

## 2022 Rule Proposal #3 - Amended

### PURPOSE OF RULE PROPOSAL:

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

- ✓ Malabar spinach cultivars ~~are~~ green stem (*Basella alba* L.) and red stem (*B. alba*, syn. ~~*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. 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.**

Addition of seed quality test methods viz., Pure Seed Unit, Germination and Tetrazolium procedure on Malabar spinach (*Basella alba* ~~spp.~~) to the AOSA/SCST for testing seeds.

- 1) Addition of Pure Seed Unit (PSU) for *Basella alba* L. ~~spp.~~
- 2) Tetrazolium Test procedure methods to *Basella alba* L. ~~spp.~~ **(Please note: This will be added to the AOSA/SCST Tetrazolium Handbook and is not part of this AOSA Rule Proposal.)**
- 3) Standard germination method to *Basella alba* L. ~~spp.~~

**PRESENT RULE:** No existing rule for Malabar Spinach in AOSA/SCST/ISTA Rules

**PROPOSED RULE:**

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

**a) Table 2A. Weights for working samples**

Pure Seed Unit	Chaffy (C) or Super chaffy (SC)	Kind of seed	Min. wt. (g) for Purity analysis	Min. wt. (g) for bulk exam	Approx. No. seeds/g	Approx. No. seeds/oz
<del>42</del> <b>38</b> <del>42</del>	<del>NA</del>	<i>Basella alba</i> L. Malabar-spinach Green stem types Red stem types (Green)	80 67	800 670	31 37	879 1,049
	<del>NA</del>	<del><i>Basella rubra</i> L. (Red)</del>	<del>67</del>	<del>670</del>	<del>37</del>	<del>1049</del>

**b) 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	<del><i>Basella rubra</i></del> <i>Basella alba</i> L.	*Malabar-Spinach, Ceylon-spinach, Indian-spinach, Malabar-nightshade, Malabar-spinach, vine-spinach	Basellaceae	V, F									
					W	C	W	W	W	W	W	C	

- ✓ **PSU-Pure Seed Unit ~~42- 38~~ 42** Half seed rule method (Ref: AOSA. 2019, Rules for Testing Seeds Volume 1)
- ✓ ~~Intact utricle with or without perianth . . . .~~
- ✓ Intact dry indehiscent one-seeded fruit with accessory structures, whether or not a seed is present. Piece of broken dry indehiscent one-seeded fruit larger than one-half the original size, unless no seed is present.
- ✓ Seed with or without seed coat. Piece of broken seed, with or without seed coat, larger than one-half the original size. . . .

“From: Price, Robert@CDFA

Sent: Wednesday, May 4, 2022 11:01 AM

Dear Roopa: To follow up on my recent message, according to Arthur Cronquist. 1981. Integrated System of Classification of Flowering Plants. Columbia University Press, New York. The fruit of the Basellaceae is a utricle surrounded by the persistent often fleshy corolla or persistent sepals. ***This would appear to match well to PSU 38 in the AOSA Rules for either green or red types of Basella spp.***”

c) **Table 6A. Methods of Testing for laboratory germination.**

Kind of seed	Varieties	Substrata	Temperature(°C)	First count (d)	Final count (d)	Specific requirements and notes	Dormant Seed
<i>Basella alba</i> <i>Species</i> Malabar-spinach	Red stem	RT T	20-30	7	14	Light	<del>No dormancy was noted</del>
	Green stem			7	14		<del>No dormancy was noted</del>

- d) **First and Final count** for Malabar spinach is recommended 7 and 14 days at 20-30° light, +2 days extension. The 21 day may lead to seedling rot/mold infection due to ideal temperature at 20-30° light.
- e) 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.
- f) **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.~~

**SUPPORTING EVIDENCE:**

**(Please note: This will be added to the AOSA/SCST Tetrazolium Handbook and is not part of this AOSA Rule Proposal.)**

Family—Basellaceae

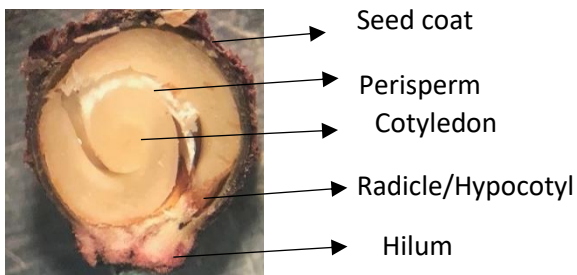
Genus: *Basella rubra* L., *Basella alba* L. spp.

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


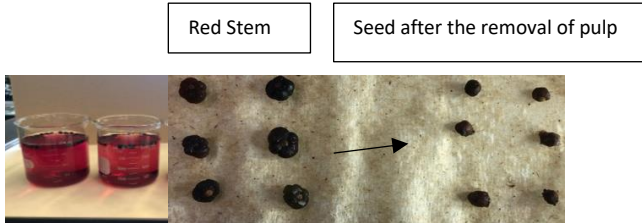


**1. PRECONDITIONING:**

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



## 1. Morphology



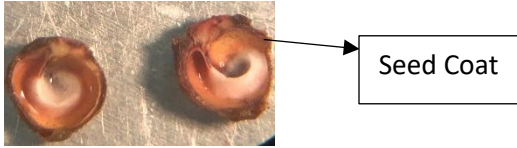
### i) Morphological difference between two varieties of Malabar Spinach

Seed Characteristic	Green stem- <i>Basella alba</i> L.	Red stem- <i>Basella rubra alba</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 tweezers 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
<b>Procedure</b> Bisect the seeds in the center with two halves <b>left attached</b> at one end (embryos stay inside the seed coat). This is easy for evaluation because of evenly staining of the embryo.		Gentle slit on the Seed coat with embryo <b>still</b> firmly attached at the hilum base	
<b>After staining</b> The seed coat is still attached to the two halves of the embryo. This makes easy to evaluate the viable and non-viable seed		Point of attachment of the two halves of the embryo	

**\* 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



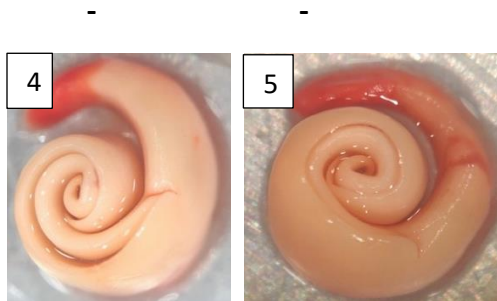
+ (1) viable      + Viable (2 & 3 ) extend these sample for 2 hours to ascertain the whole embryo is stained.

#### Non-Viable (ABNORMAL OR NO STAINING):

Any essential part of embryo unstained

Embryo not completely stained

Watery, flaccid embryo



- Nonviable

- Nonviable

### III. Objective II: Germination Test results Malabar Spinach seeds

#### a) The objective of the study

Determine germination period for the standard germination test of Malabar spinach, with the intent of developing a rule proposal next year.

#### Germination Study

The analysis of variance (ANOVA) indicated that germination of 7-day, 14-day, and 21-day germination counts varied significantly among labs, seed samples and between green and red Malabar spinach (Table 1). In addition, the interactions among labs and samples, labs and varieties, samples and varieties, as well as three-way interaction among labs, samples and varieties were highly significant as well (Table 1).

**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 (*B. alba*, *syn. 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	***	***	***
<b>Interaction</b>					
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.

**Table 2.** Germination of four green and four red Malabar Spinach seed samples at **7-day count** tested in seven different labs. Seeds tested at **(20-30°C)**.

Variety	Sample 1		Sample 2		Sample 3		Sample 4	
	Green	Red	Green	Red	Green	Red	Green	Red
Labs	Germination (%)							
1	6	0	0	0	0	0	7	0
2	70	65	55	77	47	74	38	78
3	41	32	29	33	45	45	35	44
4	75	69	60	62	35	60	38	46
5	38	46	27	57	17	57	19	29
6	9	28	16	28	12	29	11	22
7	82	71	76	82	50	85	44	78
Mean	46	44	38	48	29	50	27	42
Max	82	71	76	82	50	85	44	78
Min	6	0	0	0	0	0	7	0
Max-Min	76	71	76	82	50	85	37	78

**Table 3.** Germination of four green and four red Malabar Spinach seed samples at **14-day count (accumulative counts of 7 and 14 day counts)** tested in seven different labs. Seeds tested at **(20-30°C)**.

Variety	Sample 1		Sample 2		Sample 3		Sample 4	
	Green	Red	Green	Red	Green	Red	Green	Red
Labs	Germination (%)							
1	75	79	81	78	41	77	45	77
2	90	87	93	93	78	88	72	90
3	91	96	86	86	74	91	67	89
4	78	76	68	76	53	68	48	68
5	75	69	67	71	47	72	45	74
6	66	89	84	84	63	89	67	86
7	88	87	88	87	64	89	65	84
Mean	80	83	81	82	60	82	58	81
Max	91	96	93	93	78	91	72	90
Min	66	69	67	71	41	68	45	68

Max-Min	78	27	26	22	37	23	27	22
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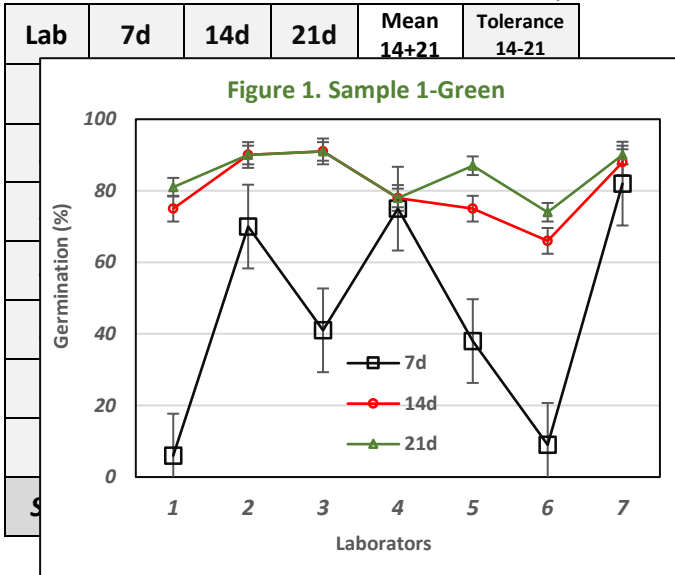
**Table 4.** Germination of four green and four red Malabar Spinach seed samples at 21-day count (accumulative counts of 7, 14, and 21 day counts) tested in seven different labs. Seeds tested at (20-30°C).

Variety	Sample 1		Sample 2		Sample 3		Sample 4	
	Green	Red	Green	Red	Green	Red	Green	Red
	Germination (%)							
Labs	81	82	82	84	65	78	56	82
2	90	88	95	93	80	88	75	91
3	91	97	87	89	78	92	71	89
4	78	76	71	80	56	70	51	70
5	87	81	77	86	54	86	49	84
6	74	93	85	88	70	88	73	89
7	90	90	90	90	67	92	70	86
Mean	84	87	84	87	67	85	64	84
Max	91	97	95	93	80	92	75	91
Min	74	76	71	80	54	70	49	70
Max-Min	17	21	24	13	26	22	26	21



**Table 5.** Germination of one **Green** Malabar Spinach sample-1 after 7, 14, and 21 day at 20-30°C.

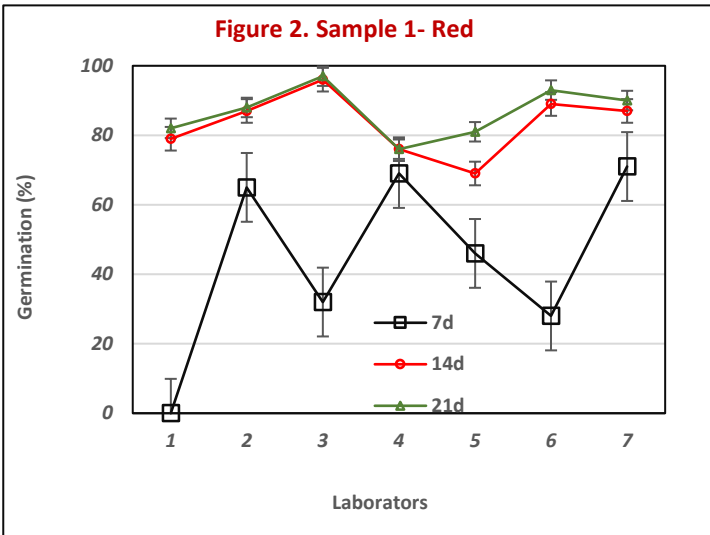
Seeds were tested at 7 different labs.



**Figure 1.** Germination of one **Green** Malabar Spinach sample -1 after 7, 14, and 21 day at 20-30°C. Seeds were tested at 7 different labs. Overlap error bars indicate non-significant means.

**Table 6.** Germination of one **Red** Malabar Spinach sample -1 after 7, 14, and 21 day at 20-30°C.

Seeds were tested at 7 different labs.

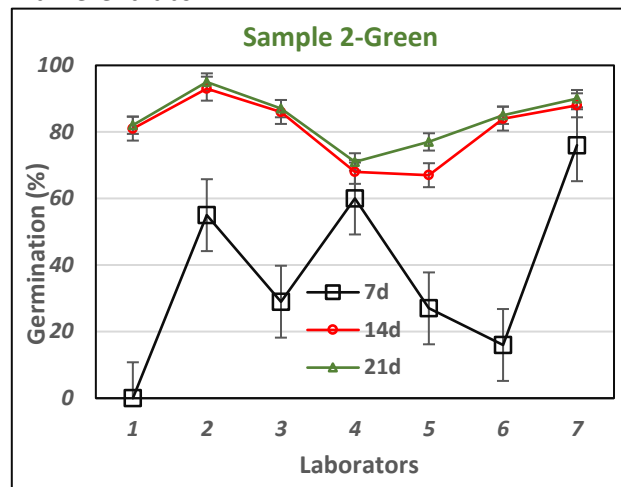


<b>SD</b>	<b>26.2</b>	<b>9.1</b>	<b>7.4</b>		
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**Figure 2.** Germination of one **Red** Malabar Spinach sample -1 after 7, 14, and 21 day at 20-30°C. Seeds were tested at 7 different labs. Overlap error bars indicate non-significant means.

**Table 7.** Germination of one **Green** Malabar Spinach sample -2 after 7, 14, and 21 day at 20-30°C. Seeds were tested at 7 different labs.

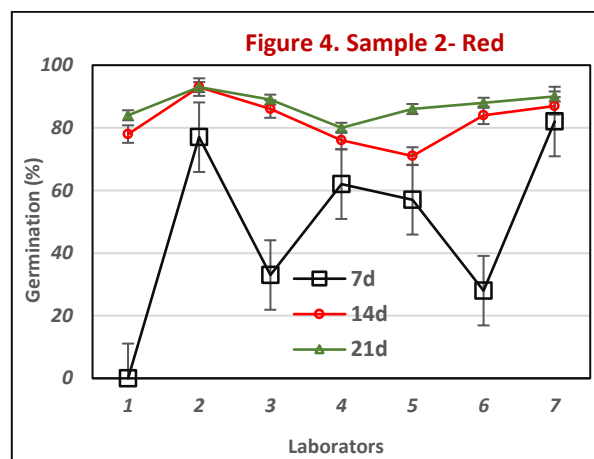
Lab	7d	14d	21d	Mean 14+21	Tolerance 14-21
1	0	81	82	82	7
2	55	93	95	94	4
3	29	86	87	87	6
4	60	68	71	70	9
5	27	67	77	72	9
6	16	84	85	85	7
7	76	88	90	89	6
<i>SD</i>	<i>26.9</i>	<i>9.9</i>	<i>8.1</i>		



**Figure 3.** Germination of one **Green** Malabar Spinach sample -2 after 7, 14, and 21 day at 20-30°C. Seeds were tested at 7 different labs. Overlap error bars indicate non-significant means.

**Table 8.** Germination of one **Red** Malabar Spinach sample -2 after 7, 14, and 21 day at 20-30°C. Seeds were tested at 7 different labs.

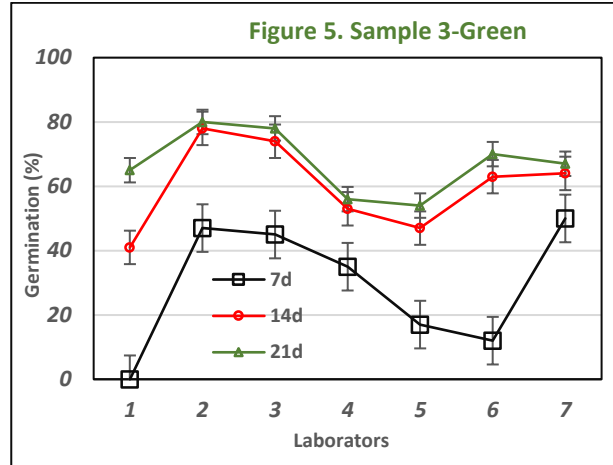
Lab	7d	14d	21d	Mean 14+21	Tolerance 14-21
1	0	78	84	81	8
2	77	93	93	93	5
3	33	86	89	88	6
4	62	76	80	78	8
5	57	71	86	79	8
6	28	84	88	86	7
7	82	87	90	89	6
<i>SD</i>	<i>29.4</i>	<i>7.5</i>	<i>4.3</i>		



**Figure 4.** Germination of one **Red** Malabar Spinach sample -2 after 7, 14, and 21 day at 20-30°C. Seeds were tested at 7 different labs. Overlap error bars indicate non-significant means.

**Table 9.** Germination of one **Green** Malabar Spinach sample -3 after 7, 14, and 21 day at 20-30°C. Seeds were tested at 7 different labs.

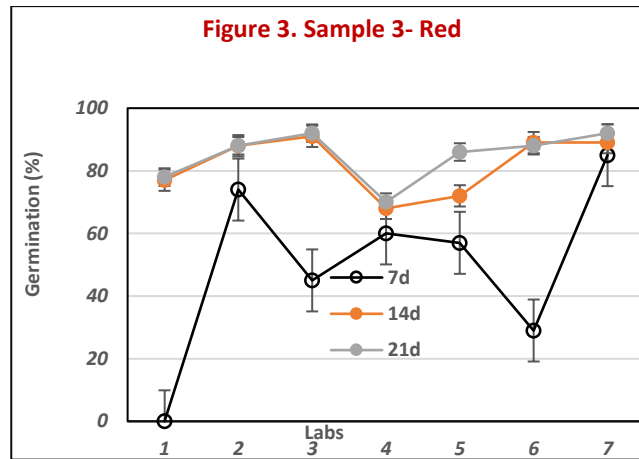
Lab	7d	14d	21d	Mean 14+21	Tolerance 14-21
1	0	41	65	53	11
2	47	78	80	79	8
3	45	74	78	76	8
4	35	53	56	55	11
5	17	47	54	51	11
6	12	63	70	67	10
7	50	64	67	66	10
<i>SD</i>	<i>19.7</i>	<i>13.7</i>	<i>9.9</i>		



**Figure 5.** Germination of one **Green** Malabar Spinach sample -3 after 7, 14, and 21 day at 20-30°C. Seeds were tested at 7 different labs. Overlap error bars indicate non-significant means.

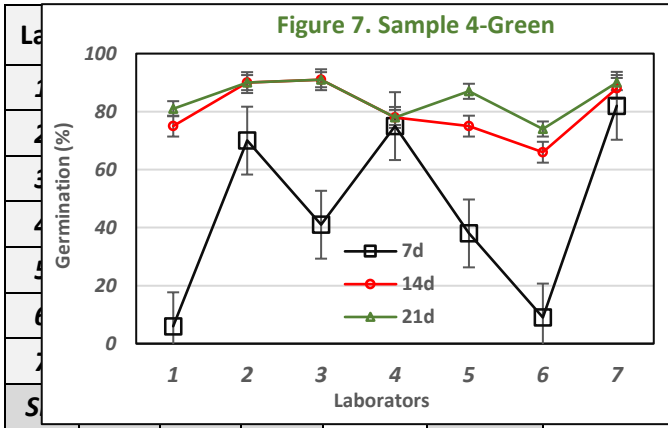
**Table 10.** Germination of one **Red** Malabar Spinach sample -3 after 7, 14, and 21 day at 20-30°C. Seeds were tested at 7 different labs.

Lab	7d	14d	21d	Mean 14+21	Tolerance 14-21
1	0	77	78	78	8
2	74	88	88	88	6
3	45	91	92	92	5
4	60	68	70	69	10
5	57	72	86	79	8
6	29	89	88	89	6
7	85	89	92	91	5
<i>SD</i>	<i>28.6</i>	<i>9.5</i>	<i>8.1</i>		



**Figure 6.** Germination of one **Red** Malabar Spinach sample -3 after 7, 14, and 21 day at 20-30°C. Seeds were tested at 7 different labs. Overlap error bars indicate non-significant means.

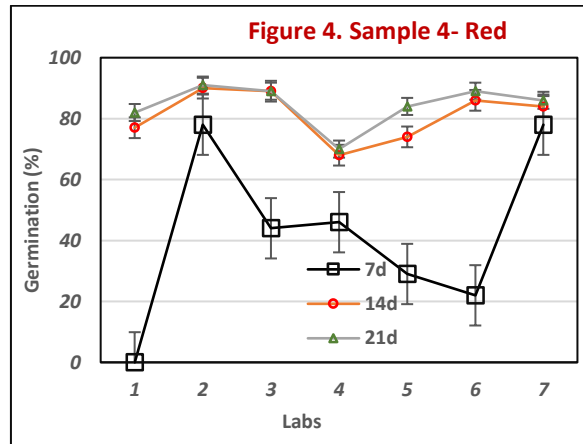
**Table 11.** Germination of one **Green** Malabar Spinach sample -4 after 7, 14, and 21 day at 20-30°C. Seeds were tested at 7 different labs.



**Figure 7.** Germination of one **Green** Malabar Spinach sample -4 after 7, 14, and 21 day at 20-30°C. Seeds were tested at 7 different labs. Overlap error bars indicate non-significant means.

**Table 12.** Germination of one **Red** Malabar Spinach sample -4 after 7, 14, and 21 day at 20-30°C. Seeds were tested at 7 different labs.

Lab	7d	14d	21d	Mean 14+21	Tolerance 14-21
1	0	77	82	80	8
2	78	90	91	91	5
3	44	89	89	89	6
4	46	68	70	69	10
5	29	74	84	79	8
6	22	86	89	88	6
7	78	84	86	85	7
SD	28.7	8.3	7.1		



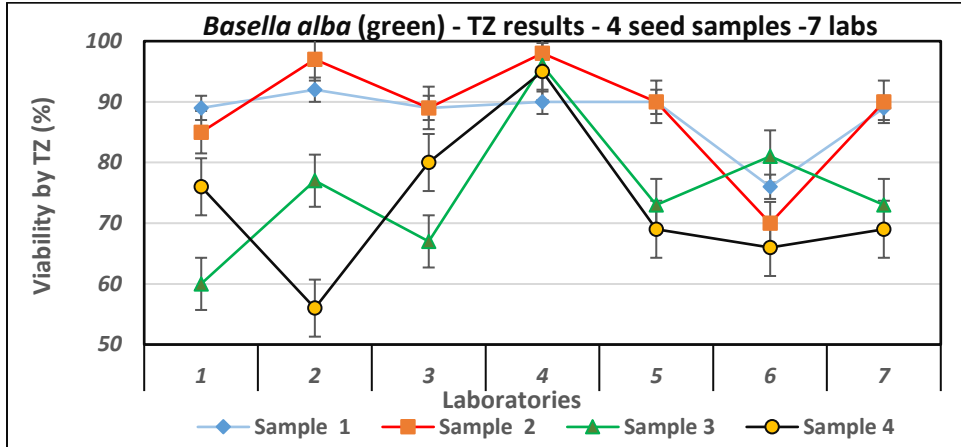
**Figure 8.** Germination of one **Red** Malabar Spinach sample -4 after 7, 14, and 21 day at 20-30°C. Seeds were tested at 7 different labs. Overlap error bars indicate non-significant means.

### V. III objective III: Tetra Zolium test results

#### *Basella alba* L. (green)

##### Laboratories

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

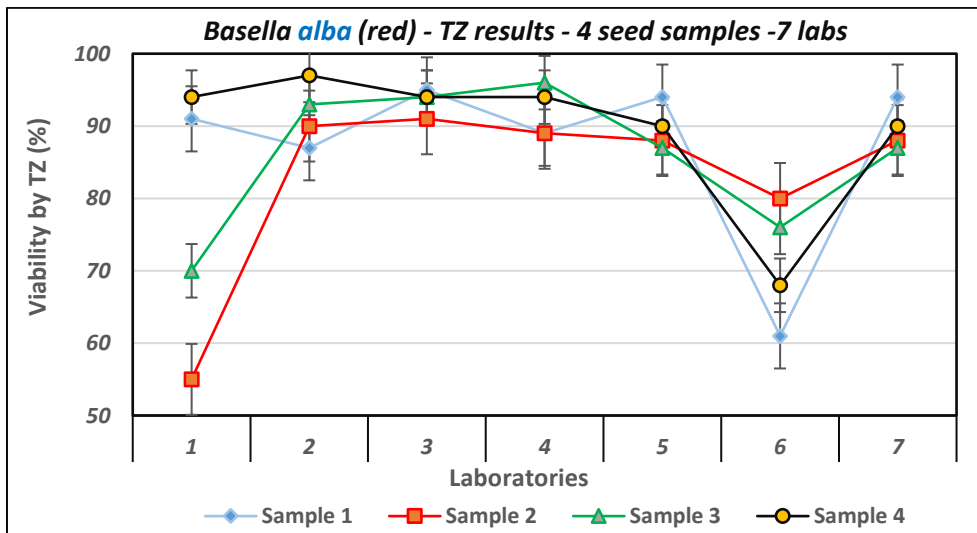


**Figure 1.** Tetrazolium test results of four green Malabar Spinach samples tested in seven seed labs. Overlapped error bars indicate non-significant means.

#### *Basella rubra-alba* L. (red)

##### Laboratories

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



**Figure 2.** Tetrazolium test results of four red Malabar Spinach samples tested in seven seed labs. Overlapped error bars indicate non-significant means.

## REFERENCES

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3. [Basellaalba.pdf \(tamu.edu\)](#) Copyright 2012 by Michael A. Arnold with all rights reserved; intended for future inclusion in Landscape Plants For Texas And Environs, Fourth Edition
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**DATE SUBMITTED: 10/15/21 Amended 5/5/2022; Sue Alvarez, co-author added**