INCREASING EFFICIENCY IN EVALUATING SEED VIABILITY OF GENEBANK MATERIALS USING WALD'S SEQUENTIAL SAMPLING

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INTRODUCTION

Due to the great number and original ecological diversity of materials maintained in genebanks, obtaining large amounts of seeds to perform viability tests as recommended by ISTA norms (2009) is a recurrent challenge. CIAT genebank developed a viability test using the principles of Wald's Sequential Sampling (Wald, 1945) which gives results using fewer seeds. Sequential sampling does not require a prefixed sample size. It takes samples until a desired precision level is reached. Estimates of very high or very low viability are promptly obtained. Viability estimates near to the limit need more sampling. The objective of this study was to further evaluate the performance of this method (it was proposed in 1980 by Ellis et al.) to meet recent FAO's Standards for Genebanks (2014) of 85% minimum germination using fewer seeds.

MATERIALS AND METHODS

Accessions of three bean species of the Rugosi section (Freytag and Debouck, 2002): *Phaseolus angustissimus* (accessions G40685 and G40704), *Phaseolus carteri* (G40675) and *Phaseolus filiformis* (G40501, G40507 and G40547) and one species of Phaseoli section: *Phaseolus dumosus* (G35758 and G3587) all with untested seed storage behavior were used (Figure 1). Seeds exhibiting orthodox behavior are known to remain highly viable after periods of storage involving drying and freezing. Most accessions were stored at -18ºC for 10 years, except the accession G40704 that was stored at 7 ± 2ºC for the same period of time.

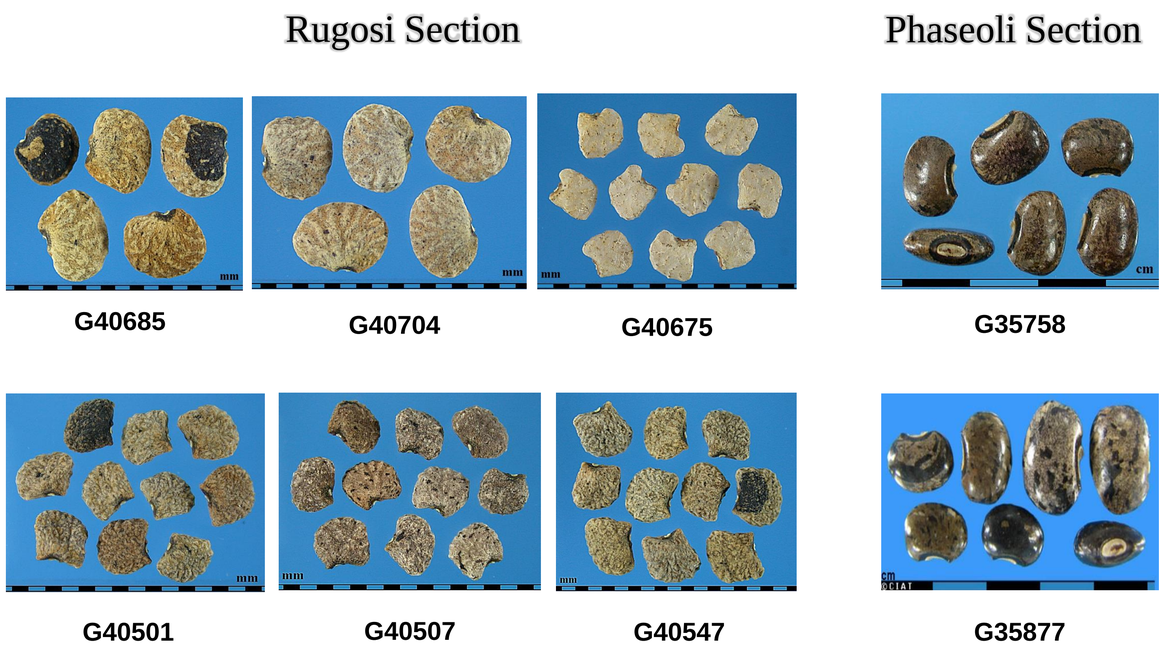

Figure 1. Bean accessions used in this study.

For the Wald's Sequential Sampling method we used the following parameters:

AQL: Acceptable Quality Level: it is fixed at 5%. This percentage is an internal standard used by CIAT genebank, where we allow to have some non-viable seeds in the sample, meaning a ceiling of 95% of viability to conserve the seeds.
LQL: Lowest Quality Level: fixed at 15%. This is the maximum percentage allowed of non-viable seeds in order to accept a batch, or the minimum quality level to conserve seeds in long term.

H_0: it refers to the first scenario where the batch of seeds is of good quality and accepted (% non-viable seeds <= AQL).
H_1: it refers to the second scenario where the batch of seeds is of bad quality and thus rejected (% non-viable seeds >= LQL).

There are then two types of errors:

Error type I: α = 20%; this error means the rejection of a batch of seeds while it is of good quality, and as a consequence, the seed accession is sent back for multiplication in the field generating ineffectiveness in time, labor, costs, etc.
Error type II: β = 5%; this error means acceptance (and thus conservation) of a batch of seeds while it is of low viability, and as a consequence, the risk is there to lose the accession.

The Sequential Sampling builds a set of equations from the parameters AQL, LQL, α and β for determining the course of the sampling. In this method, the sample is taken sequentially and seed viability is evaluated at each step. Depending on the accumulated evidence (found by sampling) and compliance with the permitted levels of error (α and β), courses of action are defined. If the assessments made provide enough evidence, one can draw conclusions and the process ends; otherwise one continues with the sampling process.

The experiment consisted of evaluating 560 seeds of each of eight accessions using germination paper and water imbibition along with supplemental Tetrazolium tests for viability. Following data collection, 1000 sequential sampling simulations were performed, by evaluating batches of 20, 25 and 30 seeds each to determine the total number of seeds required to obtain reliable results about viability for each accession.

RESULTS AND DISCUSSION

The germination tests demonstrated that all evaluated accessions had orthodox seed storage behavior, having viability ranging from 88% to 98.4% after 10 years of storage at -18ºC (and 7 ± 2ºC in one case), while they were conserved with moisture contents between 4.2% and 8.0% (Table 1).

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

Table 1. *Phaseolus* accessions evaluated for viability and respective percentage of non-viability.

From the real data, the 1000 simulations using Wald's Sequential Sampling indicated (Table 2) that at least in 700 cases of them, two consecutive independent tests using 30 seeds each, rather than 20 or 25, already sufficed to identify accessions with high viability (and thus sent to long term storage), and accessions with low viability (sent to regeneration). Accessions in-between (close to LQL) require to continue with sequential sampling. In this case, if there is any dormancy (often found in wild species), the seeds are kept in temporary storage (+5ºC) for five years, before continuing with the sampling. All cases can be resolved using a maximum of 160 seeds if we use 8 batches of 20 seeds, or 150 seeds in batches of 25 or 30 seeds, respectively.

Implementation of this method of seed viability testing can improve the efficiency of seed storage in the genebank and avoid wasting large amount of seeds.

Quantity of seeds using in testing	Percentage of non-viable seeds								Step	Batch
	1,60	2,10	2,90	3,20	3,20	4,10	4,60	12,00		
	G40685	G40675	G40507	G40547	G40704	G35877	G35758	G40501		
	Percentage of resolved simulations									
20	0,0	0,1	0,0	0,1	1,5	0,0	0,1	4,1	1	20
40	77,5	54,4	68,4	69,2	47,4	34,9	55,4	68,6	2	20
60	98,4	97,3	90,9	95,7	89,7	83,5	88,4	81,9	3	20
80	100,0	99,6	99,1	100,0	98,4	98,6	95,4	87,3	4	20
100	100,0	100,0	99,5	100,0	98,9	99,8	96,2	90,3	5	20
120	100,0	100,0	99,9	100,0	99,7	99,8	98,1	93,2	6	20
140	100,0	100,0	100,0	100,0	100,0	99,8	99,2	94,1	7	20
160	100,0	100,0	100,0	100,0	100,0	100,0	100,0	100,0	8	20
25	88,4	47,3	19,9	50,0	22,0	74,3	22,5	42,3	1	25
50	99,1	97,3	64,6	86,0	64,6	89,9	64,6	61,7	2	25
75	100,0	100,0	98,3	98,4	88,7	96,5	88,7	73,5	3	25
100	100,0	100,0	99,6	99,9	97,8	98,6	97,8	83,7	4	25
125	100,0	100,0	99,9	100,0	99,6	99,8	99,6	89,8	5	25
150	100,0	100,0	100,0	100,0	100,0	100,0	100,0	100,0	6	25
30	39,8	36,8	28,3	58,0	32,1	39,4	27,1	20,2	1	30
60	99,6	95,1	94,4	96,8	93,6	87,2	83,2	69,9	2	30
90	100,0	100,0	99,8	99,6	98,3	96,4	90,3	85,0	3	30
120	100,0	100,0	100,0	100,0	100,0	99,2	98,2	89,8	4	30
150	100,0	100,0	100,0	100,0	100,0	100,0	100,0	100,0	5	30

Percentage of resolved simulations
White: <50%
Yellow: (50%-90%)
Green: ≥90%

Table 2. Percentage of resolved simulations with data of the evaluated accessions, depending on the total number of seeds used in the testing, size of batch and percentage of non-viable seeds.

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