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Seed Academy

How Adventitious Presence Contamination Level is Estimated by Seedcalc Application

Seed tested by PCR, for GMO, is routinely conducted by amplification of the common promoter and terminator genes which are used in the insertion of the transgenic DNA. Known as 35S and TNOS, at least one of these genes are utilized for the insertion of current transgenic events of corn. After DNA is extracted from the ground seed material 35S and TNOS specific primers, under correct conditions, will only amplify DNA that has a complementary DNA code to detect a transgenic event.

In 2001, the International Seed Testing Association (ISTA) Statistics Technical Committee released the Seedcalc Excel program. This application was developed by industry statistical experts to estimate the purity/impurity percentage in a lot or sample when results are available. Version 8 is used globally as a standard in GMO contamination estimates and is available at https://www.seedtest.org/en/statistical-tools-for-seed-testing-_content---1--3449--1102.html.

Definitions

Pool size – Number of seeds that are ground at one time, typically 200, 300, 500 or 1000

Qualitative test– Result for a pool is either positive or negative for the presence of 35S or TNOS

Seedcalc- Statistical application in Excel published on ISTA website available to industry to estimate the purity/impurity percentage in a lot or sample based on number of seeds tested, pool size, total number of pools and number of pools testing positive

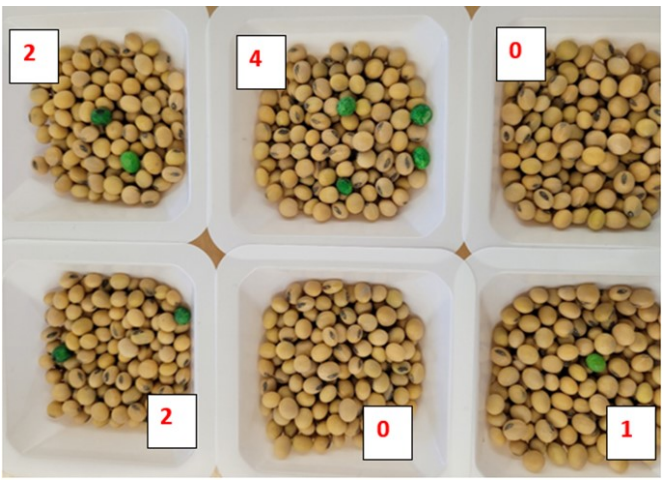

Figure 1. Examples of 6 pools of 200 seed pools and contaminate level (demonstrated by the contrasting color seed representing the contaminate)

Key Requirements for Estimating GMO Contamination by Seedcalc

One pool needs to be negative for the qualitative test to estimate a GMO contamination level. When determining the number of seeds per pool, the contamination level needs to be considered to attain a negative pool (Table 1).

Table 1. The estimated number of transgenic seeds for a given pool size based on theoretical GMO contamination level.

Pool Size/# seeds	GMO Contamination Level		
	0.6%	0.9%	1.5%
100	<1	<1	1
200	1	2	3
334	2	3	5
500	3	>4	7

In Table 2, a seed lot is tested with nine pools of 334 seed for a total of 2997 seed tested. Eight pools are positive and Seedcalc estimates the GMO contamination level as 0.66%. If the number of pools decrease to six and one pool is negative, the Seedcalc estimate would be 0.54%.

Table 2. Estimated Seedcalc GMO% of different seeds/pool and number of pools with at least 1 negative pool detected.

Seeds/ Pool	Scenario1 # pools	Max Seedcalc Est GMO% with 1 neg pool	Scenario2 #pools	Max Seedcalc Est GMO% with 1 neg pool
500	**6**	**0.36**	**--**	**--**
334	**9**	**0.66**	**6**	**0.54**
200	**15**	**1.34**	**6**	**0.89**

When using pools, it is important to select a pool size that will likely yield at least one pool being negative. In Table 3, it shows that a 0.5% GMO contamination level seed lot was able to be estimated with nine pools of 334 seed but not when six pools of 500 seed was tested.

Table 3. Example of 3000 seed sample with 0.5% level of contamination, different pool sizes, the number of contaminates per pool and estimated Seedcalc GMO % contamination level.

Pool Size	Number of Pools	Number of contaminates per pool	# positive pools/ # pools	Seedcalc GMO % Estimated
200	15	0/4/0/1/0/2/2/0/1/0/2/1/1/0/1	9/15	0.46
333	9	2/1/2/2/0/2/1/1/4	8/9	0.66
500	6	3/1/1/3/3/4	6/6	No Negative Pool