], **Philippines**

Pacific

NORTH-CENTRAL PACIFIC: **United States** [Hawaii, United States Minor Outlying Islands]
NORTHWESTERN PACIFIC: **Palau**
SOUTH-CENTRAL PACIFIC: **French Polynesia**

Southern America

CARIBBEAN: **Barbados, St. Lucia**
CENTRAL AMERICA: **Panama**
NORTHERN SOUTH AMERICA: **French Guiana, Guyana, Suriname**
BRAZIL: **Brazil**
WESTERN SOUTH AMERICA: **Colombia, Ecuador, Peru**

Other (probable origin paleotropics)

Reference –Volume 3 classification



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Taxon: *Basella alba* L.

Nomenclature

Common Names

Distribution

Economic Uses

Economic Uses

Environmental

ornamental – [Reference\(s\)](#)

Food additives

coloring – [Reference\(s\)](#)

Human food

vegetable – [Reference\(s\)](#)

Medicines

folklore – [Reference\(s\)](#)

Materials and Methods

- 1. Malabar Spinach- two seed varieties viz., **Green variety** vs **Red variety**
- 2. Eight seed lots used for the experiment

Kitazawa seeds-CA



Green variety

Red variety

Burpee seeds-PA

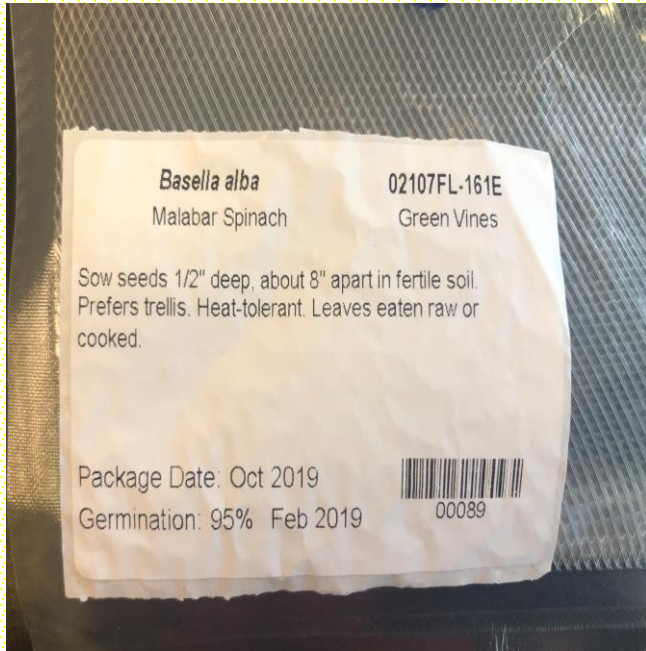


Green variety

Red variety

Eight seed lots used for the experiment

Seed Eco Bank -Florida

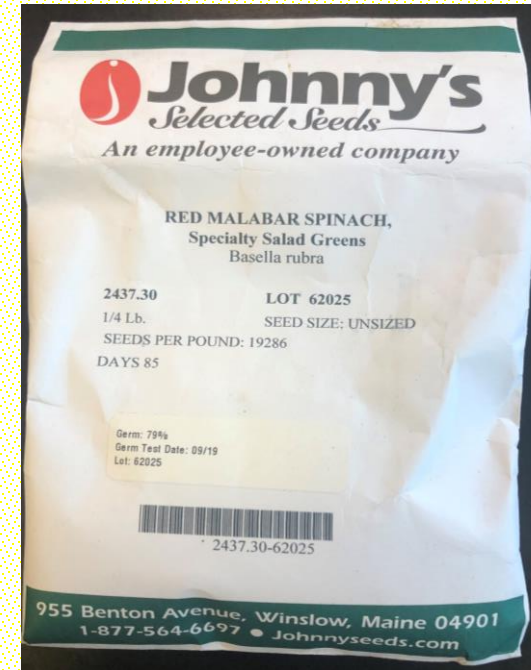


Green variety



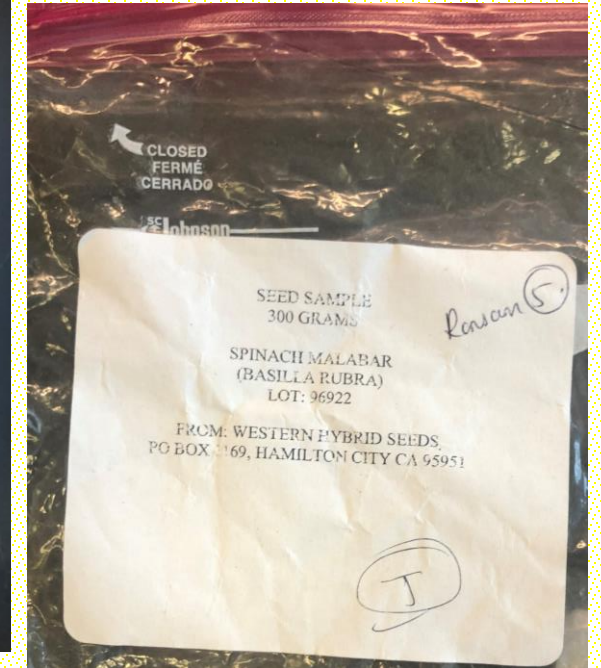
Green variety

North Carolina



Red variety

California



Red variety

Experimental Design

Statistical method : RCBD with three factorial design
(**ISTA: Multi laboratory validated test method**)

1. Labs – Total 7 Labs (L)

A Ransom Seed Lab, California

B. Federal Seed lab, Gastonia, NC

C. North Carolina State Seed Lab, NC

D. Wisconsin crop Improvement seed lab, WI

E. Oregon state university seed lab, OR

F. CFIA, Saskatoon Canada

G. Pennsylvania, Department of Agriculture, Seed lab, Harrisburg,17110

2. Seed Samples-Total 8 seed samples (S) Green samples (1-4) and Red samples (1-4)

3. Varieties (V) – Total 2 seed varieties viz., green and red stem

Referee/validation study on Malabar Spinach Test 2020-2021

I. Eight seed lots of Malabar spinach seeds were collected and referee testing conducted involving six (7) AOSA/SCST seed lab accredited by AOSA/SCST/ISTA.

The parameters collected for the study were Germination(%), 100 seed weight(g) and Tetrazolium Test (%).

II. Following seed labs were sent the referee sample of Malabar Spinach in the month of September-2020.

- A Ransom Seed Lab, California
- B. Federal Seed lab, Gastonia, NC
- C. North Carolina State Seed Lab
- D. Wisconsin crop Improvement seed lab
- E. Oregon state university seed lab, OR
- F. CFIA, Saskatoon Canada
- G. Pennsylvania, Department of Agriculture, Seed lab, Harrisburg,17110

III. The referee/validation data report on germination, 100 seed weight, Tetrazolium testing results were received during the first week of **February-2021.**

Response Sheet

Lab#: _____ Date of sample received:Date of the completed test:.....

- 1 Name and address of the participating lab
2. How many years have you been in seed testing?
3. Are you certified Seed Analyst? If yes, germination, purity, or both? If yes, how many years?
4. Are you a registered seed technologist? If yes, how many years?
5. How often do you test Malabar spinach seeds for Germination, Tetrazolium test or both?

i) Please provide the following information:

Yes	No	
		I do have experience in testing >50 <i>Basella</i> spp. samples by TZ.
		I have experience in testing < 50 <i>Basella</i> spp. samples by TZ.
		I have never tested any <i>Basella</i> spp. samples by TZ before.
Comment:		

6. Complete the report of the purity sample weight per grams of eight 100 pure seed units, germination and TZ study on *Basella alba* and *Basella rubra* in the enclosed Excel sheets (Table 1, Table 2 and Table 3).
7. Any comments/ suggestions, you would like to share while conducting the germination and/or TZ referee on Malabar Spinach is highly appreciated.

Thank you for your participation in the referee. Conducting this referee is tedious and time-consuming work, so I am very grateful for your kind participation and sharing the results of this referee at the earliest (within 45 days after the receipt of this sample).

If you have any question. Suggestions or comments please feel free to contact me ranantared@pa.gov, cell number-6105745284

Please email the results- ranantared@pa.gov

Referee Test plan on Malabar Spinach-Germination test

a) Name and address of the test organizer: Dr. Roopa Anantareddy,

Pennsylvania Department of Agriculture

Bureau of Plant Industry 2301 North Cameron Street

Harrisburg, PA 17110 Phone: 717.772.5230| Cell: 610.574.5284

i) Introduction of the species: *Basella* or **vine spinach** is a popular versatile leafy-green vegetable that originated in the monsoon fed tropical regions of Malabar Coast of India and Sri Lanka. It is revered in some East Asian cultures for its wholesome phytonutrient profile. Malabar spinach ***Basella alba* and *Basella rubra*** cultivars are slowly gaining popularity in some of the tropical and temperate climates of America, Australia, and Europe, and are known for their lush, nutritious greens, and tender stems in addition to abundant **vitamin C** compared to English spinach.

ii) Scope of the method: There are no prescribed standard methods for testing Malabar seed (*Basella spp.*) in the AOSA and ISTA rules. Therefore, referee study on seed germination, tetrazolium studies along with purity analysis for this crop species will attempt to provide supporting evidence for standardizing the seed quality testing procedure for this crop.

Preliminary studies revealed the following germination method best for *Basella Species*.

S No.	Species	Varieties	Substrate	Temperature(°C)	First count (d)	Final count (d)
1	<i>Basella Species</i>	Red stem	RT	20-30 light	7	21
2		Green stem			7	21

iii) Test protocol to be followed:

Total of eight samples are sent for referee test. Each sample variety of the seeds (400) should be planted in rolled towel (50 seeds x 8 replicates) at 20-30°C light and evaluated for first count at 7 days for respective variety seed. Note down the intermediate count on 14 days in separate column in excel sheet. Finally, record the final normal seedlings that are counted for each of the varieties at 21 days along with number of abnormal and dead seeds in the excel sheet.

Chronological order of the procedure performed by referee participated labs

PSU → Germination → Tetrazolium test

Conduct the referee test (seed count, germination, Tetrazolium test) for two varieties of Malabar Spinach (Green stem variety-coded seed lots 1-IV, Red stem variety- coded seed lots V-VIII) by following three steps.

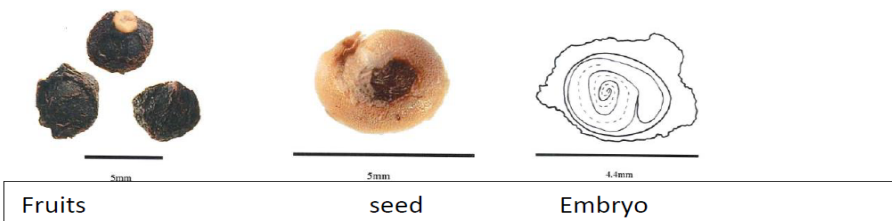
1. Each lot has >800 seeds. Pure seed units are used in seed count to determine the appropriate working weights for purity analysis. Randomly count eight replicates each of 100 seed units. Weigh each replicate in grams to four significant figures and record the data in the excel sheet (Table 1.)



2. Conduct the Germination- number of the seed to be used are 400 seeds. Record the result in excel sheet (Table 2.)



3. Conduct the Tetrazolium seed test- number of the seed to be used are 400 seeds. Record the data in excel sheet (Table 3). For the purpose of validation testing for tetrazolium seed test requires 400 seeds to be tested. (Reference: Appendix 5 ,AOSA rules for testing seed: Volume 1. Principles and Procedures)



Tetrazolium test-Malabar Spinach

Tetrazolium Test -Malabar spinach

Family–Basillaceae

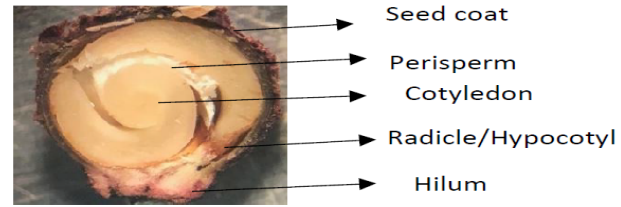
Genus: *Basella rubra* L., *Basella alba* L.

Procedure - Red stem variety (Green stem variety does not have pulpy seed coat)


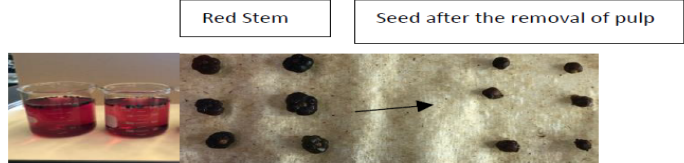


1. PRECONDITIONING:

Method	Time (h)	Temp (°C)
Seeds soaked in beaker of water	24	30

1.Morphology





i) Morphological difference between two varieties of Malabar Spinach

Seed Characteristic	Green stem- <i>Basella alba</i> L.	Red stem- <i>Basella rubra</i> L.
After imbibing in water for 24 hours	Seed coat do not exhibit pulpy layer in green stem. 	Pulpy seed coat noticed. Therefore, remove this pulp from seed coat by gently plucking with tweezer and softly rubbing the surface with tissue paper. 
Color change	Water turns mild red in some green stem varieties	Water turns deep red
Seed after preconditioned	 Green stem seed	 Red Stem Seed
** Referee Coded Sample	I-IV sample	V-VIII sample

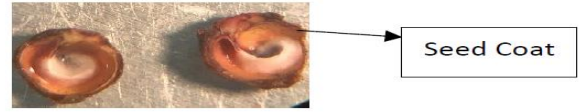
2. PREPARATION AND STAINING

Methods	TZ Conc (%)	Time(h)	Temp (°C)
1.Bisect longitudinally near the hilum end through the embryo, and retain half for staining	1.0	16 (overnight)	30

Procedure-TZ study

<p>Procedure Bisect the seeds in the center with two halves left attached at one end (embryos stay inside the seed coat). This is easy for evaluation because of evenly staining of the embryo.</p>	 <div data-bbox="1783 35 2191 149" style="border: 1px solid black; padding: 5px;"> <p>Gentle slit on the Seed coat with embryo still firmly attached at the hilum base</p> </div>
<p>After staining The seed coat is still attached to the two halves of the embryo. This makes easy to evaluate the viable and non-viable seed</p>	 <div data-bbox="1783 178 2191 264" style="border: 1px solid black; padding: 5px;"> <p>Point of attachment of the two halves of the embryo</p> </div>

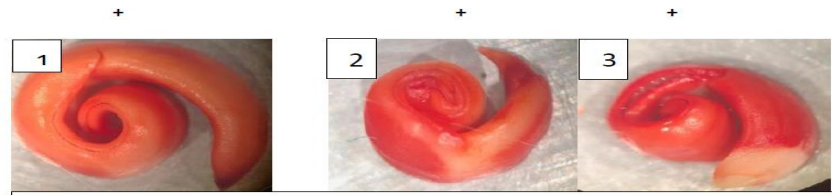
* **Note- Post staining** Sometimes the embryo gets separated from the seed coat. Pay attention in counting viable and non-viable seeds.



3. Evaluation:

Viable (Normal staining):

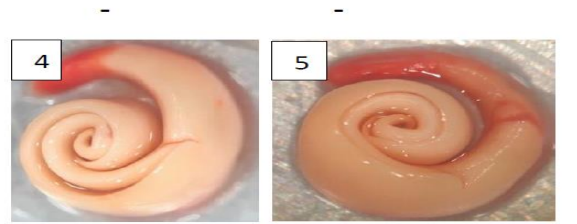
Entire embryo evenly stained
Perisperm not stain



+ (1) viable + Viable (2 & 3) extend these sample for 2 hours to ascertain the whole embryo is stained.

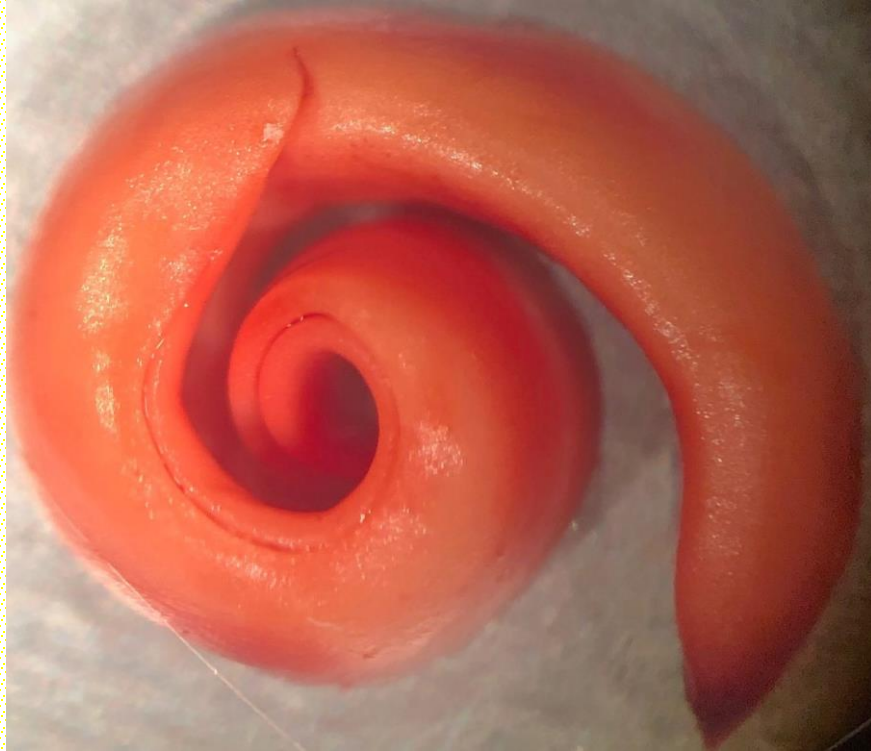
Non-Viable (ABNORMAL OR NO STAINING):

Any essential part of embryo unstained
Embryo not completely stained
Watery, flaccid embryo



- Nonviable - Nonviable

Tetrazolium Test-Malabar Spinach seeds



Answer: Viable

Plate 1. Viable / Non viable



Plate 4. Viable / Non viable

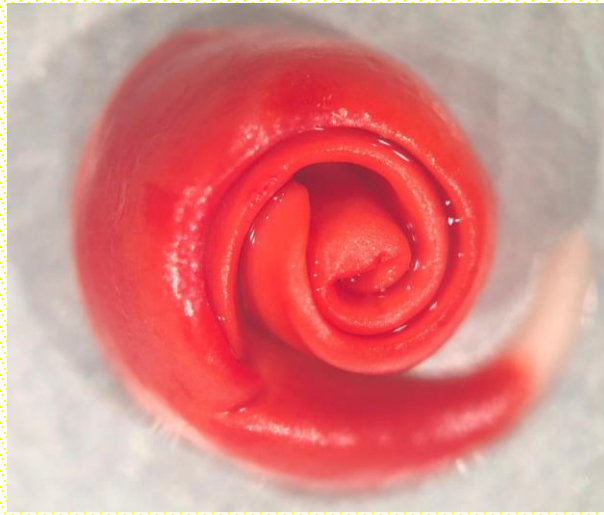


Plate 3. Viable / Non viable

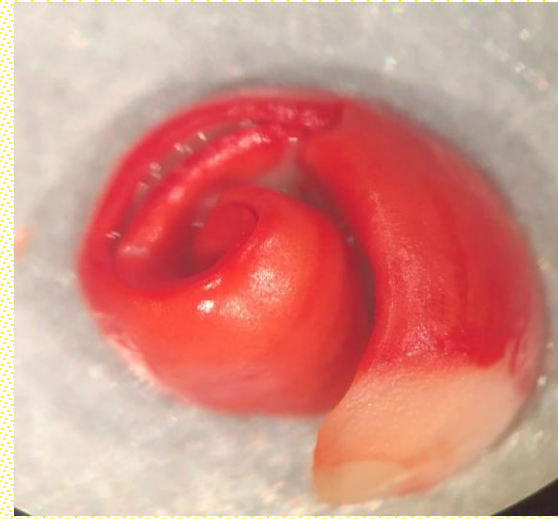


Plate 2. Viable / Non viable



Plate 7. Viable / Non viable



Plate 6. Viable / Non viable

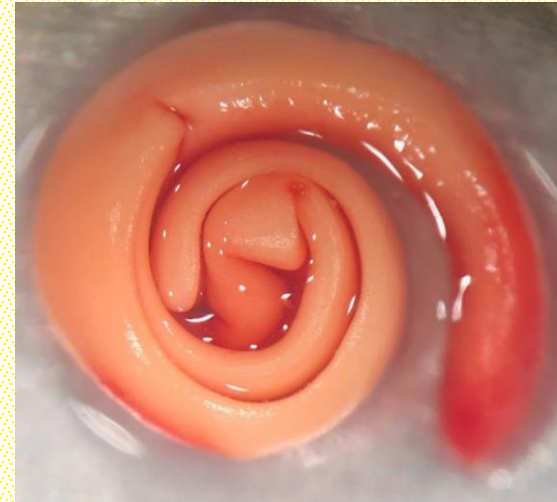


Plate 5. Viable / Non viable

Results

Result on objective I: PSU-Pure Seed Unit (g) on Malabar Spinach seeds

Table 2A. Weights for working samples

Pure Seed Unit	Chaffy (C) or Super chaffy (SC)	<u>Kind of seed</u>	<u>Min. wt. (g) for Purity analysis</u>	<u>Min. wt. (g) for bulk exam</u>	<u>Approx. No. seeds/g</u>	<u>Approx. No. seeds/oz</u>
		<i>Basella alba</i> L. (Green)	80	800	31	879
		<i>Basella rubra</i> L. (Red)	67	670	37	1049

Vol. 3 Uniform classification of weed and crop seeds

UNIFORM CLASSIFICATION SORTED BY SCIENTIFIC NAME

NOMEN #	Species	COMMON NAME	FAMILY	SPP. CLASS	CONTAMINATING CLASSIFICATION						
					A	F	H	R	S	T	V
6531	<i>Basella alaba</i> L.	*Malabar-Spinach Ceylon-spinach Indian-spinach Malabar-nightshade Malabar-spinach vine-spinach	Basellaceae	V, F	A	F	H	R	S	T	V
					W	C	W	W	W	W	C

USDA United States Department of Agriculture
Agricultural Research Service

GRIN-Global U.S. National Plant Germplasm System

Taxon: *Basella alba* L.

Economic Uses

- Environmental**
ornamental – [Reference\(s\)](#)
- Food additives**
coloring – [Reference\(s\)](#)
- Human food**
vegetable – [Reference\(s\)](#)
- Medicines**
folklore – [Reference\(s\)](#)

*Reference: [Basella alba L. GRIN-Global \(ars-grin.gov\)](http://ars-grin.gov)

Objective II: Germination Test results Malabar Spinach seeds

Result on objective II: Germination Results

Germination Test results on Malabar Spinach seed

- Comparison of **max & min mean value** of four green Malabar spinach seed sample with seven labs for 7, 14 and 21 days
- Comparison of four green Malabar spinach seed sample with seven labs
- Comparison of four red Malabar spinach seed sample with seven labs

Germination Test results Malabar Spinach seeds

Table 1. Analysis of variance (ANOVA) of germination counts after 7, 14, and 21 days of four green Malabar Spinach, *Basella alba* and four red *Basella rubra* Malabar Spinach seed samples tested in seven seed laboratories.

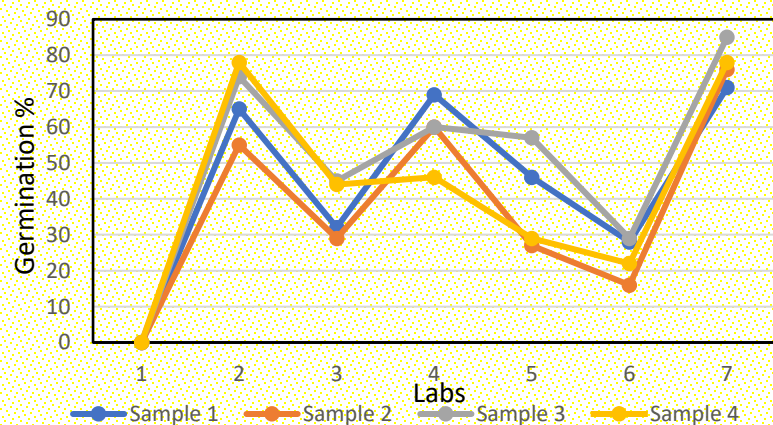
Source of variation		df	7-d germ†	14-day germ	21-day germ
Laboratories	(L)	6	***†	***	***
Seed Samples	(S)	3	***	***	***
Varieties (Green/red)	(V)	1	***	***	***
Interaction					
LS		18	***	***	***
LV		6	***	***	***
SV		3	***	***	***
LSV		18	***	***	***

*** Highly significant at the 0.001 probability level.

† Significant difference among the germination of 7-day, 14-day, and 21-day were found as well.

Germination result of Red Malabar Spinach sample vs labs

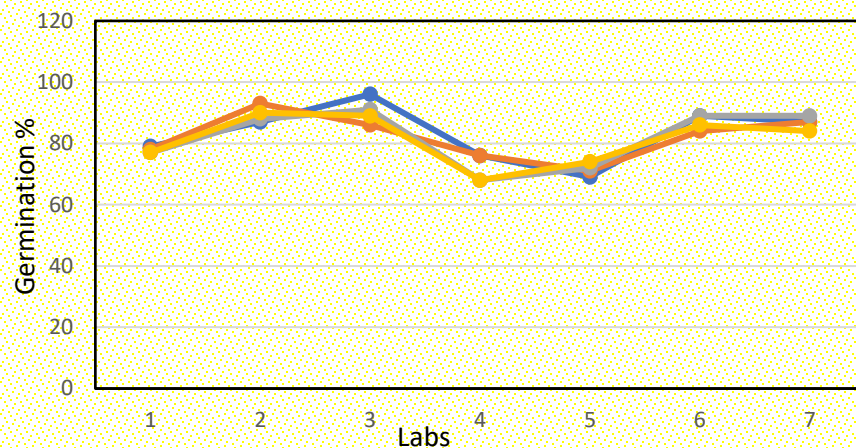
7-Day



● Sample 1 ● Sample 2 ● Sample 3 ● Sample 4

7- Day				
Labs	Sample 1	Sample 2	Sample 3	Sample 4
1	0	0	0	0
2	65	55	74	78
3	32	29	45	44
4	69	60	60	46
5	46	27	57	29
6	28	16	29	22
7	71	76	85	78
SD	26.25	26.91	28.62	28.70
SE	9.92	10.17	10.82	10.85

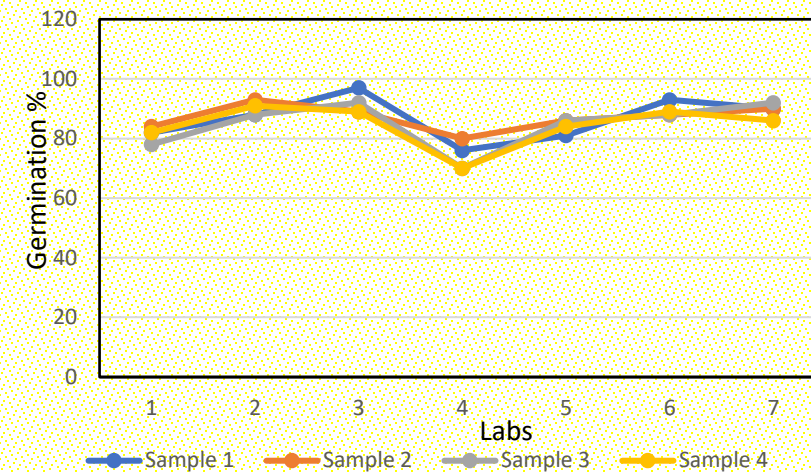
14-Day



● Sample 1 ● Sample 2 ● Sample 3 ● Sample 4

14-Day				
Labs	Sample 1	Sample 2	Sample 3	Sample 4
1	79	78	77	77
2	87	93	88	90
3	96	86	91	89
4	76	76	68	68
5	69	71	72	74
6	89	84	89	86
7	87	87	89	84
SD	9.11	7.52	9.45	8.30
SE	3.44	2.84	3.57	3.14

21- Day

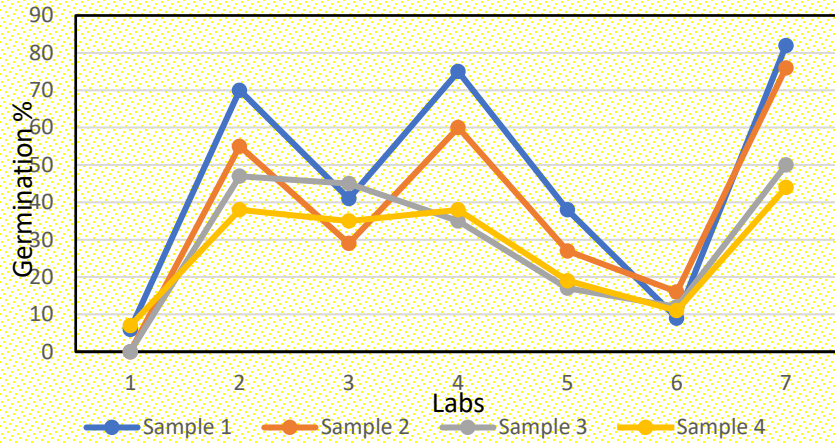


● Sample 1 ● Sample 2 ● Sample 3 ● Sample 4

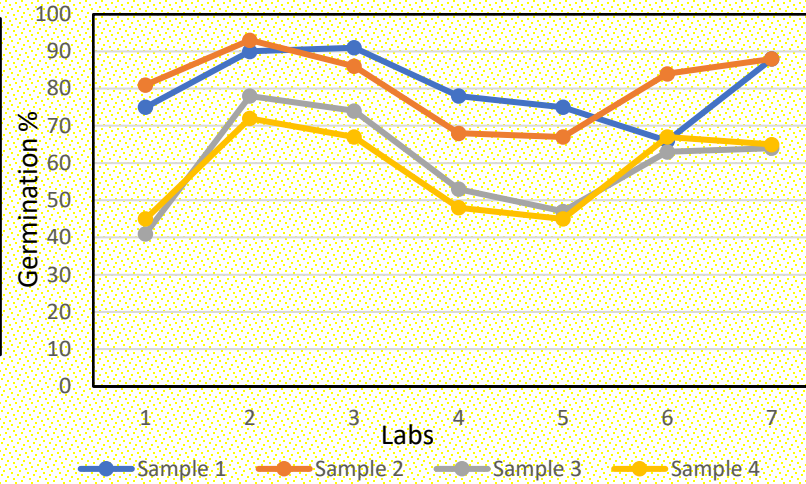
21-Day				
Labs	Sample 1	Sample 2	Sample 3	Sample 4
1	82	84	78	82
2	88	93	88	91
3	97	89	92	89
4	76	80	70	70
5	81	86	86	84
6	93	88	88	89
7	90	90	92	86
SD	7.39	4.26	8.07	7.09
SE	2.79	1.61	3.05	2.68

Germination result of Green Malabar Spinach sample vs labs

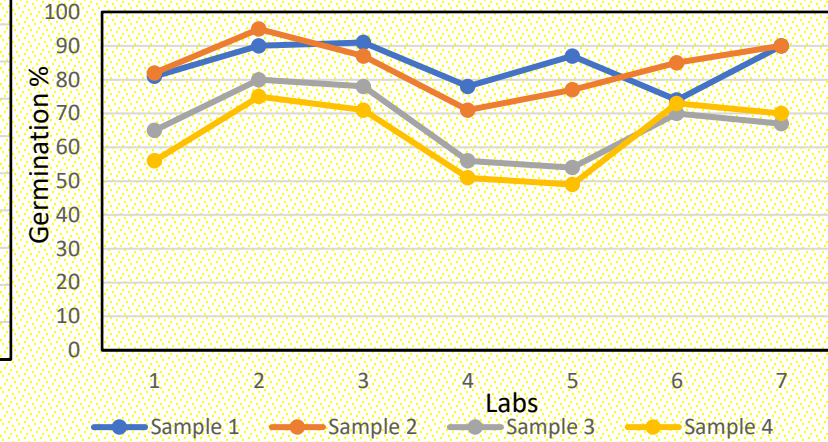
7-Day



14-Day



21-Day

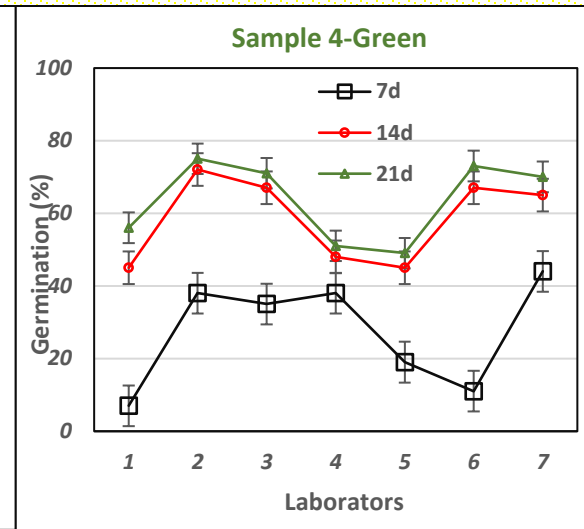
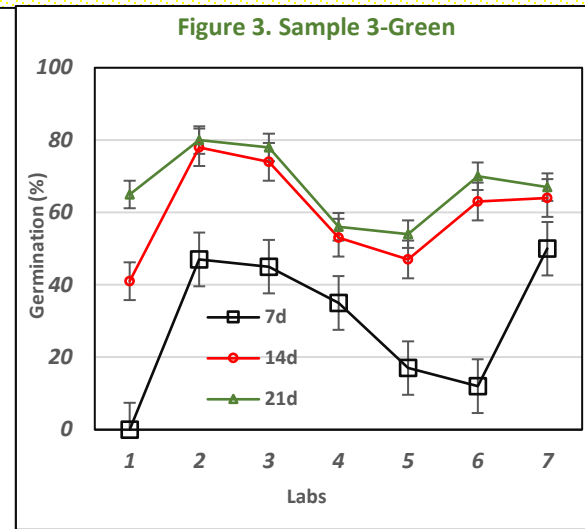
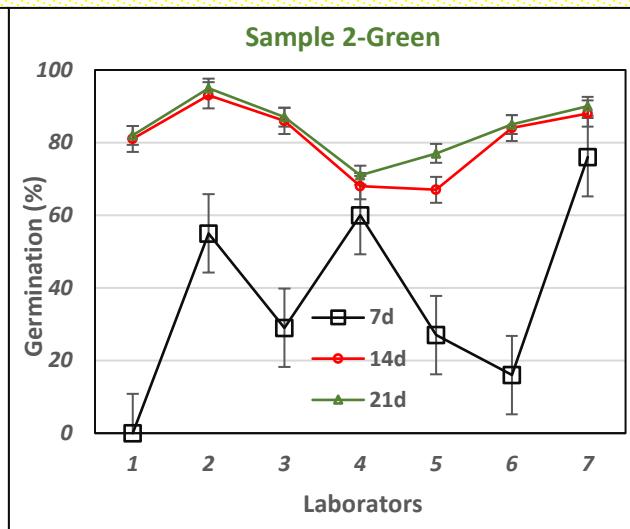
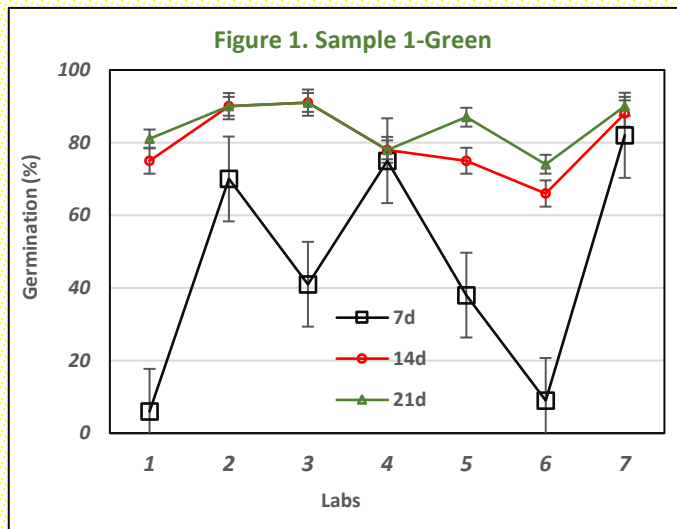


7- Day				
Labs	Sample 1	Sample 2	Sample 3	Sample 4
1	6	0	0	7
2	70	55	47	38
3	41	29	45	35
4	75	60	35	38
5	38	27	17	19
6	9	16	12	11
7	82	76	50	44
SD	31.01	26.91	19.70	14.80
SE	11.72	10.17	7.45	5.59

14-Day				
Labs	Sample 1	Sample 2	Sample 3	Sample 4
1	75	81	41	45
2	90	93	78	72
3	91	86	74	67
4	78	68	53	48
5	75	67	47	45
6	66	84	63	67
7	88	88	64	65
SD	9.43	9.93	13.69	11.86
SE	3.56	3.75	5.17	4.48

21-Day				
Labs	Sample 1	Sample 2	Sample 3	Sample 4
1	81	82	65	56
2	90	95	80	75
3	91	87	78	71
4	78	71	56	51
5	87	77	54	49
6	74	85	70	73
7	90	90	67	70
SD	6.75	8.05	9.94	11.13
SE	2.55	3.04	3.76	4.21

Germination result of Green Malabar Spinach sample vs labs



	7d	14d	21d	Mean 14+21	Tolerance 14-21	Lab	7d	14d	21d	Mean 14+21	Tolerance 14-21	Lab	7d	14d	21d	Mean 14+21	Tolerance 14-21	Lab	7d	14d	21d	Mean 14+21	Tolerance 14-21
1	6	75	81	78	8	1	0	81	82	82	7	1	0	79	82	81	8	1	7	45	56	51	11
2	70	90	90	90	6	2	55	93	95	94	4	2	65	87	88	88	6	2	38	72	75	74	9
3	41	91	91	91	5	3	29	86	87	87	6	3	32	96	97	97	3	3	35	67	71	69	10
4	75	78	78	78	8	4	60	68	71	70	9	4	69	76	76	76	8	4	38	48	51	50	11
5	38	75	87	81	8	5	27	67	77	72	9	5	46	69	81	75	9	5	19	45	49	47	11
6	9	66	74	70	9	6	16	84	85	85	7	6	28	89	93	91	5	6	11	67	73	70	9
7	82	88	90	89	7	7	76	88	90	89	6	7	71	87	90	89	6	7	44	65	70	68	10
SD	31.0	9.4	6.8			SD	26.9	9.9	8.1			SD	26.2	9.1	7.4			SD	14.8	11.9	11.1		

Germination of Red Malabar Spinach sample vs lab

Figure 2. Sample 1- Red

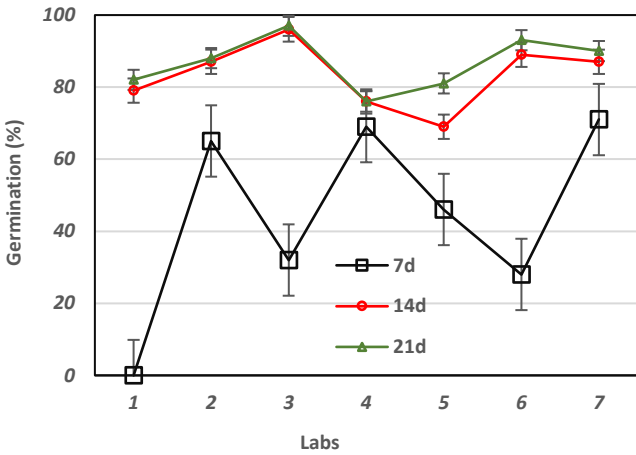


Figure 2. Sample 2- Red

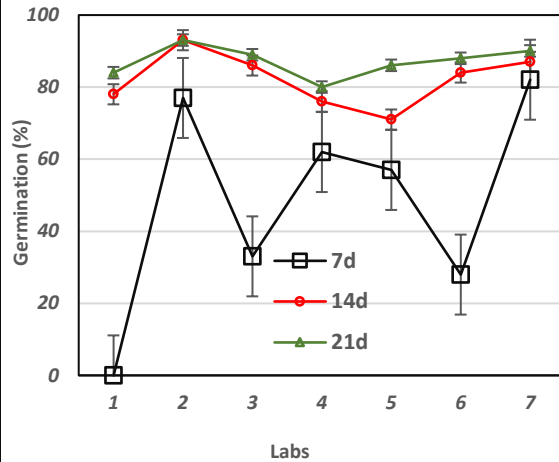


Figure 3. Sample 3- Red

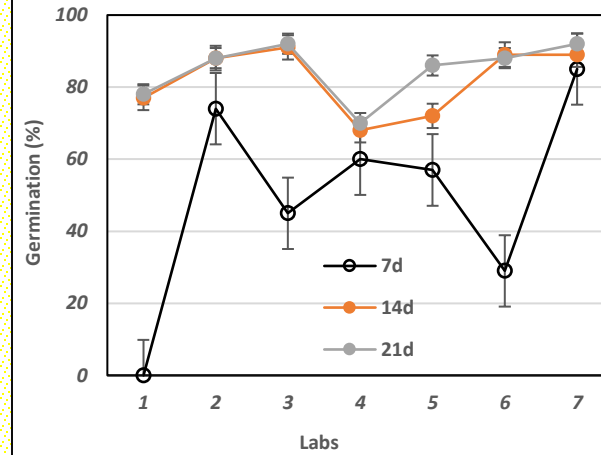
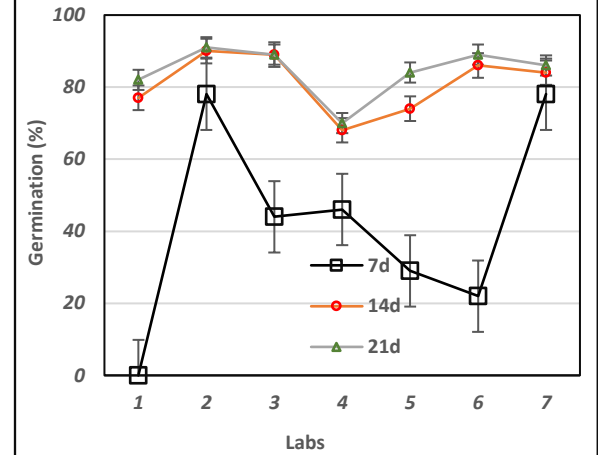


Figure 4. Sample 4- Red



Lab	7d	14d	21d	Mean 14+21	Tolerance 14-21	Lab	7d	14d	21d	Mean 14+21	Tolerance 14-21	Lab	7d	14d	21d	Mean 14+21	Tolerance 14-21	Lab	7d	14d	21d	Mean 14+21	Tolerance 14-21
1	0	79	82	81	8	1	0	78	84	81	8	1	0	77	78	78	8	1	0	77	82	80	8
2	65	87	88	88	6	2	77	93	93	93	5	2	74	88	88	88	6	2	78	90	91	91	5
3	32	96	97	97	3	3	33	86	89	88	6	3	45	91	92	92	5	3	44	89	89	89	6
4	69	76	76	76	8	4	62	76	80	78	8	4	60	68	70	69	10	4	46	68	70	69	10
5	46	69	81	75	9	5	57	71	86	79	8	5	57	72	86	79	8	5	46	68	70	69	10
6	28	89	93	91	5	6	28	84	88	86	7	6	29	89	88	89	6	6	29	74	84	79	8
7	71	87	90	89	6	7	82	87	90	89	6	7	85	89	92	91	5	7	22	86	89	88	6
SD	26.2	9.1	7.4			SD	29.4	7.5	4.3			SD	28.6	9.5	8.1			SD	28.7	8.3	7.1		

Objective III:

**Tetrazolium Test results Malabar
Spinach seeds**



Table 2. Tetrazolium test results of four green Malabar Spinach samples tested in seven seed labs

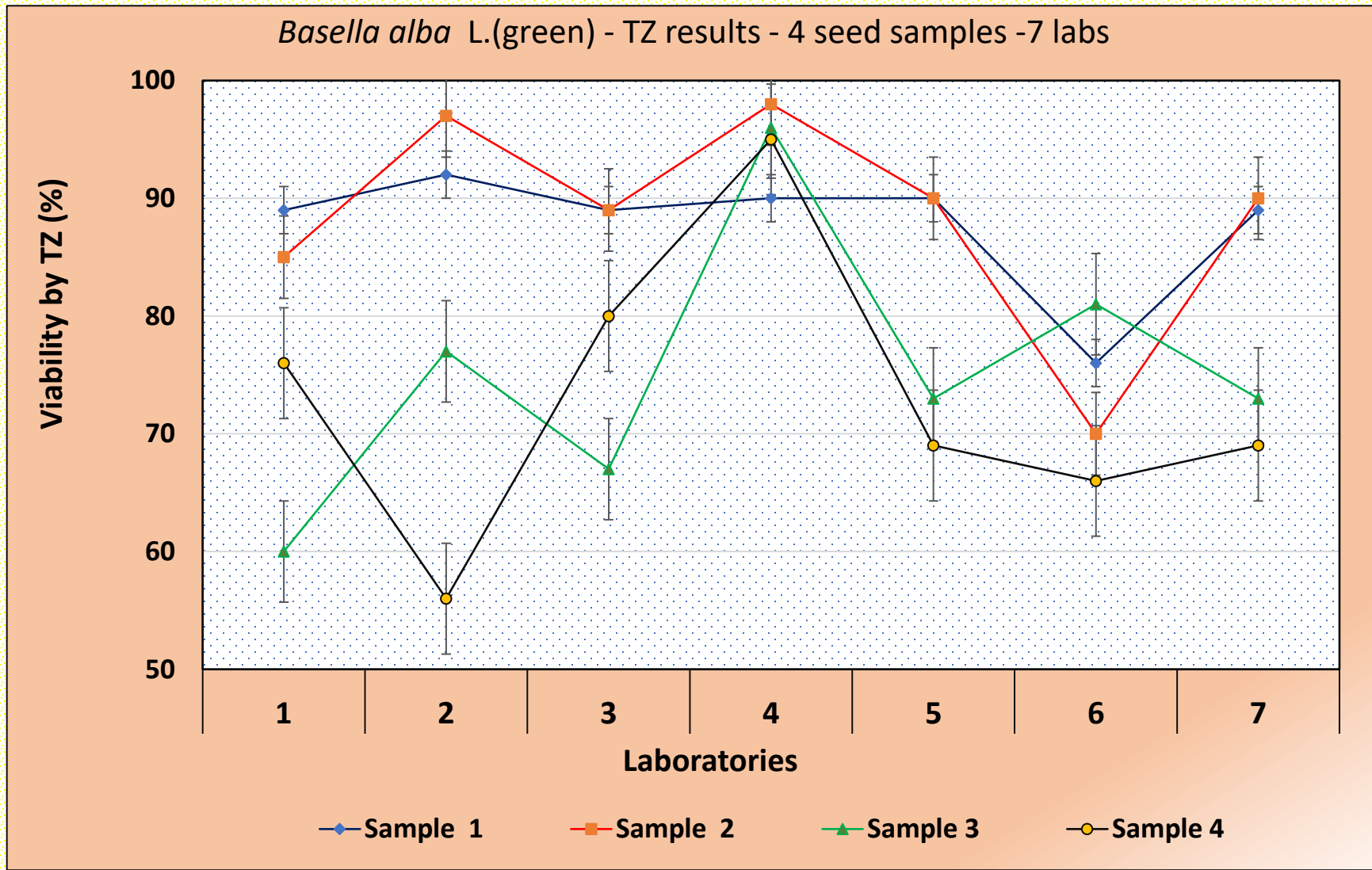
Basella alba L. (green variety)



Laboratories

<i>Samples</i>	1	2	3	4	5	6	7	SD	SE
1	89	92	89	90	90	76	89	5.3	2.0
2	85	97	89	98	90	70	90	9.3	3.5
3	60	77	67	96	73	81	73	11.4	4.3
4	76	56	80	95	69	66	69	12.3	4.7

Tetrazolium test results of four green Malabar Spinach samples tested in seven seed labs.



Samples	SD	SE
1	5.3	2
2	9.3	3.5
3	11	4.3
4	12	4.7

Figure 1. Tetrazolium test results of four green Malabar Spinach samples tested in seven seed labs.

Over laped error bars indicate non-significant means.

Table 3. Tetrazolium test results of four red Malabar Spinach samples tested in seven seed labs

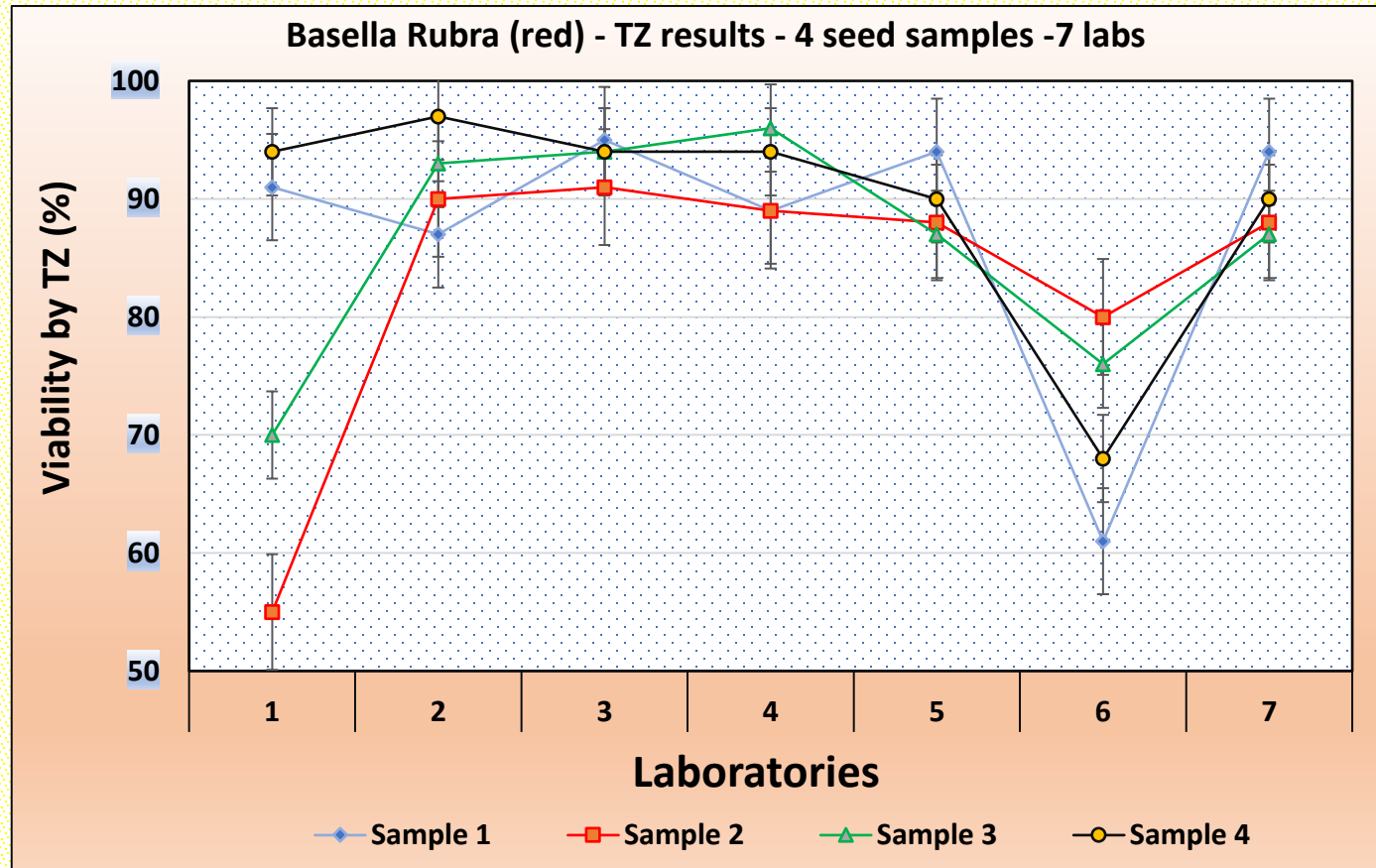
***Basella rubra* L. (red variety)**

Laboratories



<i>Samples</i>	1	2	3	4	5	6	7	SD	SE
1	91	87	95	89	94	61	94	12.0	4.5
2	55	90	91	89	88	80	88	12.9	4.9
3	70	93	94	96	87	76	87	9.8	3.7
4	94	97	94	94	90	68	90	9.8	3.7

Tetrazolium test results of four red Malabar Spinach samples tested in seven seed labs



SD	SE
12.0	4.5
12.9	4.9
9.8	3.7
9.8	3.7

Figure 2. Tetrazolium test results of four red Malabar Spinach samples tested in seven seed labs.

Overlaped error bars indicate non-significant means.

Discussion

- Miles(1963), Germination tolerance follow the binomial distribution applied to percent NS (germination tolerances were computed to avoid the random sampling variation) between the interlaboratory bias. (**Table 14 J tolerance germination test and TZ test**)
- The cause of significant differences between /among for in germination tests might be due
 1. Random sampling variation (cannot be eliminated)
 2. Inaccurate counting or recording
- **Type I Error:** It represents the chance that the researcher detects difference between two groups when in reality, no difference exists.
- A chance of **false positive** conclusion/incorrect decision
- SD-standard deviation (reflects the range of values) . Noticed wide range in case of Samples Vs Labs for 7day interval (Type I error/random sampling error that is happening by chance)
- The Standard Error Estimate (SEE)- reflects variation around the mean.
- Lower SEE indicates solid sample size.

Accomplished : 2019-Future line of work

- 1 Proposal for inclusion of new species in the AOSA& ISTA Rules VZ., includes
 - a) scientific name of proposed species
- 2 Lot and sample weights
- 3 Pure seed definition-distinguishing characteristics of the species
- 4 Validated germination test methods
- 5 Validated tetrazolium test procedure

Validated varietal identification method

Publication results:

- ✓ The supportive results would be used as part of a **2022-AOSA Rule proposal**
- ✓ The research investigation met the all the criteria of **ISTA Validation method**, the results would also be used for addition of this crop species in the ISTA rule proposal.

Recommendations & Conclusions

- ✓ PSU-Pure Seed Unit- Half seed rule method
- ✓ First and Final count for Malabar spinach is recommended **7** and **14** days at 20-30⁰ light, **+2** days extension. The 21 day may lead to seedling rot/mold infection due to ideal temperature at 20-30⁰ light.
- ✓ Although significant difference were noticed among the labs with eight seed lots for 7, 14 and 21 days for germination, the results indicates that 7 and 14 days recommended irrespective of the varieties of Malabar Spinach spp.
- ✓ **Tetrazolium Test**: The recommended method for green stem and red stem, worked out with respect to **repeatability and reproducibility** of the results across the 7 labs. Therefore, the same method is recommended for rule proposal in 2022.

Bibliography

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3. S. A. Deshmukh and D. K. Gaikwad, A review of the taxonomy, ethnobotany, phytochemistry and pharmacology of (*Basella alba* Basellaceae). J App Pharm Sci, 2014; 4 (01): 153-165.
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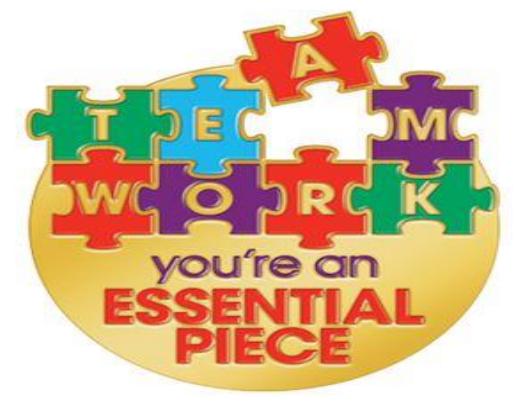
Abstract

Seed germination, purity, and Tetrazolium methods for Malabar spinach (*Basella spp.*).

The Malabar spinach seed germination, purity and tetrazolium investigation was conducted to standardized the seed testing protocol for this crop species. It involved with two *Basella spp.* varieties (red stem and green stem) across seven laboratories. The germination study indicated that the 20-30°C rolled towel with 7 as first and 14 days as final count for this species. Further, for purity weight analysis for the green stem and red stem varieties are 80 g and 67g respectively. However, the Tetrazolium protocol slightly varied for both the varieties, but the method was repeatable and reproducible across all the seven labs.



Acknowledgement



Sue Alvarez Dr. Ruojing	STRF-Fund Team Tim & Andrae team	Statistical Analysis, Germ and TZ	1	Dr. Sabry Elias
Elizabeth, Ruojing, David, Dr Riad team				
Seed Lab	Validation/Referee study	State		
Ransom Seed Laboratory	Sue Alvarez, Michael Aberle team	California		
Federal seed lab	Elizabeth Stewart, Charlene Burton, Anitra Walker, Laura Ortiz team	North Carolina		
North Carolina Seed Lab	Roger Cross, Nikki Holland team	North Carolina		
WI Crop Improvement Assoc.	Shaminder Miranpuri team	Wisconsin		
Oregon state University Seed Lab	Dr. Sabry team	Oregon		
CFIA/ACIA	Dr. Ruojing, Dr. Steve Jones, Nicole and Canadian Food Inspection Agency, Seed Science and Technology team	Canada		

Pennsylvania Seed Lab-PA
Johnny Zook & Nishit Patel team

Seed & Miscellaneous
1 Roger Cross
2 Carolyn Langley –Idaho seed lab, Seed Eco Florida
3 Melissa Phillips,

Western hybrid seeds CA

Burpee Seed , PA

Tetrazolium Test-Method
1 Michael Aberle
2 Shaminder

Pure Seed Unit (PSU)
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2 Dr. Robert Rice-CFIA

Germination
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2 Dr. Riad B

Tetrazolium test-review
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2 Jones, Steve K (CFIA/ACIA)
3 Laura Carlson-Sodak lab

Any
Questions

