APPROACHES TO THE INDIRECT EVALUATION OF GERMINATION AND VIGOUR

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ABSTRACT: In comparisons of six seed lots of different F_1 hybrid cultivars of cauliflower with similarly high laboratory germinations (above 90%) separation in germination was achieved after controlled deterioration (C.D.) at 24% moisture content (m.c.) and 45 ^{0}C for 24 hours. This measure of vigour was related to the position of the lots on the seed survival curve and was highly predictive of the longevity of the lots when stored at 15% m.c. and 20 ^{0}C for 12 and 16 weeks. When each seed lot was deteriorated at 24% m.c. for increasing times (from 0 to 36 hours) a reduction in the subsequent percentage germination was seen, which, using probit transformed percentages, was significantly and linearly related to the leakage of electrolytes into seed soak water over 24 hours. The case is made for an approach to the indirect evaluation of germination and vigour using C.D. followed by measurements of leakage that could be more discerning and rapid than the present laboratory germination test.
Key Words: Cauliflower, controlled deterioration, leakage, electrical conductivity, storage

ALTERNATIVAS PARA A AVALIAÇÃO INDIRETA DA GERMINAÇÃO E DO VIGOR

RESUMO: Diferenças na qualidade de sementes de seis lotes de híbridos F_1 de couve-flor, com germinação semelhante (superior a 90%), foram verificadas pelo teste de deterioração controlada, a 45^{0}C/24 horas, em sementes hidratadas até atingir grau de umidade de 24%. Os resultados dessa avaliação de vigor foram relacionados à posição dos lotes na curva de sobrevivência de sementes e permitiram estimar, com segurança, a longevidade dos lotes durante o armazenamento (grau de umidade de 15%, a 20^{0}C, durante 12 e 16 semanas). Quando as sementes de cada lote foram deterioradas artificialmente, com grau de umidade de 24% durante períodos crescentes (de zero a 36 horas), observou-se redução na percentagem de germinação. Verificou-se, com a utilização de probit transformado em percentagem, que esses resultados relacionaram-se linear e significativamente com a lixiviação de eletrólitos de sementes embebidas em água durante 24 horas. Desta forma, a avaliação indireta da qualidade, através da germinação e do vigor (deterioração controlada), seguido pela avaliação da liberação de eletrólitos, pode ser mais eficiente e rápida que o teste padrão de germinação.
Descritores: sementes, couve-flor, deterioração controlada, lixiviação de eletrólitos, condutividade elétrica, armazenamento

INTRODUCTION

Laboratory germination is the universal measure of seed quality which indicates to growers the field emergence ability of seed lots, the units of production, testing and sale used for crop seeds. The internationally recognised rules of seed testing aim to describe acceptable methods for conducting the laboratory germination test to ensure repeatability both within and between laboratories. However, one further aspect of seed quality not clearly described by the test is seed vigour which affects both emergence, especially under less than optimum field conditions, and longevity in storage. Furthermore the laboratory germination test takes several days and is time-consuming to set up and assess. The objectives of the work described here was to evaluate an approach to evaluating germination and vigour of seed lots without directly testing for germination.

THEORETICAL BACKGROUND

Death, seen as failure to germinate, is normally distributed in populations of seeds such as is represented by a seed lot. The result is the sigmoid survival curve (Figure 1) of germination over time of samples drawn from the population.This curve consists of three phases. There is a relatively long period at the start when few seeds lose the ability to germinate, a rapid fall when most (the mode of the normal distribution) fail to germinate and finally another relatively long period when the last few "die". The shape of the curve is essentially the same for all seed populations but differs in the length of the three phases between species and between different storage conditions. For example, in the case of orthodox seeds like cereals and Brassics, with increasing temperature and moisture the time scale is shortened and can occur over a period of hours (high seed temperature and moisture) or decades (low temperature and moisture). A further influence on the curve is the initial starting point, which in Figure 1 is illustrated by positions A, B and C. The seed lot starting at C would not go through a prolonged slow initial fall but would very soon show a rapid fall in germination, well before seeds at positions B and A.

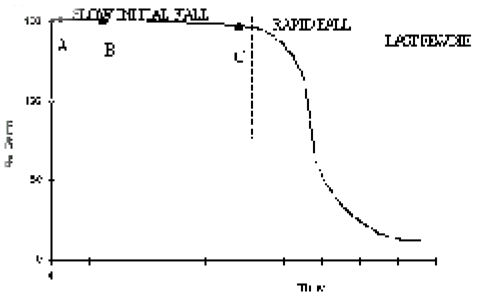

Figure 1 - Generalised seed survival curve.

When seeds at positions A, B and C (Figure 1) are subjected to the same period of severe deterioration, for example 24 hours at 20% moisture content and 45^{0}C as in the controlled deterioration (C.D.) test (Matthews, 1980) and subsequently germinated, what were similar germination levels on the slow initial decline of the curve become widely separated on the survival curve. A first step in developing an indirect measure of germination and vigour would be to relate germination after C.D. to a consequence of differences in vigour, for example, differences in longevity in storage.

The second step would be to provide an indirect measure of these widely separated germinations after C.D. without going through the process of actually germinating seeds. The inability of deteriorated tissues and non-germinating seeds to retain solutes, such as electrolytes, when soaked in water is well recognised. Seeds that fail to germinate, or do so only slowly, release higher levels of electrolytes into soak water, as measured by electrical conductivity readings, than do vigorous seeds (Hepburn et al., 1984). Thus it may be possible to predict germination after periods of deterioration from the leakage of electrolytes into seed soak water. The third step would be to compare seed lots and see if electrical conductivity measurements of the soak water of seeds following a period of severe deterioration can be used to predict germination and vigour.

The objective of this paper is to present results illustrating the first two steps: firstly, that germination after controlled deterioration can measure vigour as indicated by longevity in storage and, secondly, that germination after controlled deterioration can be predicted from electrical conductivity measurements of soak water. Work on the third step is in progress.

MATERIAL AND METHODS

The seeds used to illustrate the approach to the indirect testing were seed production lots of six single F_1 hybrid cauliflower cultivars obtained from the Organisation de selection Bretonne (OBS) in Plougoulm, France. All had high levels of normal germination (TABLE 1) but, according to the seed company's own assessments differed in vigour. The lots represented four different production years (1990, 1993, 1994 and 1996). After receipt in our laboratory in June 1997 the seeds were stored in foil packets at 5^{0}C from which samples were drawn for experimentation.

Germinations were determined following International Seed Testing Association (I.S.T.A.) rules for four replicates of 25 seeds which were set to germinate at 20^{0}C on a moist seed circle. Counts were made after 10 days of total germination (defined as the appearance of the radicle) and of normal seedlings using I.S.T.A. definitions. In the controlled deterioration (C.D.) test a weighed sample of a seed lot of known moisture content (m.c.), determined by the I.S.T.A. recommended method, was placed on a moist seed circle and allowed to imbibe to an elevated m.c. In the results reported here the m.c. was either 20 or 24%. The attainment of this m.c. was checked by frequent weighing at which time the seeds were sealed in aluminium foil packets overnight at 5^0C to allow time for the even distribution of moisture (Powell and Matthews, 1981). The packets were then held in a water bath at 45^0C for 24 hours or other stated times and subsequently germinated, counts of total germination were taken after 10 days.

Longevity in storage was tested by raising the m.c. of each of the six lots to 15%, sealing in Aluminium foil packets and holding at 20^0C in an incubator. Germinations both before and after C.D. (at 20% m.c.) were conducted periodically for up to 16 weeks (Table 1).

Measurements of electrolyte leakage were done on 100 weighed seeds at their original m.c. by soaking in 40ml of deionised water at 20^0C for 24 hours. Seeds that had been subjected to C.D. before leakage was measured were allowed to dry back to their original m.c. in open dishes in the laboratory before being placed in the soak water. Conductivity readings were taken with a single probe meter (Jenway 4010) and expressed as micromhos g^{-1} seed.

RESULTS

The germination and vigour levels of all six lots at the time of receipt were high with counts of normal seedlings of at least 92% and counts of total germination after C.D. at 20% m.c. for 24 hours ranging from 85 to 99%. Only after C.D. at 24% M.C. was differentiation between the lots in total germination observed, from 51 to 99% (Figure 2).

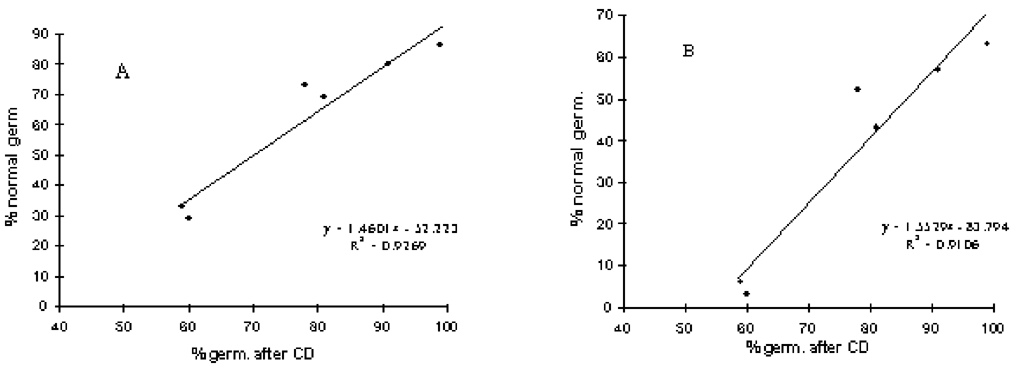

Figure 2 - Relation between % germination after CD at 24 % m.c. and normal germination after (A) 12 weeks and (B) 16 weeks storage of six cauliflower F_1 hybrid seed lots at 15 % m.c. and 20^0C.

Although vigour levels of all the lots were high, differences were detected in germination after storage at 15% m.c. and 20^0C (TABLE 1) but not until 12 weeks when lots 3 and 4 germinated at 33 and 29%, respectively, whilst lot 6 remained at 86% and at 16 weeks when lots 3 and 4 gave normal germinations of 6 and 3% and lot 6, 63%. Seed lots 3 and 4 were also seen to be the least vigorous earlier in the storage period since their total germinations after C.D. at 20% m.c. were 47 and 38% after 4 weeks and 33 and 43% after 6 weeks compared to lot 6 at 92% (4 weeks) and 88% (6 weeks).

These differences in response to storage between the lots indicated vigour differences that were not suggested by the laboratory germination tests at the start of storage (TABLE 1). However, germination after C.D. at 24% m.c. was highly significantly related to the germination of the lots after both 12 and 16 weeks storage (Figure 2). Thus C.D. germination successfully predicted differences in storage potential even though the seed lots were indistinguishable in their similarly high percentages of normal seedlings in the laboratory germination test.

The next experimental step was to determine if the germination after C.D. could be predicted from the leakage of electrolytes. Seed samples from each lot were raised to 24% m.c. and deteriorated at 45^0C for 0, 18, 24, 28, 32 and 36 hours. After deterioration sub-samples of 100 seeds from each deterioration duration were germinated and a further 100 seeds from each duration were dried back to their original m.c. and soaked in deionised water for 24 hours. The relationship between the electrical conductivity readings and percentage germination after C.D. for seed lot 2 (Figure 3A) showed an increasing level of leakage as germination declined following the increase in the deterioration time from 0 hours on the left (Figure 3A) to 36 hours when germination was down to 49% as, shown by the point furthest to the right in Figure 3A. The relationship was not linear since, although deterioration durations up to 28 hours produced progressive increases in leakage, germination declined only slightly in percentage terms from 99 to 91%. However, transforming the percentages into probits (normal deviate plus 5) resulted in a highly significant negative linear regression (Figure 3B) which could be used to readily predict germinations from electrolyte readings of leakage. Similar linear regressions were produced for the other five seed lots but the constants of intercept and slope differed between the lots.

DISCUSSION

The initial assessments of the six seed lots suggested that the lots were of equally high quality with similarly high laboratory germinations and little difference in germination after C.D. at 20% m.c. (TABLE 1). In terms of the survival curve (Figure 1) the lots would have been to the left of the slow initial decline, towards the hypothetical lot A in Figure 1. However, a more severe controlled deterioration at 24% m.c. showed that, despite similarities in their initial laboratory germination, the seed lots differed in their vigour and, location on the survival curve. For example, lots 3 and 4 would have been nearer B than would lot 6 which would have been nearer A. Thus even high quality seed with normal germinations above 90% were distinguished in terms of vigour using C.D. This was a more demanding discernment than had been previously achieved using C.D. (Powell & Matthews, 1981) when seed lots more contrasted in laboratory germination were used.

In previous work on commercially available seed lots, germination after C.D. was significantly correlated both with field emergence (Powell & Matthews, 1981) and longevity in seed company stores (Powell & Matthews, 1984) which supported two contentions: (1) that C.D. could be used to measure vigour and (2) that differences in the extent of deterioration was the major cause of differences in vigour. The present work adds further support since even these apparently similarly high quality seeds differed in storage over weeks in a manner that was predictable from germinations after C.D. at 24% (Figure 2).

Step one of the approach to the indirect evaluation of germination and vigour was achieved in these comparisons of F_1 hybrid seed lots. The separated germinations after C.D. proved indicative of one outcome of differences in vigour, namely longevity in storage. The next step, the measurement of germination after C.D. indirectly from electrolyte measurements was also seen to be possible for each F_1 hybrid seed lot using probit transformed percentages (Figure 3). Linear relationships applied to all the F_1 seed lots but the slope and the intercepts differed. Hence the same predictive equation could not be used for all the lots. This may be a genotypic difference so that a single predictive equation could only be used for all seed lots of the same cultivar. This is currently being investigated and would provide the basis for the third step enabling electrical conductivity leakage measurements after C.D. to predict germination and vigour before C.D.

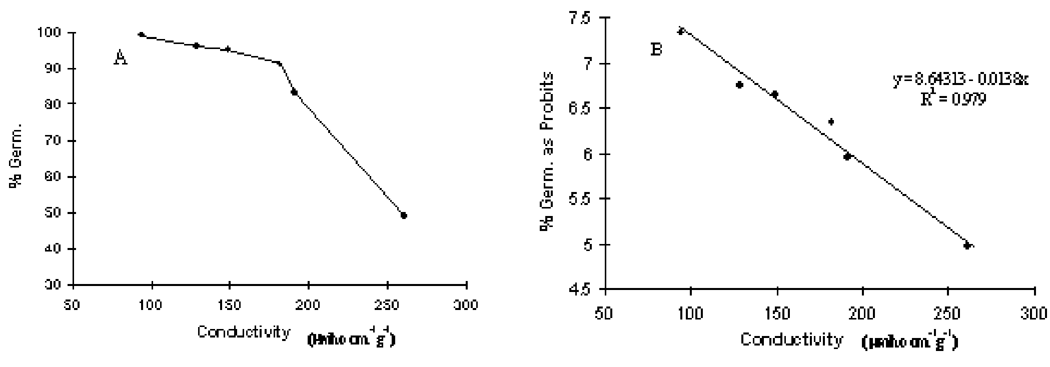

Figure 3 - (A) Relationship of % germination after increasing durations (from 0 to 36 hours) of deterioration at 24% m.c. and the leakage of electrolytes into seed soak water for cauliflower seed lot 2 and (B) the same relationship after transformation of percentages to probits.

TABLE 1 - Percentage normal laboratory germinations of cauliflower seed after storage at 15% moisture content (m.c.) and 20^{0}C. Figures in brackets are % C.D. germinations at 20% m.c.

	0	2	4	6	12	16
1	94(97)	87(91)	80(74)	80(73)	73(33)	52(3)
2	94(89)	87(88)	79(59)	79(79)	69(35)	43(8)
3	94(91)	91(69)	79(47)	82(33)	33(0)	6(0)
4	92(85)	89(88)	88(38)	72(43)	29(5)	3(0)
5	94(99)	95(90)	90(84)	82(81)	80(31)	57(5)
6	93(99)	95(94)	93(92)	91(88)	86(16)	63(0)

CONCLUSIONS

The methods described here of deterioration followed by germination or leakage measurements are more discerning than the laboratory germination test in separating seed lots but are still time-consuming. However, manipulation of deterioration and methods of leakage measurement might well lead to quality tests that take hours rather than days to provide a truly rapid evaluation of seed quality. The work reported here points towards an approach to the indirect and, possibly, rapid evaluation of germination and vigour.

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