NEW APPROACHES TO SEED VIGOR TESTING

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ABSTRACT: Among the principles used for the evaluation of seed vigor, two approaches are predominant: seed resistance to stresses and the direct or indirect identification of the "current" state of the seeds, e.g., enzymatic activity, integrity of the cellular membranes, growth parameters. In this presentation recent innovations are discussed in procedures for seed vigor evaluation emphasizing the methodologies of accelerated aging with salt saturated solutions, controlled deterioration and potassium leachate tests. These tests, performed at seed the laboratory of ESALQ/USP, in function of their simplicity and efficiency, deserve the attention of seed technologists.
Key Words: seed testing, vigor, accelerated aging, controlled deterioration, potassium leachate

INOVAÇÕES PARA A AVALIAÇÃO DO VIGOR DE SEMENTES

RESUMO: Dentre os princípios utilizados para a avaliação do vigor de sementes, duas abordagens são predominantes: a resistência das sementes a estresses e a identificação direta ou indireta do "estado atual" das sementes (atividade enzimática, integridade das membranas celulares, parâmetros de crescimento). Neste trabalho são discutidas recentes inovações em procedimentos para avaliação do vigor, de âmbito nacional e internacional, destacando-se as metodologias do teste de envelhecimento acelerado, com uso de soluções saturadas de sal, de deterioração controlada e de lixiviação de potássio. Esses testes, em função de sua simplicidade e eficiência, reveladas em estudos realizados no laboratório de sementes da ESALQ/USP, merecem atenção dos tecnologistas de sementes para observações futuras.
Descritores: sementes, testes de vigor, envelhecimento acelerado, deterioração controlada, lixiviação de potássio

INTRODUCTION

Seed quality comprises the sum of all properties or characteristics which determine the potential level of the seed or seed lot performance and crop establishment. Seed quality components include genetical, physical, physiological and soundness (microorganisms and insects) aspects. These four components may be adversely affected during seed production, processing, storage, and transport. Seed technologists have directed great efforts to identifying the main problems occurring throughout seed production and developing appropriate practices to assure high quality levels of the seeds produced.

Although research considers all characteristics of seed quality, literature shows that the physiological component has received more attention with an emphasis on studies involving the identification of the relationship between seed vigor and seedling emergence and the development of reliable methods for seed vigor evaluation. Farmers require such information to quickly determine the expected rapidity and uniformity of seedling emergence when purchasing seeds. Seedsmen also request this information because they are aware that loss in vigor precedes loss in viability and that vigor tests could aid in monitoring seed quality after maturity (McDonald, 1975), such as detecting where loss in physiological quality occurs during and after harvesting. Seed companies could then take preventive measures by improving specific procedures to guarantee the best levels of seed quality instead of just being satisfied when seed lots only attain the minimum quality standards.

It is essential, however, that seed analysts and consumers understand that vigor tests are not designed to predict the exact number of seedlings that will emerge and survive in the field, although many of the vigor test results are well related to field emergence. One of the primary purposes of vigor tests is to indicate whether or not trouble may be expected from a high germinating seed lot if the lot is placed under adverse environmental conditions in the field, storage or during transportation. It is desirable that vigor tests results provide reliable information to rank seed lots according to seed quality level and to eliminate those lots that fall below company standards, since many seed companies have their own in-house vigor test procedures (Ferguson, 1993).

According to McDonald (1975) and Hampton (1993), to be accepted by the seed testing community a vigor test must: a) Provide a more sensitive index of seed quality than the germination test; b) Consistently rank seed lots in terms of potential performance in the field and/or storage; c) Be objective, inexpensive, uncomplicated, rapid, reproducible, interpretable and related to seedling field emergence.

Establishment of the ISTA Biochemical and Seedling Vigor Committee proposed by Franck at the 1950 ISTA Congress in Washington D.C./USA, provided impetus for the concept of vigor testing. Over the past 50 years many different vigor testing methods have been proposed, studied and used by seed technologists. However, research refinement has demonstrated that the approach of vigor testing has become excessively diversified leading to a trend to redirect the goals since only a few methods are internationally used. Although more than 60 different vigor test procedures have been proposed during the period mentioned, the ISTA Vigor Testing Committee has concentrated its efforts on the standardization of nine vigor tests (Hampton & TeKrony, 1995), while AOSA has suggested or recommended procedures for seven methods since 1983. Methods considered efficient by ISTA and AOSA are almost the same.

Two views predominate in most seed vigor evaluation. First, some vigor tests were designed to identify seed performance (mainly germination) after the exposure to different stresses. These adverse conditions, such as low temperatures and moisten substratum (cold test), high temperature and high relative humidity (accelerated aging) or high seed water content (controlled deterioration) are followed by a germination test.

Second, the vigor "per se" can be evaluated, for instance, through enzyme activity, changes in cell membranes organization, seedling growth rate. These components are regarded as facets of the physiological complex that determine the expression of the physiological potential and to some extent the physical and healthy condition of seeds. The latter approach can be applied not only under unfavorable conditions but applies equally well under favorable conditions (Delouche & Caldwell, 1960). Indirect evaluation of cell membrane integrity (electrical conductivity test) has been used intensively in seed vigor testing research. Although those tests have been internationally accepted and standardization has almost been achieved, some refinements of methodology still constitute a challenge for Seed Technology research.

The main objective of this presentation is to discuss new approaches for the accelerated aging test compared to the controlled deterioration and to provide information on the potassium leachate test as an alternative methodology for the evaluation of the loss of cell membrane integrity. These approaches should be considered as new from an international and/or Brazilian point of view.

SATURATED SALT ACCELERATED AGING AND CONTROLLED DETERIORATION TESTS

The accelerated aging test is the second most popular seed vigor test in the United States, surpassed only by the cold test (Ferguson-Spears, 1995). According to Jianhua & McDonald (1996) the primary use of this test has been limited to large-seeded agronomic crops and has been less studied for small-seeded vegetable, flower and turf crops. They mentioned that for these species correlation of accelerated aging results with small-seeded quality have been poor. This is probably due to a large variation in seed water content. Small-seeded crops absorbed water rapidly and achieved maximum water content after only one day of accelerated aging.

In contrast, controlled deterioration was initially developed as a test for poor field performance potential and/or storability in seed lots of small-seeded vegetable species, e.g., carrot, onion, lettuce, brassicas (Matthews, 1980). Subsequent work has demonstrated the ability of this test to rank seed lots of a large number of species for potential performance. The controlled deterioration test can consistently identify low and high vigor seed lots (Powell et al., 1984). One of the most important aspects of this test is that seed water content must be precisely controlled at the same level prior to seed exposure to high temperature since slight differences of + 1^{o}C can have major effects on controlled deterioration results (Powell & Matthews, 1981).

There is no doubt that the controlled deterioration and accelerated aging tests have demonstrated a proper degree of efficiency to be used in seed quality control programs; however, Jianhua & McDonald (1996) emphasized that other alternatives to aging small-seeded crops appear to be desirable. Therefore they proposed a method to delay seed water uptake using the accepted accelerated aging test principle and main procedures except for substituting water for salt solutions (NaCl, KCl or NaBr) in the accelerated aging box. Their research with high and low quality impatiens (Impatiens wallerana Hook) seed lots showed that useful accelerated aging results were obtained with either KCl or NaCl, after four days germination, by aging for 72 h and 96 h at 38°C for KCl and NaCl, respectively, or 48 h at 41°C for both salts. They considered this test as applicable to impatiens and likely to other small-seeded crops.

The controlled deterioration has not been a common procedure for Brazilian seed technologists. Rossetto & Marcos-Filho (1995) showed that this test was efficient to evaluate the physiological quality of soybean seeds; this is the only reference found in Brazilian seed literature. In this research the controlled deterioration appeared to be less drastic than accelerated aging. The authors emphasized that it is necessary to carefully select the correct procedure to adjust seed water content to prevent or minimize seed deterioration prior to sealing the seeds in the aluminum foil packet.

The salt accelerated aging test is also a new approach for seed vigor testing in Brazil. The efficiency of this test and that of controlled deterioration were compared by Panobianco & Marcos- Filho (1998) using high and low quality bell pepper (*Capsicum anuum* L.) seed lots.

Agroflora-Sakata Seeds provided five bell pepper seed lots for this study. Seed water content (fresh weight basis) and germination (20-30°C; rolled paper towels) were performed as recommended by the Rules for Testing Seeds (Brasil, 1992).

Electrical conductivity of the leachate from whole imbibed seeds was performed on four replications of 50 seeds weighed to 0.01g. Seeds were placed in plastic disposable cups with 25ml distilled water, and kept in a germinator at 25°C. After 24 h the electrical conductivity of leachate was determined using a conductivity meter, and mean values were expressed in μmhos/cm/g for each seed lot.

Two procedures were used for the accelerated aging test. Two gram seed samples from each for each seed lot were placed on a wire mesh screen and suspended over 40ml water or 40ml saturated NaCl solution inside a plastic accelerated aging box. The boxes were kept at 41°C and near 100% relative humidity for 72 h. After aging, the seeds were removed from the accelerated aging box, germinated at 20-30°C and evaluated after 7 and 14 days.

Controlled deterioration was conducted using seed samples whose water content was adjusted to 18% (W1) and 24% (W2) according to Rossetto et al. (1995). Seed samples (2.5g each) were placed in aluminum foil bags and kept at 41°C for 24 h in a water bath. After deterioration, the bags were opened and seeds immediately removed to determine seed water content. Four replications of 50 seeds were then tested for germination as above.

Results showed that water content of seeds aged in 100% relative humidity was not uniform after the aging period, varying from 29.7% to 37.8% (TABLE 1). Seeds from Lot 4, possessing the lowest water content before aging, exhibited lower increases in water content during the aging period, but the non-uniformity was observed even for those seed lots with similar water contents prior to aging. It was also observed that use of a saturated solution of NaCl delayed water uptake and seed deterioration mainly for Lot 4, which had the poorest physiological quality.

TABLE 1 - Seed water content (fresh weight basis) of four bell pepper seed lots exposed to: a) 100% R.H. and NaCl saturated salt solutions at 41°C for 72h (accelerated aging test), and b) controlled deterioration test, at 41°C for 24 h. Piracicaba, SP, 1998.

Seed lots	Accelerated aging			Before controlled deterioration		After controlled deterioration	
	Initial	100% R.H.	NaCl	W1	W2	W1	W2(*)
	%water content						
1	8.0	30.7	12.0	18.1	24.5	18.1	24.3
2	8.6	35.6	11.4	18.1	24.0	18.0	24.7
3	7.9	32.6	11.2	17.9	23.9	18.2	24.2
4	6.8	29.5	9.9	18.4	24.3	18.2	24.5
5	8.1	37.8	11.5	18.3	23.9	18.3	24.5

(*) Water content prior to controlled deterioration

Seed water contents before and after controlled deterioration (TABLE 1) were considered uniform among seed lots and consistent with those reported previously (Hampton & TeKrony, 1995).

TABLE 2 shows that all tests performed indicated Lot 4 as having the lowest physiological quality. In addition, electrical conductivity, salt accelerated aging and controlled deterioration (W2) tests ranked the lots similarly.

Based on these preliminary results we concluded that the salt accelerated aging and controlled deterioration tests have potential application for seed vigor evaluation of small-seeded crops. This finding deserves more attention from Brazilian seed researchers, mainly those involved with the development of procedures to be used in seed quality control programs. Further research is needed to improve the procedures adopted here including, at least, studies to establish initial water content, temperature selection for salt saturated accelerated aging and/or controlled deterioration and size of samples to be evaluated.

POTASSIUM LEACHATE TEST

Currently, one of the main concerns of seed vigor evaluation is obtaining reliable results in order to make fast decisions with regard to harvesting, processing, storage, and marketing operations.

Poor cell membranes integrity is usually related to lower seed vigor and can be indirectly monitored by determining of the extent of electrolytes leakage during imbibition. In this regard, the electrical conductivity test has been recognized in the literature and by seed technologists as one of the best tests for the evaluation of seed vigor.

The extent of leakage of inorganic ion, sugar, inorganic acid, protein, and aminoacid molecules during seed imbibition is promoted by passive diffusion of low molecular weight solutes and by the leachate of macromolecules through cellular rupture (Duke et al., 1983). Potassium is the main inorganic ion leached by seeds during imbibition (Loomis & Smith, 1980), followed by sodium and calcium.

Amorim (1978) and Marcos-Filho (1979) demonstrated the potential of the potassium leachate test for seed testing. Other studies have been conducted at the ESALQ/USP seed laboratory to evaluate the repeatability of results, as well as to improve and standardize the methodology including temperature, sample size and presentation of results, imbibition period, and type of sample used for the test with or without selection of injured seeds.

Dias et al. (1997) concluded that the potassium leachate test showed potential to be an efficient, sensitive and non-subjective option for the evaluation of soybean seed, in addition to being faster than the electrical conductivity test. They found that the imbibition of pure seeds (without previous selection) at 30°C for 90 min was suitable to rank seed lots according to vigor level and field emergence potential.

Custódio & Marcos-Filho (1997) studied combinations between the amount of water and imbibition period, and their relationship with temperature, in order to enhance the standardization of the potassium leachate test. These relationships still require better definition, since they are directly related to the K^+ concentration of solution measured by a flame photometer.

Research was conducted with seeds of two soybean cultivars (BR-38 and IAC-15) each represented by five seed lots. On receipt, each lot

TABLE 2 - Results of germination tests, electrical conductivity, accelerated aging, and controlled deterioration vigor tests on five bell pepper seed lots. Piracicaba, 1998.

Seed lots	Germination	Electrical conductivity	Acc. aging		After C. deterioration	
			100% R.H.	NaCl	W1	W2(*)
	%	μmhos/cm/g	%			
1	96 a(**)	509.1 bc	86 a	88 b	88 a	88 b
2	99 a	500.4 b	87 a	89 ab	89 a	90 ab
3	99 a	409.8 a	86 a	96 a	87 a	94 a
4	53 b	535.2 c	38 b	67 c	56 b	60 c
5	98 a	511.5 bc	84 a	84 b	84 a	88 b

(*) Water content prior to controlled deterioration

(**) Within each column, means with the same letter are not significantly different according to the Tukey's test (P= 0.05).

was blended, sampled and evaluated for water content (105°C/24 h), standard germination (rolled paper towels, 30°C), accelerated aging (41°C, 100% R.H. for 48 h), electrical conductivity (replications of 50 pure seeds/75ml distilled water/25°C/24 h), and seedling field emergence. Tests were repeated three times at bimonthly intervals.

The flame photometer was adjusted to evaluate potassium leakage from imbibed soybean seeds, using linear regression analysis. Potassium leachate tests were then conducted with four replications of 25 seeds each/cultivar/seed lot weighed to 0.01g and placed into disposable plastic cups with 75ml or 100ml distilled water, which were kept in a germinator for 30, 60 or 90 min. Two imbibition temperatures (25 and 30°C) were used. The amount of leached potassium was determined in aliquots of 5ml withdrawn from each sample by a flame photometer adjusted to the 50ppm K^+/ml pattern and reading 100. Results were expressed in ppm K^+/g seeds.

Although reliable information has been obtained for both cultivars, this presentation only takes in to account the 'BR-38' results. All five lots of this cultivar possessed wider differences in physiological quality (TABLE 3A). The germination test was the least sensitive for detecting differences among seed lots, which occurred only at the third evaluation. The relationship among accelerated aging and electrical conductivity results was evident. In the first evaluation, the accelerated aging test showed the superior quality of Lot 1 over Lot 3. Lots 3 and 5 had lower seed vigor than Lots 1 and 2, based on electrical conductivity test results, while field emergence also ranked Lot 5 as the worst performing less than Lots 1, 2 and 4.

TABLE 3A also showed that accelerated aging and electrical conductivity tests detected the significantly lower quality of Lots 3 and 5 at the second and third evaluations. Field emergence was less sensitive and detected only the lowest physiological quality of lot 3 at the second and third evaluations.

Potassium leachate results (TABLE 3B) were related to those of accelerated aging and electrical conductivity tests, and recognized the significantly

TABLE 3 - Cultivar BR 38: results from germination and vigor tests in three evaluation times during storage at ambient laboratory conditions. A) Standard germination (Germ.), accelerated aging (A.A.), electrical conductivity (E.C.) and seedling field emergence (F.E.); B) Potassium leachate tests (ppm K+/g seed). Piracicaba, 1994.

A)

Seed lots	Jan./Feb. 1994				April/May 1994				June/July 1994			
	Germ (%)	A.A (%)	E.C µmhos/cm/g	F.E. (%)	Germ (%)	A.A (%)	E.C. µmhos/cm/g	F.E. (%)	Germ (%)	A.A. (%)	E.C. µmhos/cm/g	F.E. (%)
1	100 a(*)	97 ab	61.6 a	69 a	97 a	98 a	59.2 ab	95 a	96 ab	95 ab	57.7 a	90 ab
2	98 a	99 a	61.6 ab	71 a	96 a	95 ab	62.6 abc	92 a	93 abc	97 ab	54.6 a	92 ab
3	96 a	91 b	84.8 c	63 ab	91 a	89 b	76.4 c	85 b	85 c	84 bc	83.4 b	83 b
4	100 a	97 ab	67.3 ab	75 a	98 a	93 ab	49.7 a	96 a	97 a	98 a	58.5 a	94 a
5	96 a	95 ab	79.8 bc	59 b	93 a	92 b	70.5 bc	94 a	88 bc	78 c	79.6 b	90 ab

B)

Seed lots	January/February 1994		April/May 1994		June/July 1994	
	25ºC	30ºC	25ºC	30ºC	25ºC	30ºC
	ppm K*/g seed		ppm K*/g seed		ppm K*/g seed	
1	574 b(*)	606 b	591 b	629 b	542 b	578 b
2	541 b	581 b	553 b	607 b	514 ab	546 ab
3	675 c	671 c	658 c	719 c	663 c	656 c
4	450 a	477 a	470 a	544 a	477 a	525 a
5	665 c	676 c	666 c	717 c	669 c	696 c

(*)Within each column, means with the same letter are not significantly different according to the Tukey's test (P= 0.05).

lower quality of Lots 3 and 5 at both imbibition temperatures at all evaluation times.

In this study both cultivars showed that the relationship between accepted vigor or potassium leachate tests and field emergence was more dependant of environmental conditions than of the accuracy of each test. It was also shown that the imbibition period had a greater effect on potassium leakage than the quantity of water used (TABLE 4).

Identification of differences in seed vigor among seed lots was uniform for all the treatments using the favorable combination of 25 soybean seeds imbibed in 75 or 100ml distilled water for 30 min. This combination resulted in ranking seed lots according to field performance, and avoided the release of excess of potassium that would require previous sample dilution to be read by a flame photometer. Dias et al. (1996) considered the imbibition period of 90 min as optimum, but the excess of potassium leakage when a quantity of 100ml water was used required sample dilution. Furthermore, this procedure should include an additional variable that might alter the final results.

An analysis of mineral composition (TABLE 5) was performed to verify whether potassium leachate and electrical conductivity tests results were influenced by ion concentration of the seed lots. It was shown that ion concentration did not vary significantly among seed lots for both cultivars thus indicating that these test results were primarily determined by differences in cell membrane integrity and thus the physiological quality of seed lots.

TABLE 4 - Potassium leakage: mean values (ppm K+/g seed) obtained for five ‘BR 38’ soybean seed lots, as influenced by the amount of water, period and temperature of imbibition at three evaluation times during storage under ambient laboratory conditions. Piracicaba, 1994.

Treatments	January/February 1994		April/May 1994		June/July 1994	
	25ºC	30ºC	25ºC	30ºC	25ºC	30ºC
	ppm K*/g seed		ppm K*/g seed		ppm K*/g seed	
75 ml/30 min	490 a(*)	523 b	461 a	562 a	477 a	494 a
75 ml/60 min	552 bc	616 c	575 b	598 a	580 b	619 b
75 ml/90 min	604 cd	660 c	598 b	682 c	674 c	664 bc
100 ml/30 min	503 ab	442 a	493 a	615 ab	462 a	474 a
100 ml/60 min	656 de	633 c	609 b	653 bc	548 b	646 bc
100 ml/90 min	680 e	738 d	791 c	750 d	698 c	702 c

(*)Within each column, means with the same letter are not significantly different according to the Tukey’s test (P= 0.05).

TABLE 5 - Mineral composition of five ‘BR 38’ soybean lots.

Seed lots	N	P	K	Ca	Mg	S	B	Cu	Fe	Mo	Zn
	%						ppm				
1	6.02	0.50	1.68	0.17	0.19	0.16	33	10	52	20	31
2	6.05	0.46	1.76	0.17	0.20	0.20	30	8	74	21	36
3	5.96	0.57	1.53	0.15	0.21	0.21	30	10	100	19	38
4	6.26	0.39	1.61	0.16	0.18	0.18	32	8	104	20	39
5	5.92	0.52	1.76	0.18	0.22	0.17	22	10	101	21	37

CONCLUSIONS

The results reported here provided useful information regarding the use of potassium leachate test as an indicator soybean seed vigor, that together with salt saturated accelerated aging and controlled deterioration tests may be other options for Brazilian seed producers and technologists. Further research is needed to refine the procedures to be adopted for vigor testing of other small-seeded crops (accelerated aging and controlled deterioration) and grain crops (potassium leachate). These tests exhibit advantages of simplicity and rapidity that allow important decisions from physiological maturity to marketing in a successful seed quality control program.

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