Towards the validation of the controlled deterioration vigour test for small seeded vegetables

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The validation procedures for new laboratory tests of seed quality by ISTA have been formally laid out in the Handbook of Method Validation (ISTA, 2005) In the ISTA context, there are two main components of method validation applicable to vigour tests. First, the description of the method should be clear and complete, so as to give reliable and reproducible results, and second, the relationship between the results of the vigour tests and a practical expression of vigour should be confirmed. Currently two vigour tests (conductivity for *Pisum sativum* and accelerated ageing for *Glycine max*) have been validated by inclusion in the ISTA Rules. This article describes the work that has been completed at the University of Aberdeen and by members of the Controlled Deterioration Working Group of the ISTA Vigour Committee towards the validation of a third vigour test, the Controlled Deterioration test for small seeded vegetable species.

The history of the Controlled Deterioration (CD) vigour test goes back more than 25 years (Matthews, 1980). As the name suggests the test involves the deterioration of samples of seeds from seed lots in a precise and controlled manner at an elevated moisture content (dependent on the species, often 20%) and temperature (45°C) for a defined duration; for convenience 24h is preferred (Figure 1).

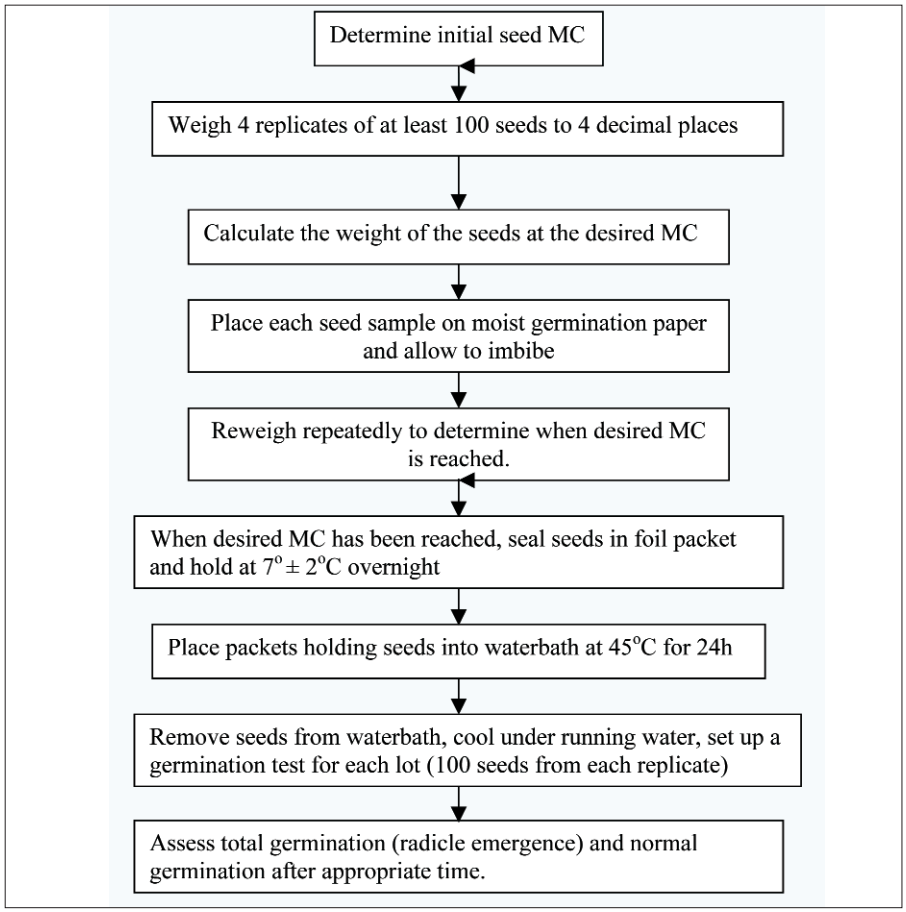

Figure 1. Stages in the completion of the Controlled Deterioration vigour test

Theoretical basis of CD:

The theoretical basis of the test lies in the seed survival curve (Figure 2). Seed lots having similarly high and commercially acceptable levels of germination would be located on the slow initial decline of the curve (e.g. A, B and C). The small differences in mean germination of such seed lots are not, however, clearly differentiated on the basis of the 400 seed sample used to estimate the population mean. However, if samples of lots A, B and C are deteriorated rapidly to exactly the same extent, then their germinations after deterioration are contrasted (Figure 2). For example, lot A compared with C: after a period of deterioration C would be placed well down the slope of the survival curve while A would remain near the top.

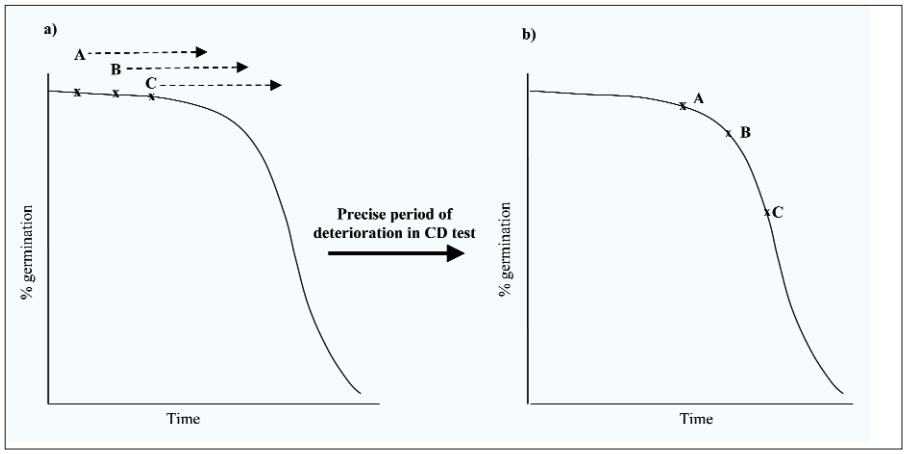

Figure 2. The theoretical basis of the Controlled Deterioration test lies in the seed survival curve and seed ageing.

Thus, CD increases the range of germination between the seed lots and the differences in germination after deterioration reflect the initial position of the seed lot on the survival curve i.e. the degree of ageing within the sample. Since ageing is the main cause of differences in seed vigour, the germination after CD reflects the vigour of the seed lot.

Relationship of CD test results to expressions of vigour:
Clear differences in germination after CD have been reported amongst commercial seed lots with high germination (Matthews, 1980). The CD results were statistically significant indicators of field emergence in each of two years in nine crops, including a number of small seeded vegetable species (turnip, swede, kale, sprouts, carrot, lettuce and onion). Standard laboratory germination was also often significantly related to field emergence, but, in general, not as closely as CD germination, which gave a much clearer separation between lots emerging well and those emerging poorly (Matthews, 1980). The relationship between field emergence and CD was supported by the work of the CD Working Group, when three laboratories carried out emergence trials in 1999, and by the work of Powell and Dutton (1984). In addition the CD Working Group noted a correlation between the CD test result and the rate of emergence, as also reported in oilseed rape (*Brassica napus* subsp *oleifera*) (Larsen et al.). Correlations between CD results and field emergence have also been seen in red clover (Wang et al, 1994), vining peas (Bustamente et al, 1984), combining peas (Powell et al., 1997) and Italian ryegrass (*Lolium multiflorum*; Marshall and Naylor, 1985) and in the emergence of cabbage in compost trays (Strydom and Van der Wenter, 1998).

Vigour differences not only cause problems in field sowings, but also under controlled glasshouse production. Comparisons of commercially acceptable lots of Brassica crops (cauliflower, sprouts, cabbage and calabrese) in glasshouse modules showed how germination after CD was related to seedling performance (emergence, rate and spread of emergence, and variation in seedling size) (Powell et al., 1991). Similar findings were reported recently for aubergine (Demir et al., 2005).

Another practical expression of seed quality indicated by the CD test is seed longevity (Figure 3) with a clear relationship reported between the results of the CD test before storage and germination after commercial storage of 15 seed lots of onions (Powell and Matthews, 1984a) and 29 lots of Brussels sprouts (Powell and Matthews, 1984b). The

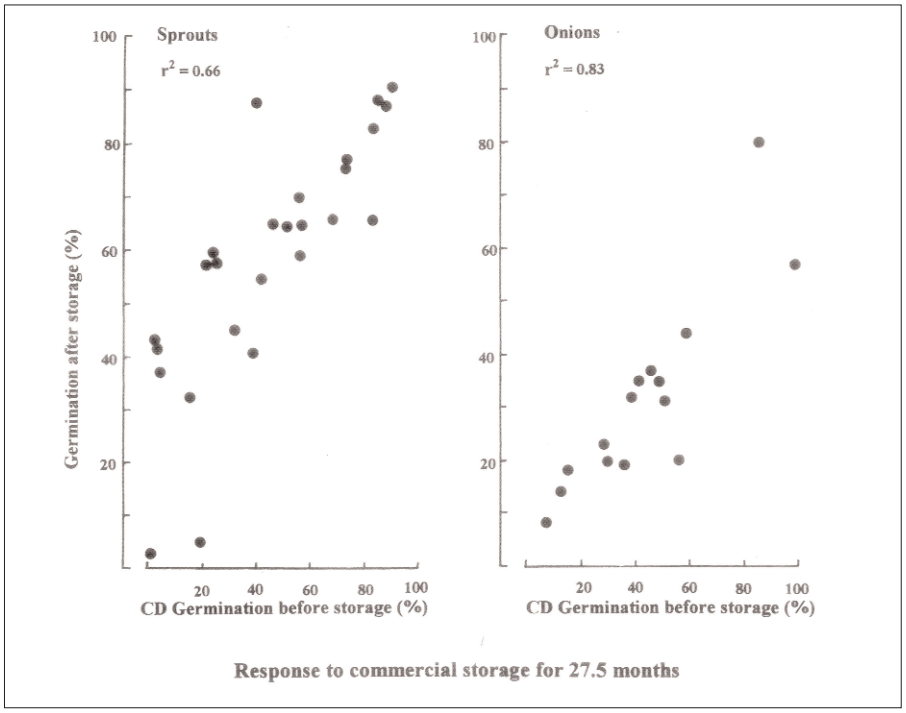

Figure 3. The relationship between the results of the CD test before storage and germination of seed lots of Brussels sprouts and onions after storage for over 2 years in commercial storage conditions.

storage potential of rye during 80 days natural storage was also predicted by CD (Steiner and Stahl, 2002), as was that of *Arabidopsis thaliana* (Tesnier et al., 2002).

Precision, repeatability and reproducibility:
The reliability of the CD test was first shown by Powell and Matthews (1981). Subsequently evidence from six laboratories from official seed testing stations and seed companies also demonstrated repeatability within, and reproducibility between, laboratories when ten seed lots each of swede and onions were tested in each of three test runs (Powell et al., 1984). Subsequent work by members of the CD Working Group over nine years (Table 1) has confirmed the repeatability and reproducibility of the test using a range of Brassica seed lots.

As in any vigour test, precision is required in aspects of the CD test. Precision is required to achieve the same seed moisture contents (MC) in seed lots at laboratory temperature **before** seeds are deteriorated rapidly at the high temperature (45°C). The adjustment of seed MC before deterioration (Figure 4) was adopted following the observation that when small seeded vegetables were deteriorated in a moist atmosphere at high temperature, as in the accelerated ageing test, the rate of increase in moisture differed between lots. This resulted in differences in the extent of deterioration (Powell, 1995). Precision is needed in the adjustment of the MC, since a 1% difference in MC has a clear and striking effect on the final germination (Table 2).

Different methods of raising seed MC content have been investigated in three series of comparative tests (Table 1). All methods require determination of the initial seed MC, then calculation of the weight that a sample of seeds would have at the desired MC. The attainment of this MC (seed weight) was achieved by imbibition from moist filter paper, or by addition of the required amount of water to achieve this weight increase, eit-

Figure 4. Raising the seed moisture content for the CD test

Table 1. Comparative tests of CD conducted by the ISTA Vigour Committee

Experimental series	Laboratories[1]	Crops	No. of lots	No. of repeat runs	Aspects of the test evaluated
1996-1998	DK, FR, H, K, GB1	*Brassica napus var napobrassica*	5	3	- Method of raising seed MC (filter paper and water added to foil packet) - Repeatability - Reproducibility· - Counts of radicle emergence vs normal germination
		Allium cepa	4		
1998-2001	DK, FR,K, GB1	*B.napus var napobrassica*	5	3	- Method of raising seed MC (filter paper and water added to foil packet) - Repeatability· - Reproducibility· - Counts of radicle emergence vs normal germination - Relationship between CD results to sto rage potential and field emergence.
2001-2004	DK, FR,GB1, GB2, IT	*B. napus subsp oleifera*	6	2	- Method of raising seed MC (filter paper and water added to revolving container) - Repeatability· - Reproducibility· - Counts of radicle emergence vs normal germination· - Relationship between CD results to field emergencece.

[1]DK: Plant Directorate Copenhagen; Fr: GEVES-SNES, Angers; H: OMMI, Budapest; K: Dept of Agronomy, Gyeongsand National University; GB1: Dept of Agriculture and Forestry, University of Aberdeen; GB2: OSTS, Cambridge; It: LaRAS, University of Bologna

her with the seeds retained in a foil packet during equilibration, or in a constantly revolving container. Comparative tests revealed that increasing the seed MC by imbibition on filter papers gave consistently the most repeatable results. This was recently confirmed by Wagner et al. (2004) who found that the filter paper method gave the overall best results for achieving any seed MC in four species, including rape seed.

A second aspect of precision that is important in the CD test is achievement of the high temperature of 45°C. Close temperature control is necessary to ensure that the same degree of deterioration is imposed on all seed samples, particularly when the seeds have a raised MC. This is accurately achieved (± 0.5°C) using a water bath, a readily available and relatively inexpensive item of equipment.

In their work on standardisation of the CD test (Table 1), the Vigour Committee have also examined the assessment criteria for germination after CD, as total germination (radicle emergence) or as normal germination. Both methods of evaluation resulted in good repeatability and reproducibility, with, predictably, a greater range in normal than total germination. Both germination assessments correlated with field emergence in 1999, with neither method of assessment giving a better correlation with field emergence, as was also found in red clover (Wang et al., 1994). The recommendation from these observations is that both normal and total germination should be reported. The spread in normal germinations is likely to be greater when the lots being compared are not very different in vigour and the moisture content during deterioration is not sufficiently high to clearly distinguish seed lots in terms of total germination (Table 2). Reporting both assessments would also confirm that differences in vigour are related to the relative position of lots on the survival curve.

Species applicability:

CD has identified differences in vigour in a range of small seeded vegetable species (Matthews, 1980; Powell et al., 1981; Powell and Matthews, 1981a,b; Demir et al., 2005), grasses (Marshall and Naylor, 1985) and red clover (Wang et al., 1994). However, the test is likely to be suitable for any species whose shape or size allows imbibition of water to an appropriate seed MC within a few hours. A number of authors have applied the test to large seeded species, such as peas and soyabean. Our experience in Aberdeen is that while this may be useful in research, the time taken to raise seed MC makes use of the test for large seeded species inappropriate in a routine seed testing context, especially when convenient alternative vigour tests are available.

Table 2. Effect of seed moisture content during CD test on total germination (% radicle emergence) of turnip seed (taken from Powell and Matthews, 1981 with permission of Seed Science and Technology).

Seed lot	Seed moisture content			
	Initial (7%)	20%	21%	22%
1	100	100	96	89
2	98	96	92	87
3	97	94	89	78
4	99	96	80	72
5	96	80	43	25
6	90	24	3	1

The future:

There is detailed evidence that the CD test satisfies the criteria required for validation as an ISTA test. The evidence summarised above is especially convincing and extensive for Brassica species. The Vigour Committee is therefore currently preparing a submission to the ISTA Method Validation Programme for application of the Controlled Deterioration test to Brassica species.

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