Sequential Sampling for Seed Viability Testing at CIAT's Genebank

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Why did we choose to test sequential sampling?

- The CIAT genebank conserves very diverse materials belonging to many species → seed production often limited due to lack of adaptation to regeneration environments.
- High cost of regenerating and producing seeds from some materials like special landraces or wild species.
- Some species don't produce many seeds → need a methodology that minimizes the number of seeds used.

The acceptance sampling problem

An **acceptance-sampling plan** consists of sample size and acceptance/rejection criteria for lot sentencing

An **acceptance-sampling scheme** is a set of procedures consisting of acceptance-sampling plans in which lot sizes, batches sizes, and acceptance/rejection criteria are related

Typical application of acceptance sampling is for **lot disposition**, sometimes referred to as **lot sentencing**:

Rejected lots are sent to be *regenerated*

Accepted lots are forwarded to be *conserved*

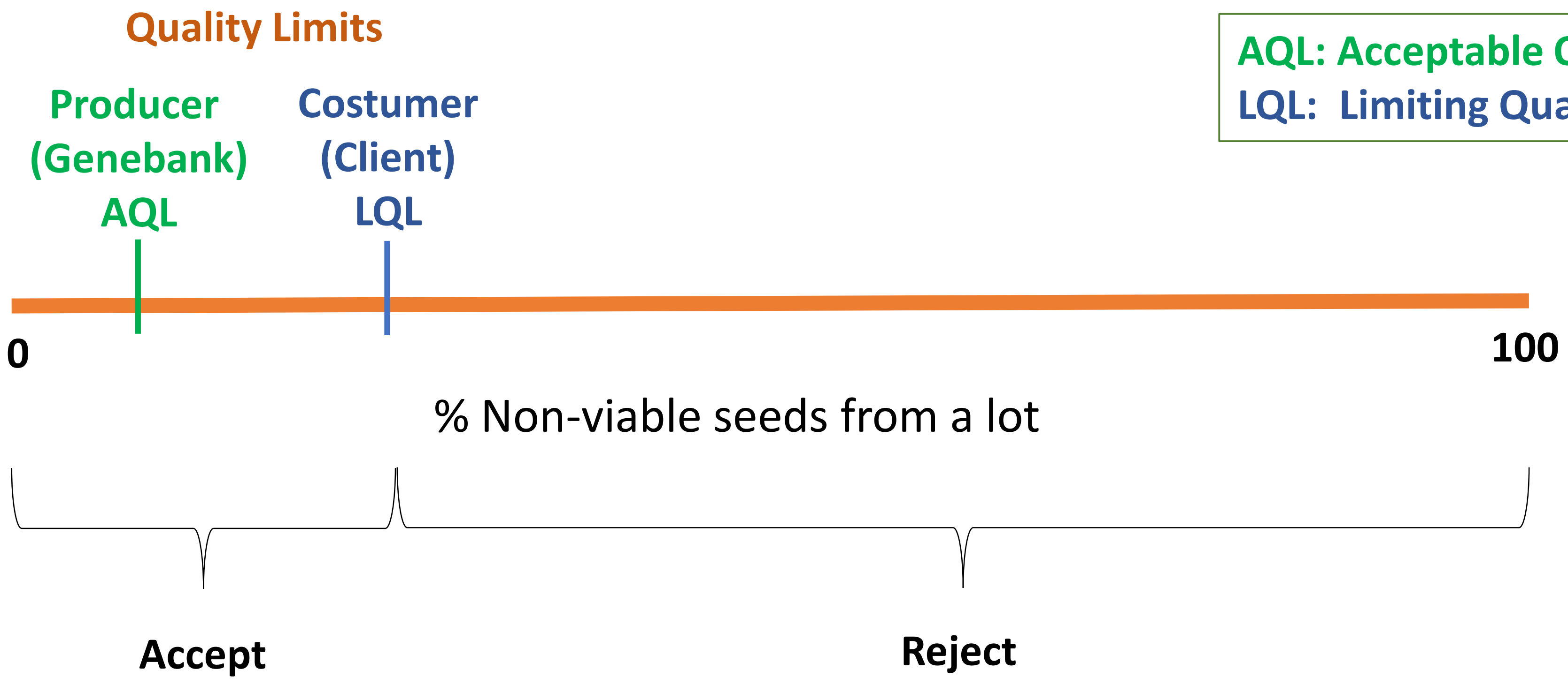
Wald's sequential sampling method

- Sampling procedure for inspection by attributes: **most widely used** acceptance sampling system for attributes
- Two key concepts: Acceptable quality level (**AQL**) and Limiting quality level (**LQL**)
- Take sequence of batches from a lot and allow the number of batches tested to be determined entirely by the results of the sampling process (until a **desired precision level** is reached)
- Estimates of very high or very low viability are promptly obtained; borderline viability estimates need more sampling
- Montgomery (2012) recommends setting up sampling at three times the number of seeds required for estimating viability using fixed-size samples (ISTA: 400 x 3 = 1,200; CIAT: 50 x 3 = 150)

Introduction to Statistical Quality Control, 7th Edition by Douglas C. Montgomery.

Sequential sampling plan:

AQL: Acceptable Quality Level
LQL: Limiting Quality Level



Minimize:

Type I error (genebank's risk): reject a "good" lot **%Non-viable seeds < AQL**

and

Type II error (client's risk): accept a "bad" lot **%Non-viable seeds > LQL**

Source: Duque M.C, 2010. CIAT

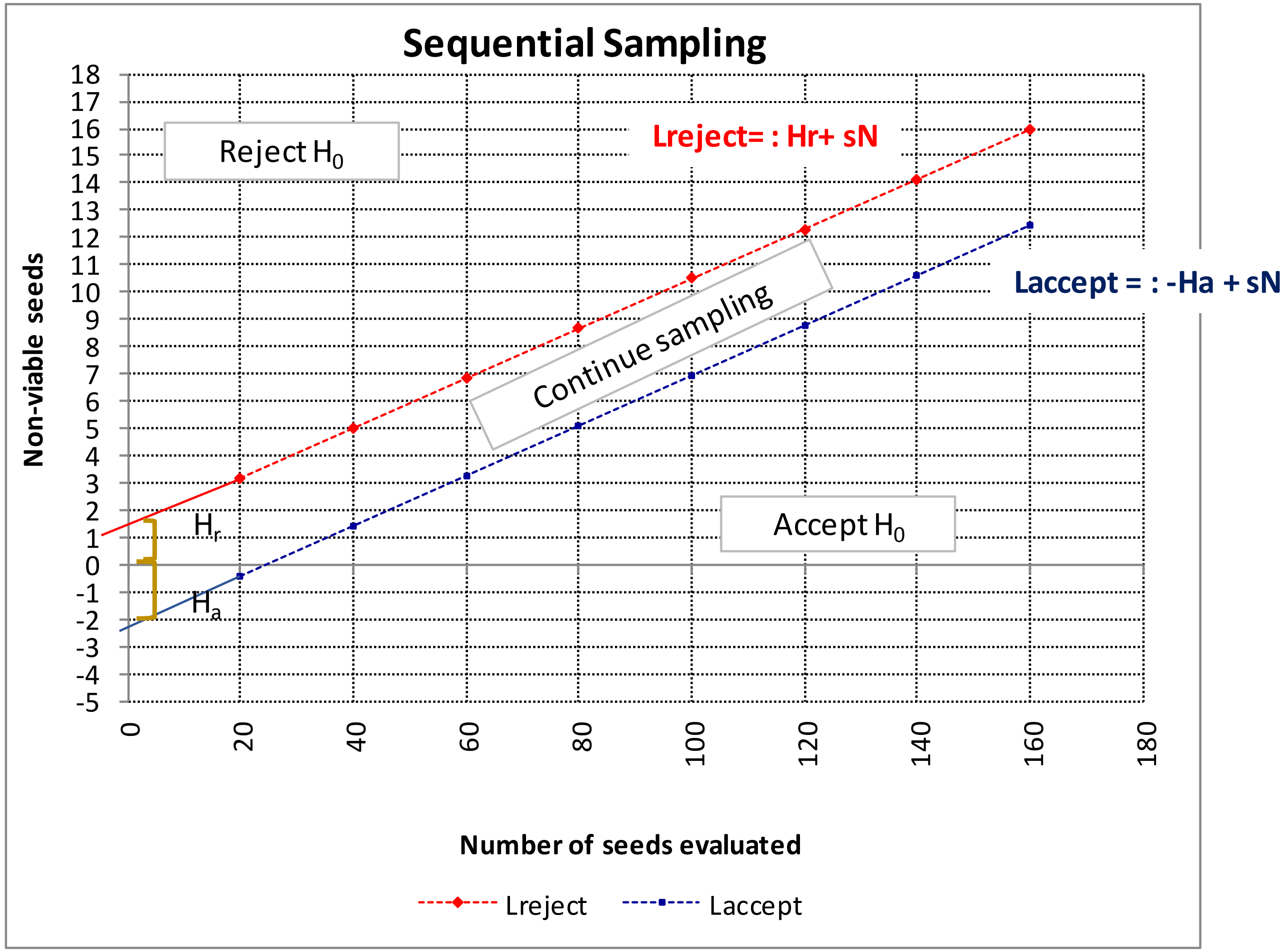
Step-by-step procedure

1. Determine the AQL depending on species and lab
2. FAO Norms (2014): LQL = 15% non-viable seeds
3. Select a preferred batch size
4. Set the null hypothesis, selecting an acceptable Type I error level (alpha = genebank risk)
5. Set the alternative hypothesis, selecting an acceptable Type II error level (beta = client risk)
6. Create table and graph using appropriate formulae
7. Perform viability test sequentially until a decision is reached

H_0: The lot is acceptable (%nV <= AQL)
Type I error
Alpha (genebank's risk):
Probability to conclude that the lot is not acceptable when it is acceptable

H_1: The lot is non acceptable (%nV > LQL)
Type II error
Beta (client's risk):
Probability to conclude that the lot is acceptable when it is not acceptable

Graph & formulae



$$Laccept = -Ha + s \times n$$

$$Lreject = H_r + s \times n$$

where

$$Ha = \frac{\ln\left(\frac{1-\alpha}{\beta}\right)}{k} \qquad H_r = \frac{\ln\left(\frac{1-\beta}{\alpha}\right)}{k}$$

$$k = \ln\left(\frac{\Pr \times (1-Pa)}{Pa \times (1-\Pr)}\right)$$

$$s = \frac{\ln\left(\frac{1-p_a}{1-\Pr}\right)}{k}$$

Implementation for testing beans at CIAT

H_0: The lot is Acceptable (%nV<=AQL)
AQL : Acceptable Quality Level
Type I error
Alpha : Genebank's risk
Probability to conclude that the lot is not acceptable when it is acceptable

H_1: The lot is non acceptable (%nV>LQL)
LQL: Limiting Quality Level
Type II error
Beta: Client's risk
Probability to conclude that the lot is acceptable when it is not acceptable

Type I error	Type II error	Pa	Pr
Alpha	Beta	AQL	LQL
0.20	0.05	0.05	0.15
1-alpha	1-beta	1-pa	1-pr
0.8	0.95	0.95	0.85

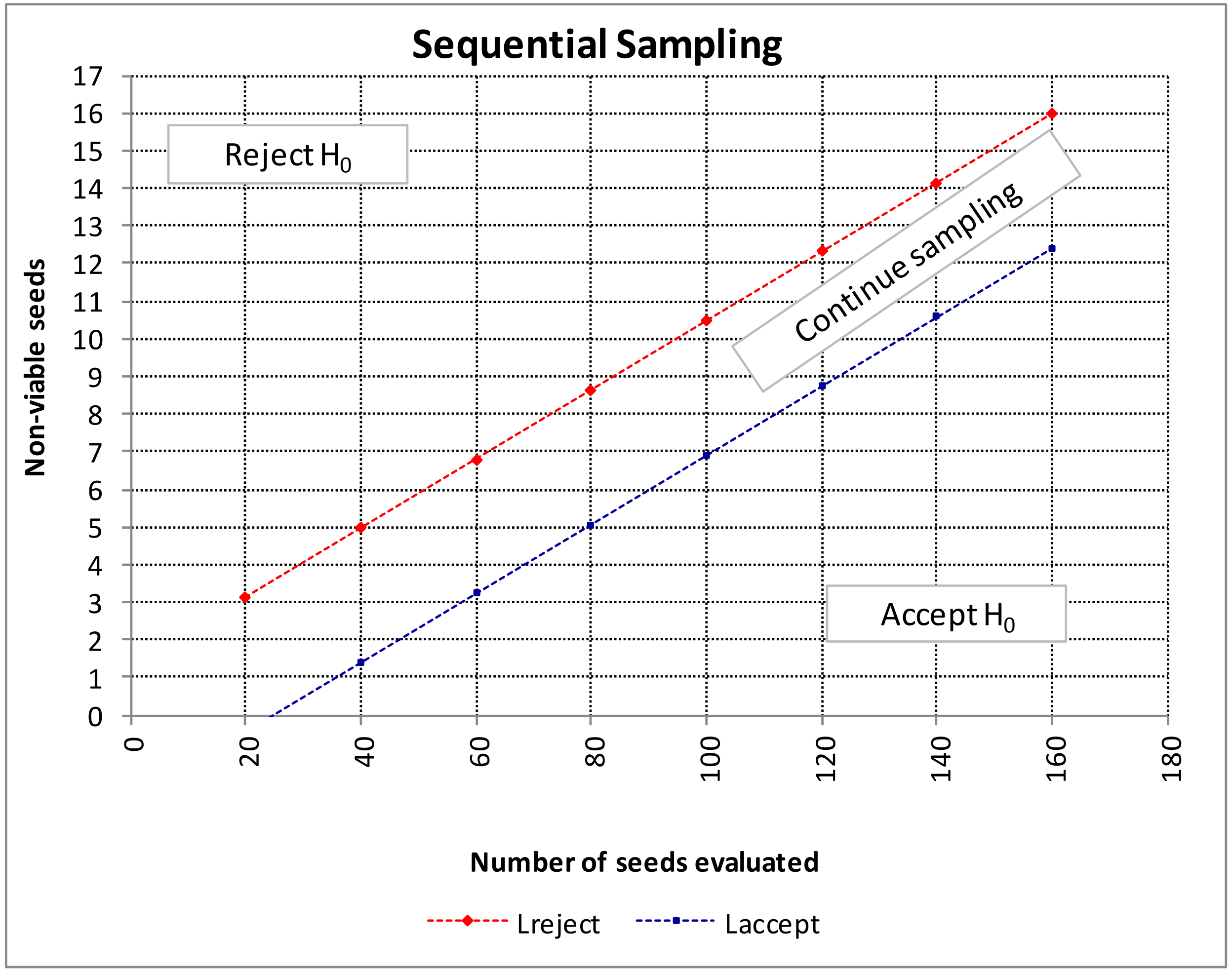
Help:	
Num Ha	1.20412
Num Hr	0.6767
Num S	0.0483
den=k	0.5254
Ha	2.2917
Hr	1.2879
S	0.0919

Wald Lines
Laccept : -Ha + sN
Lreject: Hr + sN

Source: Santos et al 2017. CIAT

Example: batches of 20 seeds…

N	Laccept	Lreject	La	Lr
20	-0,4530	3,1266	0	4
40	1,3857	4,9653	1	5
60	3,2244	6,8040	3	7
80	5,0630	8,6426	5	9
100	6,9017	10,4813	6	11
120	8,7404	12,3200	8	13
140	10,5791	14,1587	10	15
160	12,4178	15,9974	12	16
180	14,2565	17,8361	14	18
200	16,0952	19,6748	16	20
220	17,9338	21,5134	17	22
240	19,7725	23,3521	19	24
260	21,6112	25,1908	21	26
280	23,4499	27,0295	23	28
300	25,2886	28,8682	25	29
320	27,1273	30,7069	27	31
340	28,9660	32,5456	28	33
360	30,8047	34,3843	30	35
380	32,6433	36,2229	32	37
400	34,4820	38,0616	34	39



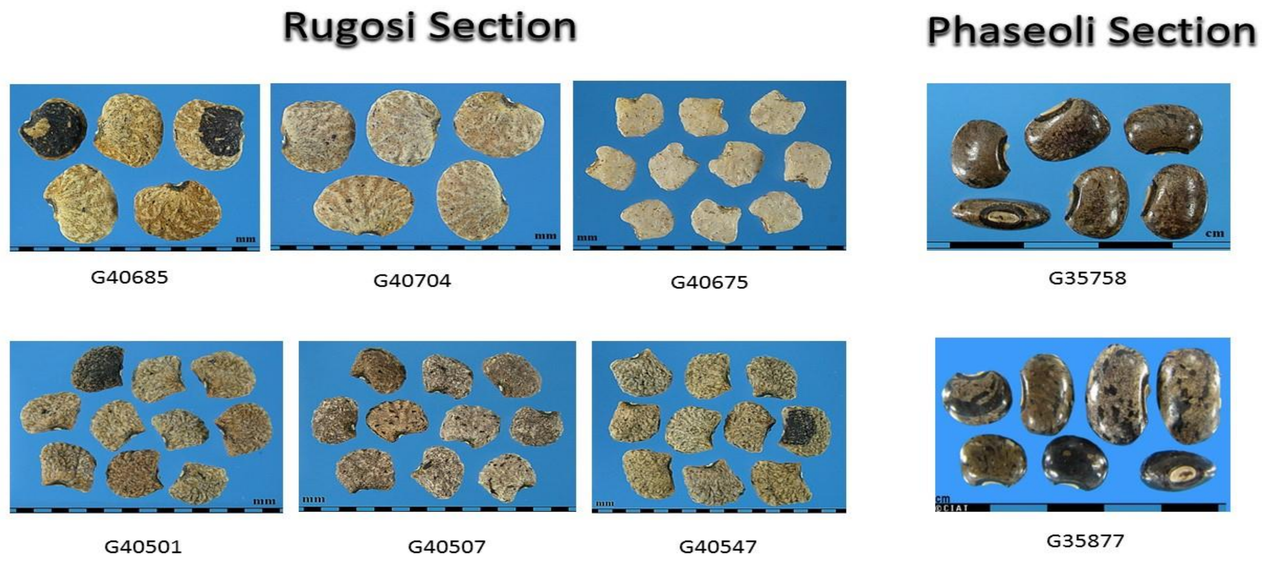
Validation of method

The experiment consisted of evaluating **560 seeds per accession**, randomly selected of each of eight accessions, using germination paper and water imbibition along with supplemental Tetrazolium tests for viability.

Three species from the *Rugosi* and one from the *Phaseoli* section of *Phaseolus*:

- *Phaseolus angustissimus* (G40685, G40704)
- *Phaseolus carteri* (G40675)
- *Phaseolus filiformis* (G40501, G40507 and G40547)
- *Phaseolus dumosus* Macfadyen (G35758 and G35877; both as wild forms)

Accessions conserved at -20ºC for 10 years (except G40704: 5ºC); all showing orthodox behavior.



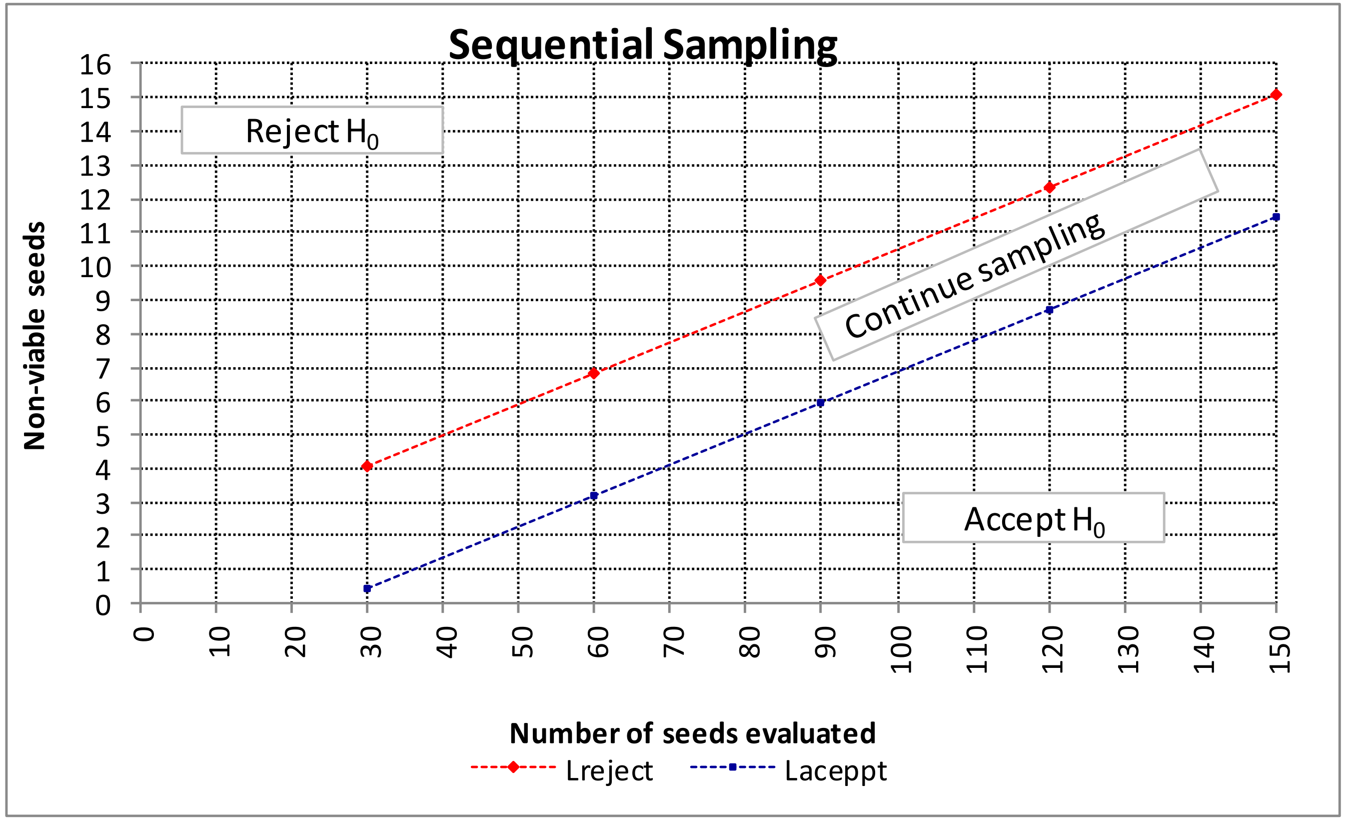
Accession	Species	Moisture content (%)	Viability (%V) /Non-viability (%nV)
G40685	*Phaseolus angustissimus*	5.1	98,4 /1,6
G40675	*Phaseolus carteri*	6.4	97,9 /2,1
G40507	*Phaseolus filiformis*	7.5	97,1 /2,9
G40547	*Phaseolus filiformis*	6.7	96,8 /3,2
G40704	*Phaseolus angustissimus*	4.2	96,8 /3,2
G35877	*Phaseolus dumosus*	8.0	95,9 /4,1
G35758	*Phaseolus dumosus*	8.0	95,4 /4,6
G40501	*Phaseolus filiformis*	5.8	88,0 /12,0

Source: Santos et al 2017. CIAT

How many seeds would have been required with sequentially sampling groups of 20, 25 or 30 seeds?

- Performed 1,000 sequential-sampling simulations with batches of 20, 25 and 30 seeds each to determine the total number of seeds required to obtain reliable viability results for each accession
- Best result: batches of 30 seeds; in many cases only 60 seeds required to accept or reject lots

N	Laceppt	Lreject	La	Lr
30	0.4663	4.0459	0	5
60	3.2244	6.8040	3	7
90	5.9824	9.5620	5	10
120	8.7404	12.3200	8	13
150	11.4984	15.0780	11	16



Second experiment: comparison fixed-sample (50 seeds) vs. sequential sampling (batches of 30 seeds)

ACCESSIONS	SPECIES	Biological Status	Number of seeds evaluated	Number of seeds Rejected	Results
G 7305	*Phaseolus vulgaris*	Cultivated	50*	4	92%
			30	0	Accepted
G 8170	*Phaseolus vulgaris*	Cultivated	50*	1	98%
			30	0	Accepted
G 8172	*Phaseolus vulgaris*	Cultivated	50*	1	98%
			30	1	Continue sampling
G 19694	*Phaseolus vulgaris*	Cultivated	50*	3	94%
			30	0	Accepted
G22949	*Phaseolus vulgaris*	Cultivated	50*	0	100%
			30	0	Accepted
G23654	*Phaseolus vulgaris*	Wild	50*	0	100%
			30	0	Accepted
G40879	*Phaseolus tuerckheimii*	Wild	50*	0	100%
			30	0	Accepted

* Fixed sample size to estimate percentages: 85% with 10% maximum permissible error and 90% confidence

Advantages & disadvantages

- In contrast to ISTA norms, Wald's sequential sampling needs fewer seeds (more seeds only used if no decision can be taken with first batch). Often, regeneration decisions can be taken with 60 seeds or less.
- Increased throughput (no. of accessions) for viability testing per staff and time.
- Enables more viability measurements over time.
- Still involves destructive testing.
- Strong dependency on random subsampling.
- Method provides binary answer: seed quality is acceptable or not. It is not designed to estimate the percentage of viable seeds.
- Ability to distinguish between low viability and procedural problems.
- This methodology is more recommended for monitoring testing.

Many thanks to Myriam C. Duque and our seed-viability team!

CIAT

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