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Communication

Problems with using mean germination time to calculate rate of seed germination

Elias Soltani[A,E], *Farshid Ghaderi-Far*[B], *Carol C. Baskin*[C,D] *and Jerry M. Baskin*[C]

[A]Department of Agronomy and Plant Breeding Sciences, College of Abourahian, University of Tehran, Tehran, Iran.
[B]Department of Agronomy, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan 49138-15739, Iran.
[C]Department of Biology, University of Kentucky, Lexington, KY 40506, USA.
[D]Department of Plant and Soil Sciences, University of Kentucky, Lexington, KY 40546, USA.
[E]Corresponding author. Email: elias.soltani@ut.ac.ir

Abstract. Seed scientists and other plant biologists are interested in the measurement of germination because seeds from different individuals, populations, seed lots and treatments can differ in germination percentages, rate (speed) and uniformity. Mean time to germination (MGT) is a measure of the rate and time-spread of germination; however, there is a problem with using this method to calculate germination rate. MGT does not show the time from the start of imbibition to a specific germination percentage. MGT has been used to compare specific pairs or groups of means and to evaluate seed vigour. However, it is not the real time to mean germination but just an index of germination speed. Using MGT is not correct for ANOVA, post-ANOVA or the other comparison tests, because it does not show time to a specific germination percentage. Thus, we recommend that t50 be used instead of MGT. The t50 has all benefits of MGT, but it does not have the problems of MGT in treatment comparisons.

Additional keywords: germination measurement, germination rate, seed dormancy loss rate, seed vigour test.

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Introduction

Seed germination is one of the most important and the first stage of plant growth, and it generally is defined as protrusion of the radicle from the tissue(s) enclosing it (Bewley *et al*. 2013; Baskin and Baskin 2014). Seed scientists and other plant biologists are interested in measurement of germination because the percentage and rate (speed) of germination can vary among and within individuals and populations of a species and for seeds in different stage of dormancy break (Baskin and Baskin 2014). Measurement of germination can provide valuable information about the start, rate, uniformity and final percentage of germination. For example, two seed lots can have the same germination percentage but differ in speed or uniformity. Therefore, total percentage germination after a specific period of time does not give a full explanation of the dynamics of germination (Joosen *et al*. 2010).

Germination rate can be defined as inverse of time to 50% germination (Soltani *et al*. 2001, 2002; Bewley *et al*. 2013), and there are different ways to calculate it. Mathematical function (such as the Hill function) fitting is one of them (El-Kassaby *et al*. 2008). These functions include meaningful parameters such as time for 50% of viable seeds to germinate. Mean germination time (MGT) is another way to calculate germination speed and is widely used by seed scientists and other plant biologists (Matthews and Khajeh-Hosseini 2006, 2007; Khajeh-Hosseini *et al*. 2009; Oliveira and Garcia 2011; Cheib and Garcia 2012; Chen *et al*. 2013; Zhang *et al.* 2014). These studies calculated MGT using the formula cited by Ellis and Roberts (1980). Another way to calculate germination rate is to estimate the time taken for cumulative germination to reach 50% of its maximum (t50 m) and interpolate from the germination progress curve versus time (Soltani *et al*. 2001, 2002, 2013*a*).

Bewley *et al.* (2013) analysed different sets of germination time courses to calculate germination rate of 50% of total seed population (t50) and of 50% of final germination percentage (t50 m). The t50 always shows time to 50% of total seed population. For a seed lot with germination lower than 50% t50 would be infinite, in which case time to another percentile can be used (e.g. time to 20% germination, t20). The t50 m does not show the time from the start of imbibition to a specific germination percentage, and it changes with the final germination percentage. For example, t50 m is equal to time to 30% of total seed population for a seed lot with 60% germination and to 40% for a population with 80% germination. Bewley *et al.* (2013) showed that when the final germination percentage differs widely among the treatments, the latter method results in the comparison of different percentiles in different populations, which, as they pointed out, is inherently misleading. Bewley *et al*. (2013) did not explain that

MGT is equal to time to 50% of final germination percentage (t50 m). Thus, they did not compare MGT and t50. They compared t50 and with t50 m, but this comparison was not performed statistically; it was just shown that the values calculated by t50 and t50 m are not equal. Now, the questions are as follows: (1) is MGT equal to t50 m or t50; and (2) if they are not equal, do germination rates calculated by MGT affect the results of statistical analysis, such as mean comparisons and regression analysis? Heretofore, these questions have not been answered. If calculating germination rate by MGT differs significantly from that calculated by t50, then the usefulness of MGT would be in doubt. The aim of the present paper is not to review and compare the different approaches for calculating germination rate. Rather, the purpose is to demonstrate that the use of MGT is not the most accurate way to measure germination rate.

Misunderstanding the definition of MGT

MGT is defined as a measure of the rate and time-spread of germination (Bewley *et al.* 2013). It is equal to $\sum(n,t)/\sum n$, where t is time from the beginning of the germination test in terms of hours (Mavi *et al.* 2010) or days (Matthews and Khajeh-Hosseini 2006, 2007; Khajeh-Hosseini *et al.* 2009; Chen *et al.* 2013; Sommerville *et al.* 2013; Zhang *et al.* 2014), and n is the number of newly germinated seeds at Time t. MGT can be thought of not only as the rate of germination of a sample of seeds, but also as the mean of the lag period for each seed between the start of imbibition and germination (Matthews and Khajeh-Hosseini 2007). However, there is a misunderstanding of this definition; it does not give time from the start of imbibition to a specific germination percentage.

Table 1 shows an example of calculating MGT for two different treatments. Final germination percentages were 98.3% and 63.3%. By definition, MGT should calculate time to 50% of germination for both treatments (t50) or time to 49% (50% of 98.3, i.e. t50 m) and 32% (50% of 63.3, i.e. t50 m) in Treatments 1 and 2, respectively. However, when time after germination reached MGT, germination percentages were ~81% and 54% for Treatments 1 and 2, respectively (Fig. 1). Therefore, MGT cannot be the mean (or median) time for germination, and it does not show the time taken to reach 50% of total seed population (t50) or 50% of final germination percentage (t50 m).

Misunderstanding the application of MGT

MGT has been used to compare specific pairs or groups of means (García-Gusano *et al.* 2009; Guma *et al.* 2010; Hacisalihoglu and Ross 2010; Oliveira and Garcia 2011; Cheib and Garcia 2012) and to evaluate seed vigour (Matthews and Khajeh-Hosseini 2006, 2007; Khajeh-Hosseini *et al.* 2009; Mavi *et al.* 2010; Matthews *et al.* 2012) and seed-dormancy loss (Chen *et al.* 2013).

Guma *et al.* (2010) used MGT for comparison of various scarification methods to break dormancy in *Cicer canariense*, and they indicated that MGT was not significantly affected by the various methods applied. Oliveira and Garcia (2011) calculated germination rate by the inverse of MGT, and evaluated the effects of temperature on germination rate of the seeds of seven species of *Syngonanthus*. Matthews and Khajeh-Hosseini (2006, 2007) and Khajeh-Hosseini *et al.* (2009) indicated that MGT was significantly related to seed vigour and field performance in maize. Mavi *et al.* (2010) found the same relationships between final emergence percentage and MGT (on the basis of hourly counting) in watermelon, melon and cucumber. Demir *et al.* (2008) showed that the MGT of seed lots of peppers predicted the final emergence, mean size and variability of seedlings in transplant production. These are just a few of the examples of the application of MGT in seed science and seed-germination ecology. Here, results of two experiments are presented to show MGT applications such as explained above. These two examples demonstrate the misapplication of MGT.

Experiment 1

Seeds of wheat were accelerated-aged at 43°C and a relative humidity of 90–95% for 0, 48, 72, 96 and 144 h (Soltani *et al.* 2008, 2009), and germination time courses of five of the treatments are shown in Fig. 2. Accelerated aging for 144 h was characterised by a longer time to the start of germination than at 0 h. MGTs were 45, 53 and 63 h for seed lots of 0, 72 and 144 h of aging, respectively (Fig. 2). These results indicated that seed aging can delay the germination rate because MGT is increased. However, a comparison of different MGTs is not an appropriate method to use to compare the seed lots; different percentiles would be compared, depending on the final germination percentages. For example, seed lots accelerated-aged for 0, 72 and

Table 1. Examples of calculating mean germination time (MGT) for two different treatments

t (days)	Treatment 1		Treatment 2	
	n	$n.t$	n	$n.t$
0	0	0	0	0
1	23.33	23.33	8.33	8.33
2	73.34	146.68	45.00	90
3	1.67	5.01	9.17	27.5
4	0	0	0.83	3.33
	$\sum n=98.34$	$\sum n.t=75.02$	$\sum n=63.33$	$\sum n.t=129.16$
	MGT = 1.78		MGT = 2.04	

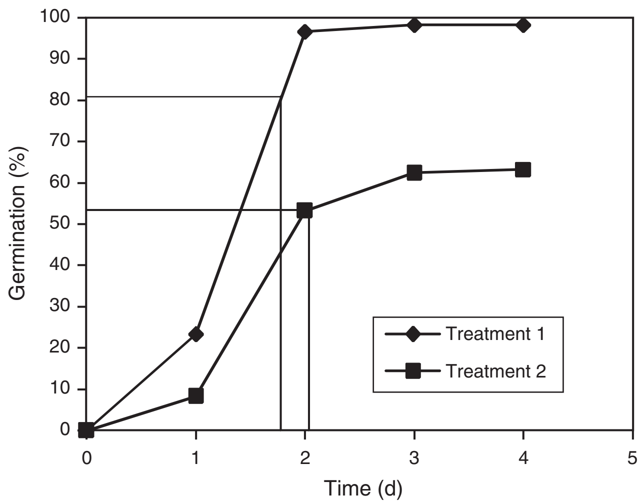

Fig. 1. Germination time course for each of the two treatments in Table 1. Mean germination time (MGT) and germination percentage at the MGT are indicated for each treatment.

144 h reached 80%, 70% and 48%, respectively, of germination at their MGTs (Fig. 2). Conceptually, comparison of different MGTs is actually a comparison of time to 80%, 70% and 48% germination. For example, it is like evaluating the time taken for three runners to reach three different distances (80, 70 and 48 m) and then saying that the runner who ran 48 m was the fastest, without considering that the second and third runners ran longer distances. It is obvious, then, that when we want to evaluate the speed of three runners, we need to do it for a fixed distance (e.g. 100 m). Likewise, a comparison of the germination rate of seeds, for example, from different treatments, cultivars, habitats, populations and taxa should be done for a fixed percentage (e.g. 50% of total population). Thus, MGT does not show the time to a specific germination percentage, and it will lead to mistakes in comparisons of means or the other post-ANOVA tests. Therefore, other indices that give the true rate of germination, such as time to 50% germination (t50), should be used to compare treatments.

The t50s for seed lots accelerated-aged for 0, 72 and 144 h were ~39, 48 and 64 h, respectively (Fig. 2). MGTs were not equal to t50s for different lots, and the differences were high in some treatments (Fig. 3). Linear regression was applied to investigate the effect of increasing the period of accelerated aging on MGT and t50, because the treatment levels were quantities (numbers), i.e. not qualities such as in studies that compare cultivars. Thus, MGT and t50 significantly increased at different rates as the aging period increased, with MGT and t50 increasing by 0.13 and 0.18 h, respectively, for every additional hour of accelerated aging (Fig. 3). Figure 4 shows the relationships between t50 and MGT. As indicated, the relationship between them was non-linear, and therefore they were not measuring the same thing. In this case, the values calculated by t50 were lower than those obtained by MGT (Fig. 3). For example, when t50s were 40, 50 and 60 h, MGTs were 46.9, 56.4 and 63 h, respectively (Fig. 4). In this case, differences in t50 and MGT were lower where seed vigour was low (144 h of accelerated aging). However, it cannot be said that this relationship is always the same; thus, sometimes MGT values may be greater than those of t50. In sum, it is clear that MGT and t50 are not calculating the same thing. MGT is not the real time to mean germination; it is just an index of germination speed. Using MGT is not correct for ANOVA, post-ANOVA or the other comparison tests, because it does not show the time to a specific germination percentage.

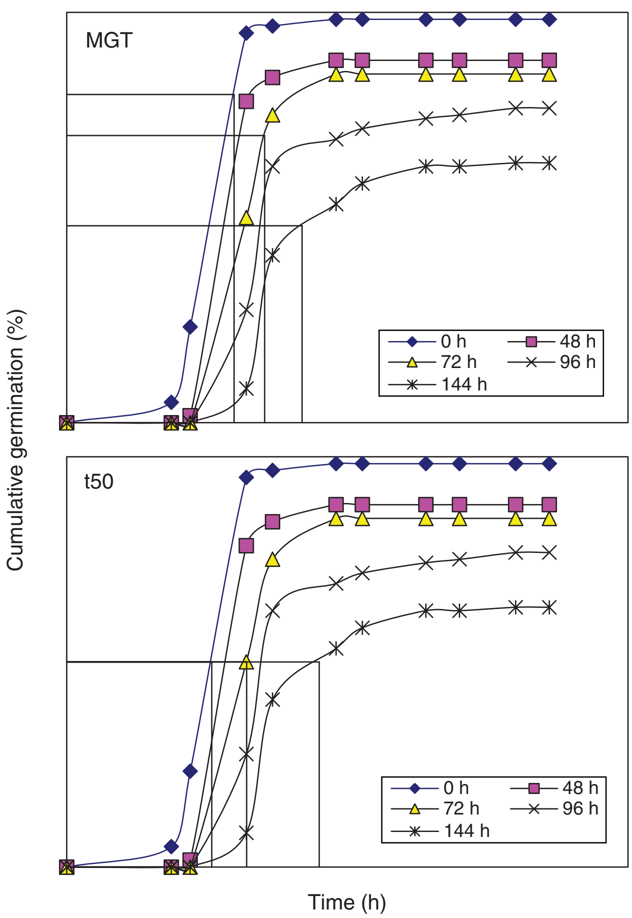

Fig. 2. Germination time course for each of five accelerated aging periods in wheat. Mean germination time (MGT), t50 and germination percentage at MGT are shown for 0, 72 and 144 h of accelerated aging.

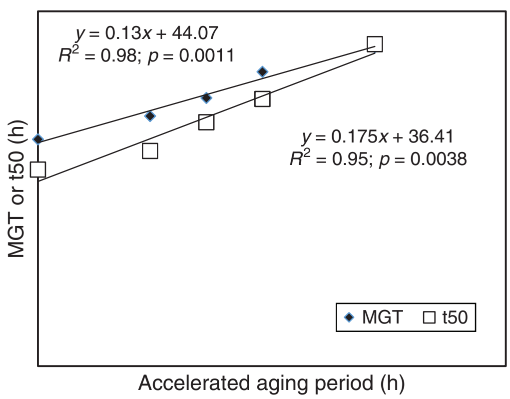

Fig. 3. Regression lines for the effect of accelerated aging on mean germination time (MGT) and t50.

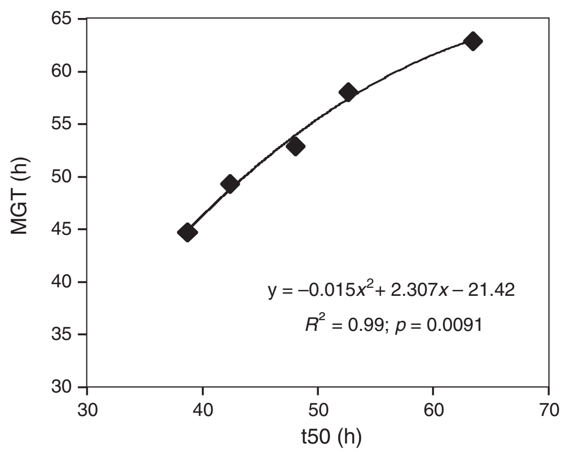

Fig. 4. Relationship between t50 and mean germination time (MGT) in Experiment 1.

Table 2. Germination percentage, mean germination time (MGT), time to 50% germination (t50), time to 20% germination (t20) and emergence percentage for different accelerated-aging periods with and without hydro-priming in cotton

Within a column and within each aging category figures followed by the same letter are not significantly different at $P = 0.05$

Accelerated aging (days)	Priming	% germination	MGT (h)	t50 (h)	t20 (h)	% emergence
0	Yes	83.5a	31.1a	28.0a	9.0a	33.5a
	No	85.5a	34.4a	28.9a	14.1a	28.5a
2	Yes	74.5a	31.6a	22.5a	11.4a	33.0a
	No	73.0a	33.3a	30.1b	20.4b	21.5b
4	Yes	38.0a	41.3a		32.4a	18.5a
	No	25.5b	60.7b	–	75.7b	8.0b

Experiment 2

Cotton seeds were subjected to accelerated aging, and all seeds then were hydro-primed and treatments were compared by t20, t50 and MGT (Soltani and Ghaderi-far unpubl. data). Accelerated-aging treatments were conducted by aging cotton seeds cultivar Siokra324 at 42°C and a relative humidity of 90–95% for 0, 2 and 4 days. Accelerated-aged seeds were primed in tap water for 16 h at 25°C. Therefore, the experiment factors were accelerated aging (three levels) and priming (two levels).

Standard germination test

Four replicates of 50 seeds for each treatment were placed on two moistened paper towels. After covering the seeds with a third paper towel, the three towels were loosely rolled to form a tube and placed in plastic bags to prevent evaporation. Seeds were incubated in dark in a controlled-temperature incubator at 20°C. Seed germination was monitored twice a day, and a seed was considered to be germinated when the radicle was 2 mm long.

Field seedling emergence

A randomised block design with four replicate blocks was used, and each contained one row of 50 seeds for each treatment. Field seedlings were counted daily until maximum emergence, and emergence percentages were calculated.

Germination and emergence (%) decreased with accelerated aging, and seed priming improved both of them slightly (Table 2). A comparison of mean MGTs showed that there was no significant difference between seed priming and control at 0 and 2 days of aging and that the difference became significant at 4 days of aging. However, a comparison of mean t50s indicated that the difference between seed priming and control was significant for 2 days of aging (Table 2). Differences in seedling-emergence percentage were significant between primed and unprimed seeds at 2 and 4 days of aging for t50 but not for MGT (Table 2). MGTs were 34, 33 and 61 h for 0, 2 and 4 days of aging, respectively; thus, seeds with 0 days of aging (without priming) had a longer time to germination than those with 2 days of aging (Table 2). This result came from misunderstanding the definition of MGT, namely, it does not show the mean germination time. The t50s (for non-primed seeds) were 29 and 30 h for 0 and 2 days of aging, respectively; thus, aged seeds had a longer period of time to germination. However, germination of seeds (without priming) aged for 4 days had a germination percentage lower than 50%, and t50s were infinite. In this situation, time to lower germination percentage can be used for comparisons of the treatments (see Bewley *et al.* 2013). Here, time to 20% germination (t20) is used, because the lowest germination percentage was 26% and all treatments had a t20 (Table 2).

Conclusions

Results from the present study showed that MGT is not the mean time to germination. It is just an index of germination speed and does not show time to a specific germination percentage. MGT is not equal to either t50 or t50 m. In contrast to other methods used to calculate germination rate, such as t50 (Soltani et al. 2013*b*, Soltani and Farzaneh 2014), MGT does not consider a fixed germination percentage. MGT as well as measures of t50 must be based on the total seed population and not scaled to 50% of final number of seeds (t50 m) that actually germinate (e. g. Soltani *et al*. 2001, 2002, 2013*a*; Joosen *et al*. 2010). MGT should not be used to compare treatments, because the comparison of different MGTs is actually a comparison of time to different percentiles of germination, thus leading to mistakes in the results of comparison tests. In total, we recommend that t50 should be used instead of MGT. The t50 has all the benefits but not the problems of MGT in treatment comparisons.

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