

Evaluation of vigour tests for determination of seed storage potential in red clover (*Trifolium pratensis* L.) and timothy (*Phleum pratense* L.)

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Abstract

Three different seed vigour tests, Accelerated ageing (AA), Germination index (GI) and Polyetyleneglycol (PEG), were evaluated with regard to ranking six reference seed lots of timothy cv. Grindstad and red clover cv. Lea according to their physiological potential for storage. The seed reference lots, which all had germination percentages between 70-100%, had sustained different degrees of ageing before examination. In timothy, both the AA-test and the GI-test managed well to separate the low-vigour seed lots, having a poor storage potential, from the high-vigour seed lots with better storage longevity. The clearest separation was obtained using the GI-test, either as complete test (six out of six seed lots was significantly separated) or as a simplified test (five out of six seed lots was significantly separated). The germination index of the two tests was based on counting of germinating seeds either on a daily basis for ten days or after counting on day 3 and day 5, respectively. Also the AA-test managed to separate five out of six seed lots of timothy after optimal treatment for 56 h at 45°C. In red clover, the AA-test gave the clearest separation between seed lots. At optimal conditions for 24 h at 43-45°C, as many as five of the six red clover seed lots could significantly be separated from each other. Also the PEG-test discriminated well between reference seed lots of both species, but was considered as more labour-intensive than the two other vigour tests. The recommended seed vigour test for timothy (simplified GI-test) and red clover (AA-test for 24 h at 43-45°C) has later been adopted for use in practical seed testing routines at the Norwegian National seed laboratory.

Introduction

In the early 2000s Norwegian seed companies had to discard large amount of forage seed, especially of timothy, due to germination losses on storage. In order to minimize losses, methods for ranking seed lots with regard to storage longevity were highly requested by the seed companies. Seed vigour tests have earlier been found as good predictors for seed longevity in storage (Wang & Hampton 1991). The maximum seed vigour occurs at physiological maturity, after which vigour decline during ageing on plant and during storage.

The aim of the present project was to evaluate three different seed vigour tests, Accelerated ageing (AA), Germination index (GI) and Polyetyleneglycol (PEG), with regard to ranking seed lots of timothy (*Phleum pratense* L.) and red clover (*Trifolium pratense* L.) according to their physiological potential for storage.

Materials and methods

Seed reference set

The seed vigour testing was performed at the seed laboratory at Bioforsk Øst Landvik in 2007. The original seed material used for vigour testing was supplied from commercial production of timothy cv. Grindstad and red clover cv. Lea. From the original seed lot, seed samples were artificially aged under humid conditions (about 100% RH in airtight plastic containers) at four different temperatures (35, 38, 41 and 44°C) for various incubation periods. After ageing, germination capacity was determined according to ISTA (2008) but with one replicate of 100 seed per treatment. In both species, only seed aged at the highest temperature (44 °C) obtained a severe loss of germination capacity during the 82 h incubation period (Figure 1a and 1b). Thus, five samples of both species with different physiological age, incubated for various lengths at this temperature, was selected as a seed reference set for later use in the seed vigour testing programme. The initial germination capacity of the selected reference seed lots (B-F) was between 70-100% as shown in Figure 1 and 2. In addition, seed from the original seed lot (A: untreated seed, 97 % germination in both species) was selected as a reference (i.e. six reference lots in total for each species). According to the different degrees of physiological ageing, reference seed lot F was ‘oldest’ (low storage potential) and seed lot A ‘youngest’ (high storage potential).

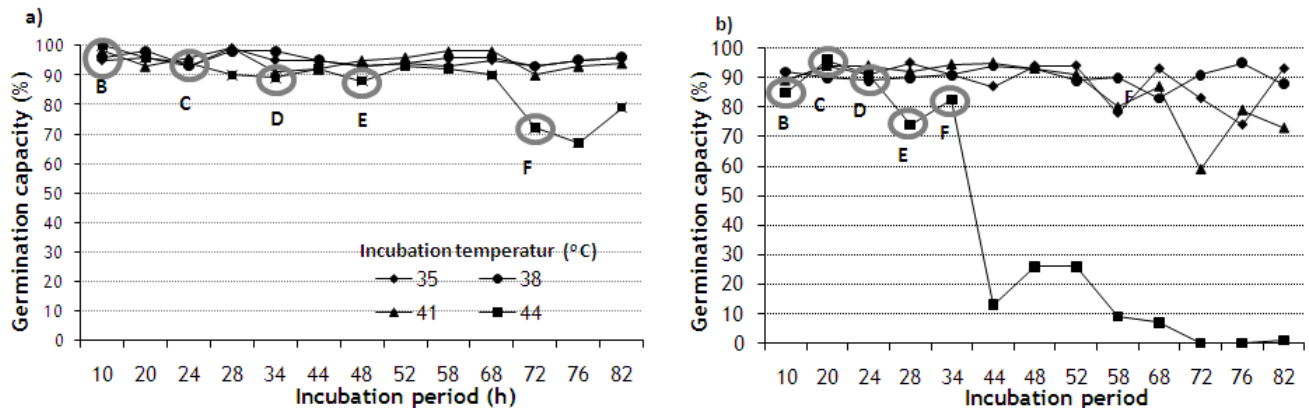


Figure 1. Germination capacity (%) of the selected reference seed lots (marked with grey circles) of a) timothy cv. Grindstad and b) red clover cv. Lea after being aged at different temperature and incubation length.

Germination index test

The germination index test was carried out as a standard germination test (ISTA 2008) where four replicates of 100 seeds of the six reference seed lots of each species were laid out on the Jacobsen germination apparatus, but with a more frequent counting regime. The day/night temperature was regulated to 25 / 20 °C and 20 / 20 °C for timothy and red clover, respectively. The first registration of emerging seedlings was performed after 2 and 3 days in red clover and timothy, respectively. New seedlings were then daily counted for 7 days before a last counting after 10 days completed the registration program. Seed was registered as germinated when the length of the emerging seedling (timothy) or the total length of radicle and seedling (red clover) was 2 mm or longer. The germination index (GI) was calculated based on the following formula: $GI = (\text{number of new seedlings after day } n_1 / n_1) + (\text{number of new seedlings after day } n_2 / n_2) + \dots + (\text{number of new seedlings after day } n_i / n_i)$.

PEG-test

The six different reference groups of each species was osmotic stressed by imbibing the seed at five concentrations (0.00, -0.15, -0.30, -0.45 and -0.60 MPa) of Polyethylene glycol (PEG 6000) based on the equation: $\psi_o = -(1.18 \times 10^{-2})C - (1.18 \times 10^{-4})C^2 + (2.67 \times 10^{-4})CT + (8.39 \times 10^{-7})C^2T$ where C is the concentration of PEG 6000 in grams per kilogram H₂O and T is the temperature in degrees Celsius (Michel and Kaufmann, 1973). Filter papers were placed in Petri dishes and moistened with 3 ml distilled water (control) or the respective concentrations of PEG solutions before seeding samples of 50 seeds per dish (total: 8 x 50 seed = 400 seed per treatment). The Petri dishes were sealed with parafilm to avoid evaporation and placed on the Jacobsen germination apparatus at the same conditions as in the germination index test. The number of germinating seeds was counted and removed after 7 and 10 d (timothy) or after 5 and 10 d (red clover).

Accelerated aging test

A preliminary AA-screening test indicated that seed from the six different reference groups was best discriminated at around 44 °C (data not shown). Thus, a more detailed accelerated ageing test was performed in both species at 43 and 45 °C for 32, 48 and 72 h (timothy) or 24, 32 and 48 h (red clover). For each combination of temperature and ageing period, seeds from the six reference seed lots were lightly dusted with the fungicide thiram and placed on a wire mesh in a plastic box 30 mm above water surface (around 100% RH). Following the incubating period, seed was dried at room temperature for 24 h and germination capacity (4 replicates x 100 seed per treatment) evaluated using standard methods (ISTA 2008).

In order to discriminate differences between the artificially aged reference seed lots, analyses of variance were performed separately for each species (timothy and red clover), always regarding each replicate (100 seeds) as a random variable for each of the three vigour tests. In the tables, significant differences are indicated by different letters according to Duncan multiple comparison test at $P < 0.05$.

Results and discussion

In timothy and red clover, six and three of the total six reference set lots of each species was significantly separated by the GI-test, respectively. In addition, a less labour-intensive model, based on the number of germinating seeds on day 3 and day 5 in the GI-test, was calculated for timothy. This simplified GI-test managed to discriminate five out of six seed reference lots (Table 1).

Table 1. Germination index (GI) for the six reference seed lots of timothy and red clover.

Reference seed lot	Timothy		Red clover
	Germination index	Simplified GI, (3 + 5 days)	Germination index
A	24.5a	20.5a	38.0a
B	23.0b	18.6b	38.4a
C	21.8c	15.5c	38.8a
D	20.0d	12.1d	38.5a
E	18.0e	12.1d	34.3b
F	11.4f	0.9e	28.3c
Sign	<0.01	<0.01	<0.01

The clearest separation by the PEG-test was found at an osmotic pressure of -0.6 MPa and -0.15 MPa for timothy and red clover, respectively. Under these conditions, significant differences in germination capacity between five of the six reference seed lots was detected in both species. However, the test was considered as more labour-intensive for practical use in seed laboratories than the two other seed vigour tests.

Table 2. Effect of osmotic pressure (PEG-concentration) on germination capacity of six reference seed lots of timothy and red clover.

Reference seed lot	Timothy					Red clover				
	0,00 Mpa	-0,15 MPa	-0,30 Mpa	-0,45 MPa	-0,60 MPa	0,00 Mpa	-0,15 MPa	-0,30 Mpa	-0,45 MPa	-0,60 MPa
A	98a	99a	96a	82a	68a	97a	96ab	83a	39bc	20a
B	95a	98ab	96a	78a	55b	98a	97a	83a	68a	13b
C	98a	98ab	87b	55b	30c	97a	91b	84a	53ab	2c
D	95a	92ab	76c	60b	12de	88b	84c	79a	30c	2c
E	93a	87b	84b	51b	15d	91b	76d	54b	23c	3c
F	63b	76c	65d	32c	4e	68c	61e	43c	21c	1c
Sign, %	<0.01	<1	<0.01	<0.01	<0.01	<0.01	<1	<0.01	<1	<0.01

The AA-test managed to separate five out of six seed lots of timothy, with regard to germination capacity, after optimal treatment for 56 h at 45°C. In red clover, incubation for 24 and 48 h at 43°C or for 24 h at 45°C gave similar separation (five out of six) (Table 3). The results indicate that red clover has a relatively tolerant optimal temperature regime (43-45 °C) when incubated for 24 h.

Table 3. Effects of accelerated aging at various temperatures and incubation periods on germination capacity of the six reference seed lots of timothy and red clover.

Ref. seed lot	Timothy						Red clover					
	43°C			45°C			43°C			45°C		
	32t	48t	56t	32t	48t	56t	24t	32t	48t	24t	32t	48t
A	93a	90a	90a	90a	85a	79a	95a	79a	73a	89a	79a	6a
B	89ab	88a	90a	88a	84a	77a	91a	79a	58b	80b	73b	10a
C	90ab	88a	85b	89a	71b	64b	80b	71a	30c	48c	14c	1b
D	88bc	81b	81bc	79b	64c	53c	63c	57b	19d	46c	16c	0b
E	85c	85ab	78c	81b	62c	34d	48d	35c	0e	14d	0d	1b
F	47d	22c	20d	19c	3d	1e	14e	4d	0e	5e	1d	0b
Sign,%	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	0.2

Conclusions

Based on the seed vigour test results (Table 1-3) and for practical usability in seed laboratories, the simplified GI-test and the AA-test for 24 h at 43-45°C was recommended for timothy and red clover, respectively. In order to rank commercial seed lots of the two species with regard to their storage potential, these two seed vigour tests have later been adopted for use in practical seed testing routines at the Norwegian National seed laboratory.

References

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