Towards automated single counts of radicle emergence to predict seed and seedling vigour

Stan Matthews[1] and Alison Powell[2]
[2]Member, ISTA Executive Committee; [2]Chair and [1]Member, ISTA Vigour Committee

School of Biological Sciences
University of Aberdeen
Aberdeen, AB24 3UU, United Kingdom
a.a.powell@abdn.ac.uk

The radicle emergence test for *Zea mays* is an ISTA-validated vigour test (Matthews *et al.,* 2011b) which was accepted into the ISTA Rules at the Annual Meeting in Zurich in June 2011. This article will:

- briefly describe the background to the radicle emergence test;
- provide evidence in support of the potential widespread use of radicle emergence as a vigour test for many species;
- consider how this test can make use of automated methods;
- compare this approach to other automated methods for seed quality evaluation and improvement.

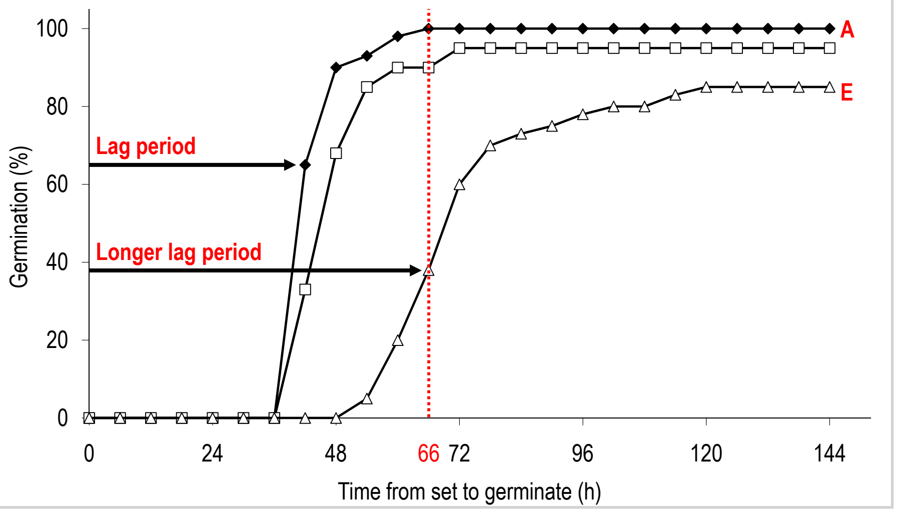

Figure 1. Germination progress curves at 20 °C for commercial seed lots of maize differing in vigour and field emergence. The vertical line at 66 h indicates the appropriate time for a single count of germination (radicle emergence).

Germination progress curves, mean germination time and seed ageing

Germination curves, produced following regular counts of germination (radicle emergence) during a germination test, are familiar to most seed scientists. Such curves differ between seed lots, even amongst those having high standard (normal) germination, as seen for three commercial seed lots of maize (Fig. 1). The regular germination counts can also be expressed as the mean germination time (MGT) (Ellis and Roberts, 1980). The MGT describes the average time for a seed to germinate, or the delay (lag period) from the start of imbibition to radicle emergence. In Figure 1, seed lot E has the longest average delay (high MGT), is the latest to start to germinate and has the greatest spread of germination over time.

The differences seen in the germination curves described by the MGT have been related to the seed vigour of maize, expressed as both the rate and final level of emergence (Khajeh Hosseini *et al.*, 2009; Matthews *et al.*, 2011a). For example, in Figure 1, lot A with a small MGT (lag period) emerged rapidly with a high final emergence, while lot E with a larger MGT emerged more slowly to a lower final emergence. This relationship between MGT and emergence performance is also significant in a wider range of species, from cotton to viola (Table 1, columns A and B). Thus, where germination was slower (high MGT), the rate of emergence was also slower and emergence was lower, i.e. the seed lot had low vigour.

Table 1. Significance of correlation coefficients between mean germination time (MGT) and A) rate of emergence (field or glasshouse transplants), B) final emergence and C) germination (%) after accelerated ageing (AA) or controlled deterioration (CD). Numbers in parentheses indicate the number of lots from which the correlation was calculated

Species	A: Rate of emergence	B: Final emergence (%)	C: germination (%) after AA or CD test	Source
Maize	*	** (9)	* (9)	1
Cotton	ND	*** (13)	*** (13)	2
Pepper	***	* (11)	** (5)	3
Watermelon	**	*** (10)	*** (10)	4
Melon	**	*** (10)	*** (10)	4
Cucumber	**	*** (9)	*** (9)	4
Oil seed rape	***	** (11)	ND	5
Viola	***	*** (9)	*** (9)	6

ND: not determined
Significances: *P < 0.05; **P < 0.01; ***P < 0.001
Source: 1. Matthews *et al.*, 2011a; 2. S. Matthews, B. Ross and P. Steele (Cotton Seed Distributors, Australia) unpublished data; 3. Demir *et al.*, 2008; 4. Mavi *et al.*, 2010; 5. McLaren *et al.,* 2010; 6. Demir *et al.*, 2011

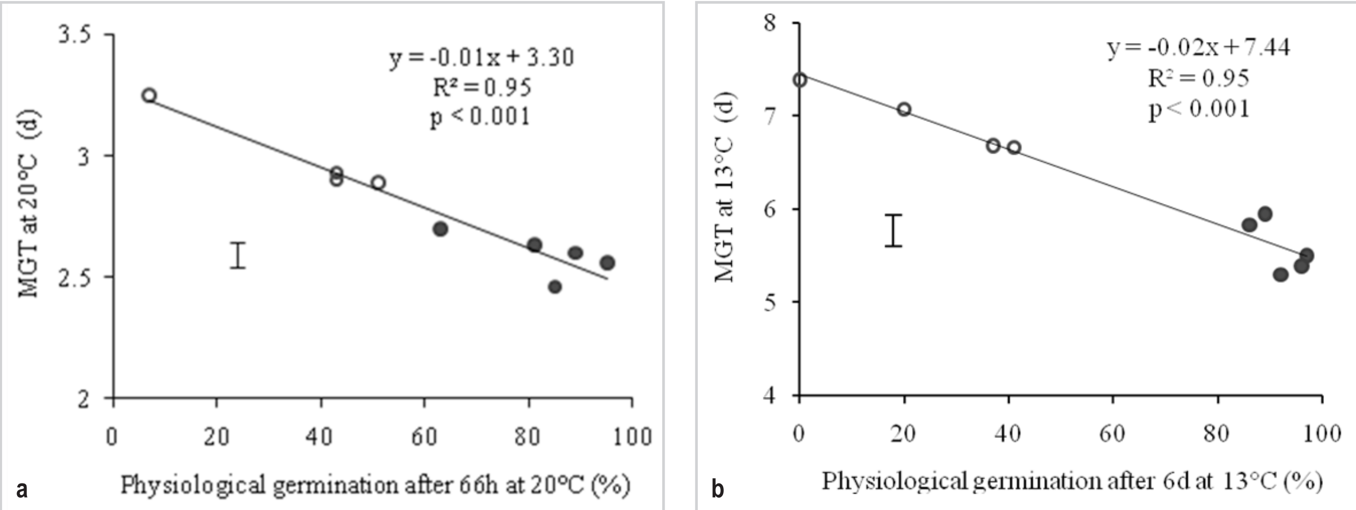

Figure 2. Relationships between physiological germination (radicle emergence) made after 66 h at 20 °C (a) and 6 d at 13 °C (b) and mean germination time at the same temperatures for nine seed lots of maize. Open circles are for four seed lots significantly lower in germination than the five highest germinating lots at each temperature, as determined by LSD ($P < 0.05$) following ANOVA of arc-sine transformed percentages. Vertical bars are the LSDs for MGT following ANOVA. Each point is a mean of 4 replicates of 25 seeds (taken from Matthews *et al.*, 2011a).

The major cause of differences in vigour is seed ageing, leading to seed deterioration. One of the first effects of ageing is an increase in MGT (Guy and Black, 1998; Bailly *et al.*, 2002). Thus, ageing and the resultant seed deterioration would result in the curve for lot A becoming similar to the curve of lot E (Fig. 1). Differences in the initial level of deterioration or ageing of seed lots can be identified by two of the ISTA-validated vigour tests, accelerated ageing (AA) for *Glycine max* and controlled deterioration (CD) for *Brassica* spp. (ISTA 2011). The levels of deterioration in commercial seed lots, measured using these ageing tests, have been consistently and significantly correlated with MGT in a large number of species (Table 1, column C). The more deteriorated the seed lots, the higher the MGT, that is, the greater the lag period from the start of imbibition and radicle emergence. This association between deterioration and the length of the lag period has been explained by the need for more time for metabolic repair in the more deteriorated seeds before germination processes can begin (Matthews and Khajeh Hosseini, 2007; Matthews *et al.*, 2011a).

Thus, the germination progress curves and MGT describe the extent of deterioration in a seed lot, and hence seed vigour.

Single counts of radicle emergence, MGT and vigour

The periodic counting of radicle emergence to produce germination progress curves and allow calculation of MGT is time consuming. However, automated methods to generate germination progress curves have been developed that involve image analysis for a time series of photographs. These are entered into a computer for storage and curve fitting (Ducournau *et al.*, 2005; Joosen *et al.*, 2010). Instead of producing a germination progress curve, a simpler approach would be to use single early counts of radicle emergence to predict MGT, and hence vigour. This was the approach behind the new radicle emergence test for maize. Close relationships were seen in maize between MGT and single counts after 66 h at 20 °C and after 6 d at 13 °C (Fig. 2), and between these single counts and emergence (Matthews, *et al.*, 2011b). These close relationships formed the basis of the new vigour test.

Evidence suggests that the relationships described for maize are common to a wide range of species. MGT has been shown to relate to the rate and final emergence in other species (Table 1, columns A and B), and the differences in the MGT, or lag period, relate to the extent of deterioration in the seed (Table 1, column C), as in maize. Furthermore, a single count is highly predictive of MGT since, as indicated by the R^2 calculations (Table 2), more than 90% of the variability in MGT was accounted for by regression on a single count for seven out of ten regressions.

Single counts of radicle emergence are also closely related to emergence performance, i.e. to seed vigour (Table 3), in the field (maize, cotton, oil seed rape, watermelon, melon, cucumber) and in seedling transplant production (viola). In canola, 2 d radicle emergence was significantly correlated with relative seedling size ($r = 0.84$, $p < 0.001$; calculated from data in Buckley and Huang, 2011).

MGT reflects the relative level of deterioration of a seed lot, as do the AA and CD tests. The close relationship between MGT and these tests (Table 1, column C), and between MGT and single counts (Table 2), highlights the possibility that single counts of germination could provide an alternative, more rapid test of seed vigour than existing vigour tests. For example, counts of radicle emergence in maize between 2 and 3 days of the standard germination test would be a more efficient use of time and space than a 10 d cold test that would be done in addition to a standard germination test (Matthews *et al.*, 2011a,

Table 2. Regression (R^2) values and significances between single counts of radicle emergence and mean germination time (MGT) for seed lots of 8 cultivated species with high standard germination (%)

Species	Lots (n)	R^2 with single counts (time of count and germination temperature)	Standard germination (%)	Source
Maize	7	0.98 (66 h, 20 °C); 0.95 (6 d, 13 °C)	>90	1
	9	0.95 (66 h, 20 °C); 0.95 (6 d, 13 °C)	>90	2
Cotton	13	0.96 (3 d, 18 °C)	>82	3
Watermelon	10	0.83 (68 h, 25 °C)	>98	4
Melon	10	0.60 (44 h, 25 °C)	>98	4
Cucumber	9	0.97 (48 h, 25 °C)	>98	4
Radish	9	0.94 (48 h, 20 °C)	>80	5
Viola	9	0.85 (48 h, 20 °C)	>82	6

Significances: R^2 all at $P < 0.001$ except cucumber where $P < 0.01$
Sources: 1. Khajeh Hosseini *et al.*, 2009; 2. Matthews *et al.*, 2011a; 3. S. Matthews, B. Ross and P. Steele (Cotton Seed Distributors, Australia), unpublished data; 4. Mavi *et al.*, 2010; 5. K. Mavi (University of Mustafa Kemal, Turkey), unpublished data; 6. Demir *et al.*, 2011

Table 3. Significance of correlations between single counts of radicle emergence and final emergence for seed lots of 8 cultivated species with high standard germination (%)

Species	Lots (n)	Time of count and temperature	Significance	Source
Maize	9	66 h, 20 °C	*	1
	9	6 d, 13 °C	**	1
Cotton	13	3 d, 18 °C	***	2
Watermelon	10	68 h, 25 °C	*	3
Melon	10	44 h, 25 °C	*	3
Cucumber	9	48 h, 25 °C	**	3
Oil seed rape	11	30 h, 20 °C	**	4
Canola	19	2 d, 20-30 °C	***	5
Viola	9	2 d, 20 °C	**	6

Significances: *$P < 0.05$; **$P < 0.01$, ***$P < 0.001$
Sources: 1. Matthews *et al.*, 2011a; 2. S. Matthews, B. Ross and P. Steele (Cotton Seed Distributors, Australia), unpublished data; 3. Calculated by Mavi from data in Mavi *et al.*, 2010; 4. McLaren *et al.*, 2010; 5. Calculated from data on seedling growth and 2 d radicle emergence in Buckley and Huang, 2011; 6. Demir *et al.*, 2011

b, c). Similarly, in the ornamental viola, a 2 d count of radicle emergence during a standard germination test can identify low-vigour lots as effectively as the saturated salt accelerated ageing test (SSAA) (Demir *et al.*, 2011). Again, this would be much quicker (2 d) than the 17-day SSAA test which would be done in addition to the standard germination test.

Seedling vigour and MGT

The contrasted germination progress curves for maize (Fig. 1) have an impact not only on emergence, but also on seedling size. Thus, there are clear differences in seedling size and uniformity of lots A and E after 14 d in rolled towels at 13 °C (Fig. 3). Lot A, with a lower MGT (from counts of 2 mm radicles), produced larger and more uniform seedlings than lot E. The same applies when an earlier criterion of germination, the first appearance of the radicle, which we have termed mean just germination time (MJGT), is used (Fig. 3). Differences in seedling growth therefore relate back to the timing of the earliest stages of germination. Differences in the length of time from radicle emergence to the measurement of seedlings seem to be the determinant of size. This general proposition was suggested by Ellis (1992) and is supported by observations on peppers (Demir *et al.*, 2008), *Brassica* spp. (Powell *et al.*, 1991) and onions (Wheeler and Ellis, 1991), as well as maize (Khajeh Hosseini *et al.*, 2009)) and canola (Buckley and Huang, 2011). In addition, the greater spread of germination over time of lower-vigour lots (e.g. E in Figure 1) results in greater variation in seedling size, as seen in Figure 3. This is particularly important in the production of uniform transplants of vegetable and ornamental species.

Development of automated methods

Computer-aided image analysis has been the most popular approach to automated germination and vigour testing (Dell'Aquila, 2007, 2009). Changes in seed dimensions and shape during imbibition have been investigated to try and identify germinable seeds before radicle protrusion

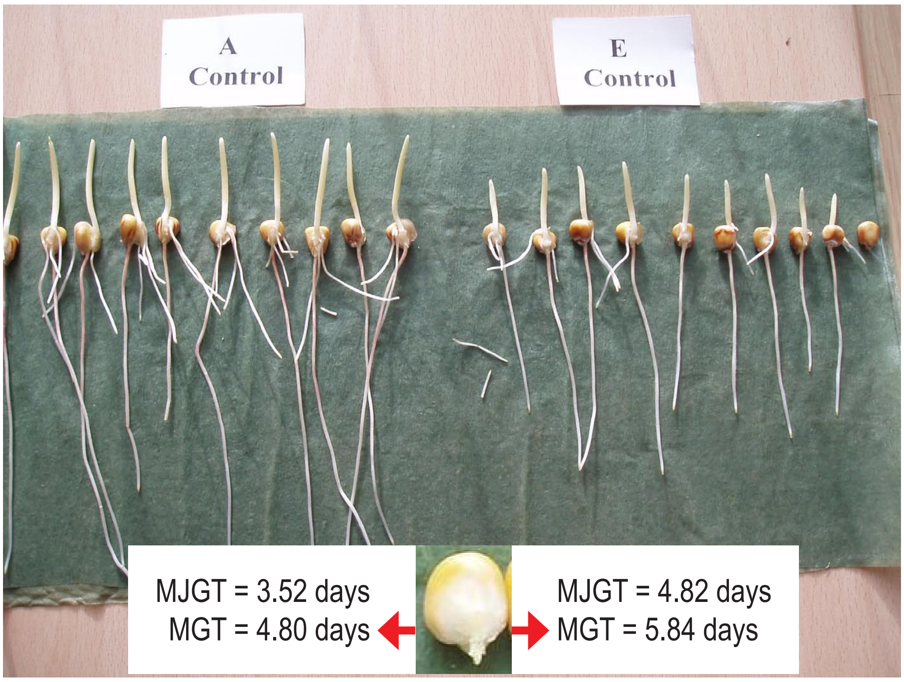

Figure 3. Seedling growth after 14 d at 13 °C of two commercial maize seed lots contrasted in the rate of germination, as indicated by MGT and the earlier stage of mean just germination time (MJGT).

(Dell'Aquila, 2003). The more direct approach of using image analysis to identify and count seeds at the time of radicle protrusion has been developed to the point of routine use in research laboratories to generate germination progress curves (Ducournau *et al.*, 2005; Joosen *et al.*, 2010).

However, the work described above on the general applicability of single counts of radicle emergence to predict vigour suggests that these automated methods could be used more simply. Our suggestion would be to identify appropriate times for single counts of radicle emergence and use a single photographic image to determine radicle emergence, and hence vigour. In many laboratories involved in routine testing, this could also be done manually to identify lots that would have a high MGT and the germination progress curve of a low-vigour lot.

A further use of image analysis in vigour testing has been on seedlings using a scanner image-capturing technique (Sako *et al.*, 2001). This type of approach has been applied to ornamentals and vegetables to give vigour scores to transplant seedlings (Geneve and Kester, 2001). The influence of the timing of radicle emergence on seedling size and uniformity described earlier raises the possibility of obtaining information on seedling quality from much earlier counts of radicle emergence.

Computer-aided use of X-rays for non-destructive sorting is coming into routine use for tomatoes. Seeds containing embryos that are morphologically malformed or reduced in size can be identified and discarded to improve the quality and uniformity of seedling transplants (Van der Burg *et al.*, 1994). The same method can also provide automated tests of the seed quality that results from differences observed in embryo morphology.

A physiologically based non-destructive method proposed for improving germination and vigour is chlorophyll fluorescence (CF) sorting. This has been under development since the 1990s, and aims to remove low-quality seeds that contain higher levels of chlorophyll. Equipment to assess CF is being sold for both sorting and quality testing. There are, however, few scientific papers that convincingly support this approach. The earliest publication (Jalink *et al.*, 1998) used a seed lot of white cabbage described by the authors as 'specially selected', since it showed a wide distribution of chlorophyll fluorescence signals and may have been harvested at an immature stage. A further seed lot of white cabbage described in two later publications (Jalink *et al.*, 1999a, b) had a normal seedling germination count before sorting of only 64.5%. This lot was effectively sorted into groups ranging from 5% normal germination with low vigour (high CF) to 87.5% normal germination with high vigour (low CF). In other work on white cabbage, the CF values of a low-germinating (77%) lot increased after artificial ageing, and the values for aged and unaged seeds correlated with both germination and vigour (Dell'Aquila *et al.*, 2002). None of the three white cabbage lots used in work on CF sorting (Jalink *et al.*, 1999a, b; Dell'Aquila *et al.*, 2002) were typical commercial seed lots. Furthermore, it would have been interesting to compare the results of CF sorting with conventional processing methods such as air separation, size and colour sorting. Conventional methods might remove smaller, immature seeds as effectively as CF sorting.

The use of CF sorting of tomato and cereals has not always been convincingly effective. When a seed lot of tomato was sorted into six groups using CF, only the group with the highest signal (3.4% of the seeds) had a significantly slower and lower (75%) germination than the unsorted seed (Jalink, 1999a). In three lots of barley, CF sorting failed to produce groups that differed significantly in total (normal plus abnormal) germination (Konstantinova *et al.*, 2002). For three other lots, the authors claimed that the total germination percentages of the groups having medium or low CF values were higher than the non-sorted grains. However, the maximum increase was only 6%. Further data, for one lot only, showed a significant increase in the normal germination of grain with medium and low CF values and a decrease for grain with high CF values (Konstantinova *et al.*, 2002). Removing the high CF grains (30% of the lot), referred to by the authors as immature seeds, would improve the germination level. In paddy rice, removal of lower germinating immature seeds (13.9% of the lot) with high CF values improved germination from 90 to 97.5% (Van der Burg, 2009). Again, for these cereal examples, comparisons with other ways of removing immature, smaller seeds would have been useful and appropriate.

The identification of radicle emergence by CF is also under investigation as a possible automated approach to germination testing (Van der Burg, 2009). Published work on many more commercially available seed lots and comparisons with existing procedures would help to confirm the general applicability of CF. This is also the case for other proposed methods and equipment that are promoted, and in some cases sold, for seed quality evaluation and improvement.

Concluding comments

- Mean germination time (MGT), calculated from many counts of germination, is the average delay or lag period from the start of imbibition to radicle emergence and describes the germination curve. MGT is highly indicative of the emergence (rate and final level) as well as seedling size and variation of commercial seed lots (Table 1, columns A and B).
- Single counts of early radicle emergence relate closely to MGT and emergence performance in a range of species. A single early count of the radicle emergence forms the basis of the new radicle emergence vigour test for maize.
- There is clear evidence that early counts of radicle emergence can be used to predict vigour differences in a wide range of species. Comparative testing is under way to extend the validation of this test to other species in addition to maize.
- Single counts of radicle emergence could be incorporated into routine germination testing and hence assess germination and vigour within one test.
- An objective of recent research in seed technology has been the development of rapid automated tests of germination and vigour (Dell'Aquila, 2007, 2009), but none have, so far, been rigorously evaluated on commercial seed.
- Development of radicle emergence as a vigour test will encourage the focused application of automated counting at an appropriate time without the need to determine the complete time course of germination.

- Much of the work on automated methods for testing germination and vigour involves complex procedures and expensive equipment. These automated methods remain to be tested on commercial seeds.
- In this review, we advocate a simpler approach, based on deterioration and its repair, for testing a wide range of species. This approach is the basis of the new vigour test for maize. As we have shown, such an approach could provide quicker, repeatable test methods for routine use on commercial seeds, with little further development or expense.

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