Seed Vigor Tests for 'Autumn King' Cabbage Seeds

Nan Kyi Yin [1] Yu Sung [2]

Key words: Cabbage seed, Vigor test, Leachate, Germination

Summary

In this study using vigor tests carried out on 'Autumn King' cabbage to assess seed vigor ability. The germination performance decreased significantly with increasing aging time. The electrical conductivity of the seed leachate and the levels of soluble carbohydrate, free amino acid, and potassium ion in the leachate from aged seeds were about double that of control seeds. The carbon dioxide of control seed produced 1.2 and 2.7 fold more than that of seed aged 36 h and 48 h, respectively. The dehydrogenase activity of control seed was significantly higher than that of aged seed. In the tetrazolium test, stained seeds were separated into five classes. The seeds number in class I of control seed was higher than aged seed. The sinapine fluorescent of control, aged and dead seeds was present under UV, while seeds were imbibed. The simplest and non-destructive vigor testing method was sinapine fluorescent detection.

Introduction

Seed vigor represents the potential level of activity and performance of the seed during germination and emergence. The seed vigor test is standard to provide information on the planting value of the seeds under a wide range of environments and/or the storage potential of seed lots (Hampton and TeKrony, 1995). There are currently many methods available for testing the vigor of *Brassica* seeds.

Accelerated aging at 42°C for different periods of time was found to result in canola (Elias and Copeland, 1997), soybean (Vieira *et al*., 1999), and pigeonpea (Kalpana and Madhava Rao,

1) Graduate student, Department of Horticulture, National Chung Hsing University.
2) Professor, Department of Horticulture, National Chung Hsing University. Corresponding author.

1995) seed of varying quality levels. This is related to the cellular membrane integrity of seeds (AOSA, 1983). Poor membrane structure and leaky cells are usually associated with seed deterioration and low vigor seed.

The solutes leaked from seeds of various plant species include free amino acids (Harman and Granett, 1972), proteins (McKersie and Stinson, 1980), sugars (Takayanagi and Murakami, 1969), the conductivity of electrolytes (Anon, 1983), and phenolics (Samad and Pearce, 1978). Hill *et al*. (1988) found that viable seeds of 'King Cole' cabbage had the lowest conductivity value and that heat–killed seeds had the highest value. The amount of K^+ increased significantly as the aging time increased (Edward and Orrin, 1980). Sinapine, the major aromatic choline ester of *Brassica* seeds, is present in both viable and non–viable cabbage seed, but the leakage of sinapine during germination is observed only from non–viable seeds (Taylor *et al*., 1988). This compound is fluorescent under UV light (Tzagoloff, 1963). Tang and Song (1999) found that sinapine leakage was higher in low–vigor Chinese cabbage seeds than in high–vigor ones.

Respiration is disturbed in seeds with reduced viability and vigor (Bewley and Black, 1994). The respiration rate during seed imbibition can be significantly correlated with germination rates, seedling growth, field emergence, and crop yield (Carver and Matthews, 1975; Hall and Wiesner, 1990; Woodstock and Grabe, 1967). Tetrazolium is a vital stain based on oxidation–reduction and widely used for estimating seed viability (ISTA, 1985). The intensity and stained position of the coloration were directly correlated with the potential level of seed vigor. The objective of this study was to determine the appropriate vigor testing method for 'Autumn King' cabbage seeds.

Materials and Methods

Acceleration ageing test

Cabbage cv. 'Autumn King' seeds were artificially aged at 42°C and 100% RH for 36 and 48 h, respectively, in a plastic box ($32 \times 22 \times 12$ cm^3). The seeds were then removed from the box and dried at room temperature until its original seed moisture content was reached. These seeds were used for germination and emergence test.

Germination test

Each treatment consisted of three replications, and each replication consisted of a petri dish containing 20 seeds on a single layer of moistened (1 ml distilled water) 55 mm thick filter paper (Advantec No.1). The petrishes were placed at 25°C in the dark. Germination was considered to have occurred when the radicle protruded more than 2 mm. The number of the

germinated seed was counted daily for 10 days. The final germination percentage (G) and the mean days to germination (MDG) were calculated using the following formulae:

$G\ (\%) = (\Sigma GNi / GN) \times 100$

$MDG = \Sigma(i \times GNi) / \Sigma GNi$

Where: GNi = the number of seeds germinating on day i

GN = the number of seeds tested

i = 1, 2, 3,……, n; where n＝the last day of the test

Emergence test

Emergence tests were conducted at 25°C in a growth chamber. Each test consisted of three replications, with each replication consisting of 20 seeds sown in approximately 1 cm deep medium (peat moss: vermiculite: perlite, 8:1:1) in a 128–cell plug tray. The number of the emerged seeds (when the hypocotyl breaks through the soil) was counted daily for 10 days. Data on the number of the normal and abnormal seedlings were collected on the last day of the experiment. The final emergence percentage (E), the mean days to emergence (MDE), and normal seedling percentage (NS) were calculated using following formulae.

$E\ (\%) = (\Sigma ENi / EN) \times 100$

$MDE = \Sigma(i \times ENi) / \Sigma ENi$

$NS\ (\%) = (\Sigma NS \times 100) / \Sigma ENi$

Where: ENi = the number of seeds emergence on day i

EN = the number of seeds tested

i = 1,2,3,……, n; where n＝the last day of the test

In accordance with the International Rules for Seed Testing (2003), the seedlings were evaluated as normal and abnormal. The characteristics of abnormal seedlings are the primary root is stunted, retarded, or missing, or it is trapped in the seed coat. The hypocotyls are bent over, forming a loop, or tightly twisted, forming a spiral. Compare with intact seedlings, the cotyledons are deformed, separated, missing, discolored, or necrotic, in more than 50% of the abnormal seedlings. The results wer expressed as a percentage of the total number of seeds with the sum of the percentages of normal and abnormal seedlings and ungerminated seeds being 100.

Leachate analysis

A 0.5 g sample of dry seeds was put into a test tube (15 mm×105 mm) containing 5 ml of deionized water at 25°C for 19 h. The leachates were analyzed by the following items.

Electrical conductivity test

Three seeds were put in one plastic cell (2cm×2cm×1.5cm) containing 3 ml deionized water at 25°C. The electrical conductivity of the water was read with an electronics conductivity

meter (Suntex 170) at two hours intervals between 1 and 17 h of imbibition for 'CA49', 1 and 19 h for '228', and 1 and 25 h for 'Autumn King'. Each treatment consisted of three replications, with ten samplings for each replication.

Free amino acids

The 0.4 ml of leachate was mixed with 1 ml of ninhydrin reagent (95 g KH_2PO_4, 43 g $NaHPO_4$, 5 g ninhydrin, 3 g fructose in 1 l distilled water) and allowed to stand in a 100°C water bath for 10 min. The solution was then cooled and mixed with 5 ml diulted color reagent (2 g KIO_3 in 600 ml distilled water mixed with 95% ethanol to a final volume of 1 l). Free amino acid content was measured by reading the absorbance at 570 nm using a UV–1201 (Shimadzu) spectrophotometer and comparing it to the standard curve for D,L–alanine (0–1 mM) (Rosen, 1957).

Soluble carbohydrate

The 0.3 ml of leachate was mixed with 1.7 ml of deionized water, 0.1 ml of liquid phenol, and 6 ml of concentrated sulfuric acid. The solution was shaken well and allowed to stand at room temperature for 30 min. Following which soluble carbohydrate content was measured by reading the absorbance of the solution at 490 nm using a UV–1201 (Shimadzu) spectrophotometer and comparing it to the standard curve for 0.5 μM/ml D–glucose (Dubois *et al*., 1956).

Potassium ion

The 0.5 ml of leachate was diluted with 4.5 ml of deionized water, and the potassium ion was measured using a Varian Techtron Atomic Absorption Spectrophotometer Model 1250.

Sinapine fluorescent method

Three replications of this experiment were carried out, with each replicate consisting of 20 seeds placed on a single layer of moistened (2 ml distilled water) 90 mm diameter filter paper (Advantec No.1) in a petri dish. Each seed was about 1 cm apart for an easier determination of fluoresence. The petri dishes were maintained at 25°C in the dark. Seeds on the filter papers were visually examined for fluorescent leakage and counted under UV light (330 nm) at two hours intervals for 24 h. The percentage of seeds that exhibited radicle emergence was recorded daily for the first 10 days (Lee *et al*., 1997). Predicted germination and the sinapine leakage index were calculated using the following formulae.

Predicted germination = $[1– (f/n)] \times 100$

Sinapine leakage index (SLI) = X_f / X_t

Where: f = the number of seeds that exhibited fluorescent leakage;

n = the number of seeds tested;

X_f = the number of non–germinable seeds exhibiting fluorescence;

X_t = the number of non–germinable seeds.

Tetrazolium chloride (TZ) staining test

Seeds were soaked in water for 4 h, then their testa were removed. Three replications of 20 seeds each were carried out. Seeds were transferred to a 0.5% TZ solution and incubated in the dark at 25°C for 8 h. At the end of the staining period, the seeds were washed by water, observed and grouped into five fractions.

Respiration rate

A 100 mg sample of seeds was allowed to imbibe for 19 h, then transferred to a conical flask and sealed for 2 h at room temperature. A 1 ml gas sample was then collected from each conical flask with a syringe. The CO_2 concentration in each sample was measured using a far–infrared CO_2 analyzer (UNOR–610). Seed respiration was calculated as [{(CO_2 concentration of sample – CO_2 concentration of empty bottle)/CO_2 concentration of standard} × container volume × standard sample conc.]/(sample weight × time).

Data analysis

The experiment design was a completely randomized design with three replicates. Germination data were arcsine- transformed. All data were analyzed using SAS (version 9.1, SAS Institute Inc, 2003). Fisher's LSD (least significant differences) was calculated where differences between means were significant at the 5% level.

Results

'Autumn King' seeds were subjected to accelerated aging for 36 or 48 h. The germination, emergence, and percentage of normal seedlings decreased significantly with increasing aging time (Table 1). The normal seedlings possessed more than 50% of healthy cotyledon, leaf and root system (Figure 1A). Abnormal seedlings are shown in Figure 1B. In cabbage abnormal seedlings, the primary root was stunted, retarded, or missing, or it was trapped in the seed coat. The hypocotyls were bent over, forming a loop, or tightly twisted, forming a spiral. Compared with intact seedlings, the cotyledons were deformed, separated, missing, discolored, or necrotic, in more than 50% of the abnormal seedlings.

The leachate increased significantly when the aging time was increased to 36 and 48 h. In seeds aged for 36 h and 48 h, electrical conductivity increased to 831 and 1060 μs/cm/5ml (1.5- and 2.0-fold); soluble carbohydrate concentration increased to about 74.2 and 91.3 μg/g (1.5- and 2.0-fold); free amino acid increased to 165.6 and 211.4 μg/g (2.5- and 3.3-fold); and

Table 1. The germination performance of controlled and aged 'Autumn King' cabbage seeds at 25°C.

Treatments	Control	Aging 36 h	Aging 48 h
Germination (%)	96.7 a[z]	63.3 b	53.3 c
Mean day to germination (Days)	1.9 b	3.3 a	3.4 a
Emergence (%)	95.0 a	58.3 b	43.3 c
Mean day to emergence (Days)	3.5 b	6.4 a	6.4 a
Normal seedling (%)	91.7 a	46.7 b	30.0 c

[z] Mean separation within rows by Fisher's LSD test at 5% level.

(A) Normal seedling (B) Abnormal seedling

Fig. 1. The seedling performance of 'Autumn King' cabbage.

potassium ion concentration increased by 80.2 and 89.5 μg/g (1.2- and 1.4-fold), respectively, compared to control seeds (Table 2).

Table 3 shows that the rate of producing carbon dioxide in control seed (18.3μl/g FW/h) was significantly higher than that in seed aged 36 and 48 h (13.1 and 6.7 μl/g FW/h, 1.2- and 1.7-fold) respectively. The dehydrogenase activity of control seed was significantly higher than that of aged seed.

Tetrazolium chloride–stained seeds were separated into five classes (Figure 2): I, seed completely stained; II, seed unevenly stained, with intense staining associated with partial and/or unevenly stained grayish or damaged tissue on the cotyledons, axis, or both; III, uneven staining, with a staining gradient associated with minor, unstained yellowish areas; IV, weak staining, in which the embryo was an unstained yellowish color or was stained with minor shades of pink; V, more than one–half of the cotyledonary tissue was unstained or the necrotic embryo was stained with minor traces of pink.

The tetrazolium test used to assay vigor revealed that a significantly higher proportion of the control seed (66.7%) showed the class I staining pattern in comparison to the aged seed: 48.3% (36 h) and 31.7% (48 h). There was no significant difference among aging treatments in terms of class II staining. However, class III and IV staining occurred the most frequently in 48–h aged seed, with control seed showing the least class III and IV staining. Class V staining increased with increasing aging time (Table 4).

'Autumn King' seed from four treatments were used to compare the actual germination percentages and to predict germination by the fluorescent leakage assay (Table 5). The differences between the predicted and actual germination percentages from the control, aged and dead seeds were ranged from 0~15%. There was a 1.7% overestimation of germination in control seed. In both groups of aged 36 and 48 h seeds, there were 10 and 15% overestimation of germination. In dead seeds, all had fluorescent and ungerminated. The sinapine leakage index (SLI) provided a measure of the fluorescent–ungerminated seeds. The SLI ranged from 0 to 1, with the percentage of fluorescent ungerminated seeds increasing as the SLI increased.

The correlation coefficients between the emergence rate and the values of the vigor tests of 'Autumn King' cabbage seed are shown in Table 6. The results of the tetrazolium staining test and the dehydrogenase activity assay were positively correlated with the emergence rate at the 5% level of significance. Germination, normal seedling evaluation tests, and respiration rate were highly significantly correlated with emergence. Sinapine content and the results of the leachate assay (electrical conductivity test, free amino acid, soluble carbohydrate, and potassium ion) were highly negatively correlated with emergence rate.

Table 2. The leachate from control and aged 'Autumn King' cabbage seeds after imbibition for 19 hours.

Leachate of seeds	Control	Aging 36 h	Aging 48 h
Electrical conductivity (μs/cm/5ml)	540.7 c[z]	831.7 b	1060.0 a
Free amino acid (μg/g)	64.5 c	165.6 b	211.4 a
Soluble carbohydrate (μg/g)	44.7 c	74.2 b	91.3 a
Potassium ion (μg/g)	62.6 c	80.2 b	89.5 a

[z] Mean separation within rows by Fisher's LSD test at 5% level.

Table 3. The amount of CO_2 producing and dehydrogenase activity of control and aged 'Autumn King' cabbage seeds.

Treatments	CO_2	Dehydrogenase activity	
	μl/g FW/ h	OD/20 embryos	OD/ embryo
Control	18.3 a[z]	0.27 a	0.013 a
Aging 36 h	13.1 b	0.22 b	0.011 b
Aging 48 h	6.7 c	0.14 c	0.007 c

[z] Mean separation in column by Fisher's LSD test, mean with different letters are significant at 5% level.

Table 4. The stained results of tetrazolium test on accelerated aging 'Autumn King' cabbage seeds.

Treatments	I	II	III	VI	V
			(%)		
Control	66.7 a[z]	18.3 a	10.0 b	3.3 b	1.7 c
Aging 36 h	48.3 b	15.0 a	13.3 ab	15.0 a	8.3 b
Aging 48 h	31.7 c	11.7 a	18.3 a	21.7 a	16.7 a

[z] Mean separation in column by Fisher's LSD test, mean with different letters are significant at 5% level.

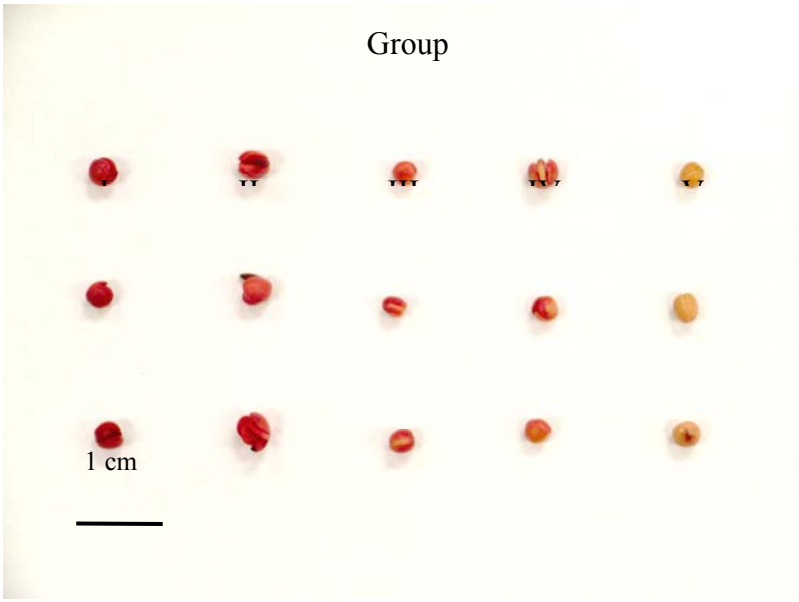

Fig. 2. Tetrazolium chloride stained seeds were separated into five groups.

I. Seed completely stained.

II. Unevenly stained: deep staining with partial uneven grayish or damaged tissue on cotyledons, axis or both.

III. Unevenly stained: a gradient of staining with minor unstained yellowish area.

IV. Weakly stained: unstained yellowish embryo, or embryo with minor shades of pink.

V. More than one–half of cotyledonary tissue unstained or necrotic embryo with minor trace of pink.

Table 5. The predicted germination using the fluorescent leakage assay and actual germination percentage of control and accelerated aging 'Autumn King' cabbage seeds.

Treatments	Germination (%)		P-A[x]	SLI[y]	Fluorescent (%)	Non-fluorescent (%)		
	Predicted	Actual	(%)		Ungerminated	Germinated	Ungerminated	Germinated
Control	98.3 a[z]	96.7 a	+ 1.7	0.00 c	0.0 c	1.7 ab	0.0 a	98.3 a
Aging 36 h	73.3 b	63.3 b	+10.0	0.53 ab	26.7 b	0.0 b	5.0 a	68.3 b
Aging 48 h	68.3 b	53.3 c	+15.0	0.73 ab	25.0 b	0.0 b	1.7 a	73.3 b
Dead	0.0 c	0.0 d	+ 0.0	1.00 a	100.0 a	0.0 b	0.0 a	0.0 c

[z] Mean separation in column by Fisher's LSD test, mean with different letters are significant at 5% level.
Aging: Seeds were artificially aged at 42°C, 100% relative humidity for 36 and 48 hours, respectively.
Dead: Seeds were soaked in water for 4h, transferred to –80°C freezer and kept for half hour.

[y] SLI: Sinapine leakage index (no. of fluorescent ungerminated seed/ total no. of ungerminated seed)

[x] P－A = predicted - actual germination percentage ('＋' means over estimated and '－' means less estimated in prediction).

Table 6. Correlation coefficients between emergence rate and the values of the vigor tests of 'Autumn King' cabbage seeds.

Traits	Emergence rate at 25°C
Germination test	0.969 **
Seedling evaluation test	0.982 **
Electrical conductivity test	– 0.956 **
Free amino acid analysis	– 0.986 **
Soluble carbohydrate analysis	– 0.980 **
Potassium ion leakage test	– 0.955 **
Sinapine content	– 0.926 **
Tetrazolium staining test	0.869 *
Dehydrogenase activity	0.869 *
Respiration rate	0.937 **

*, ** significant at p=0.05 or 0.01, respectively.

Discussion

Artificial seed ageing was initially proposed as a method by which to evaluate seed storability. It can also be used to predict the potential quality of stored seed and field emergence under environmental conditions as the metabolism of the artificial aged seed is similar to that in naturally deteriorated seed. We observed a loss of seed viability in artificial aged seeds (Table 1). This result is in agreement with the findings of other researchers on other species (Byrd and Delouche, 1971; Kalpana and Madhava Rao, 1997; Lin and Pearce, 1990; Pukacka and Kuiper, 1988). The germination performance decreased by more than 30% following a 36–h ageing treatment. In ageing experiments with canola seed, Elias and Copeland (1997) also reported a concomitant decrease in seed germination with increased ageing of the seeds. We also found that the emergence percentage increased while the germination percentage increased, which is agreement with the results of Matthews (1980), who reported that normal germination rates of cabbage seed show a closer relationship with field emergence rates.

During imbibition, the influx of water into the cells of dry seeds results in temporary structural perturbations, particularly those that affect the structure of the membranes. The membrane system changes from being a bilayer phase of a gel to a normal hydrated liquid–crystalline state (Crowe and Crowe, 1982). This alteration destroys the effectiveness of the membrane as a permeability barrier, leading to an immediate leakage of solutes and low–molecular–weight metabolites into the surrounding imbibition solution. Within a short time of hydration, the membranes return to a more stable configuration, at which time solute leakage is curtailed. The longer the time required to restore the integrity of the membrane, the lower the seed vigor (Cheng *et al*., 2005). Our study also confirmed that the conductivity test works well in determining the vigor of seed lots among seed lots with large differences in seed vigor because low–vigor seeds have poor membrane structure and leaky cells.

The membrane functions were severely altered in ageing seeds. We found negative correlation between germination percentage and quantity of leachate. As increasing the ageing duration the germination rate decreased, the leachate quantity increased (Table 2). This may be due to an increased level of protein degradation by proteinases. The increasing activity of proteinase has been associated with rapidly ageing seeds of crimson clover and perennial ryegrass (Ching and Schoolcraft, 1968) and bambarra groundnut (Sreeramulu, 1983). There is a steep K^+ concentration gradient across the plasma membrane that favors the diffusion of K^+ out of the cell. Membranes with a poor integrity cannot actively absorb K^+. In addition, these cells allow more K^+ to diffuse as solute out of the cell, which again enhances the K^+ concentration outside the cells.

Sinapine has been found in all *Brassica* seeds tested to date as well as throughout the embryo (Huang *et al*., 1991). Lee and Taylor (1995) reported that the percentage sinapine content in seeds from six varieties of cabbage, as determined by spectrophotometry, ranged from 1.5 to 2.34%. In general, large quantities of sinapine leak out of heat–killed and non–viable seeds because the cellular membranes are non–functional (Leopold, 1980; Powell and Matthews, 1981) and have lost their normal material retentive ability.

Respiration is disturbed in seeds with reduced viability and vigor (Bewley and Black, 1994). A germinating seed requires energy in the form of, for example, adenosine triphosphate (ATP) and nicotinamide adenine dinucleotide phosphate–oxidase (NADPH). The carbon dioxide producing rate can be a significant correlation between the rates of oxygen uptake and subsequent seedling growth, field emergence, and crop yield (Hall and Wiesner, 1990). We found that prior to germination the respiration rate of control seeds was higher than aged seeds (Table 3). This result was in agreement with that of Bettey and Finch–Savage (1996) who reported that during the seed aging process, the activity of antioxidant enzymes in the seed decreases, and respiratory metabolism is disrupted, leading to disturbed mitochondrial development and axis growth.

Dehydrogenase activity was significantly lower in aged seeds than that in control seeds (Table 3). This result was supported by that of Tang and Song (1999). This decrease in the activities of various enzyme systems is one of the factors affecting seed deterioration. The tetrazolium test has been recommended by the ISTA (1999) as a seed vigor test. In our study, seeds in tetrazolium staining group I were completely stained and those of group II had only small portions of dead tissue; consequently, both of these groups of seeds were considered to be viable. The other group of seeds was considered to be non–viable (Table 4) because large amounts of non–viable tissue in critical areas may have resulted in no or very poor germination. In this study, a significant correlation between the results of the vigor tests and the emergence rate of 'Autumn King' cabbage seeds was found (Table 6).

Conclusion

All the vigor tests used in this study provided sufficiently reliable data that could be used to classify seed vigor level. Among them the quickest, easiest and non destructive methods were sinapine fluorescent detecting and electrical conductivity.

Reference

Anon. 1983. Seed vigor testing handbook. Association of Official Seed Analysts.

Association of Official Seed Analysts. 1983. Seed vigor testing handbook. Contribution No. 32. Association of Official Seed Analysts, Lincoln, NE.

Bettey, M. and W. E. Finch-Savage. 1996. Respiratory enzyme activities during germination in *Brassica* seed lots of differing vigour. Seed Sci. Res. 6: 165-173.

Bewley, J. D. and M. Black. 1994. Seeds. Physiology of development and germination. 2nd edition. New York, Plenum Press.

Byrd, H. W. and J. C. Delouche. 1971. Deterioration of soybean seed in storage. Proceedings of the Association of Official Seed Analysts. 61: 41-57.

Carver, M. F. F. and S. Matthews. 1975. Respiratory measurements as indicators of field emergence ability in pea. Seed Sci. Tech. 3: 871-879.

Cheng, H. Y., G. H. Zheng, X. F. Wang, Y. Liu, Y. T. Yan, and J. Lin. 2005. Possible involvement of K^+/Na^+ in assessing the seed vigor index. J. I. P. B. 47 (8): 935-941.

Ching, T. M. and I. Schoolcraft. 1968. Physiological and biochemical differences in aged seed. Crop Sci. 8: 407-409.

Crowe, L. M. and J. H. Crowe. 1982. Hydration-dependent hexagonal phase lipid in a biological membrane. Arch. Biochem. Biophys. 217: 582–587.

Dubois, M. K., A. Gilles, J. K. Hamilton, P. A. Rebers, and F. Smith. 1956. Colorimetric method for determination of sugars and related substances. Anzl. Chem. 28: 350-356.

Edward, L. L. and E. S. Orrin. 1980. The effect of artificial aging on the concentration of Ca, Mg, Mn, K, and Cl in imbibing cabbage seed. J. Amer. Soc. Hort. Sci. 105(5): 647-650.

Elias, S. G. and L. O. Copeland. 1997. Evaluation of seed vigor tests for canola. Seed Technol. 19(1): 78-87.

Hall, R. D. and L. E. Wiesner. 1990. Relationship between seed vigor tests and field performance of 'Regar' meadow bromegrass. Crop Sci. 30: 967-970.

Hampton, J. G. and D. M. TeKrony. 1995. Handbook of vigour test methods. Zurich, Switzerland: ISTA.

Harman, G. E. and A. L. Granett. 1972. Determination of stored pea seed: Changes in germination membrane permeability and ultrastructure resulting from infection by *Aspergillus ruber* and from ageing. Physiol. Plant Pathol. 2: 271-278.

Hill, H. J., A. G. Taylor, and X. L. Huang. 1988. Seed viability determinations in cabbage utilizing sinapine leakage and electrictrical conductivity measurements. J. Exp. Bot. 39: 1439-1447.

Huang, X. L., Y. H. Cao, Y. H. Li, and J. R. Fu. 1991. The degree of seed deterioration in *Brassica juncea* (L.) coss var. foliosa and its fluorescent leakage. China. J. Bot. 3: 139-144.

International Seed Testing Association. 1985. Handbook on tetrazolium testing. International Seed Testing Association, Zurich, Switzerland.

International Seed Testing Association. 1999. International rules for seed testing. Seed Sci. Technol. Supplement.

Kalpana, R. and K. V. Madhava Rao. 1995. On the ageing mechanism in pigeonpea (*Cajanus cajan* (L.) Mill sp.) seeds. Seed Sci. Tech. 23: 1-9.

Kalpana, R. and K. V. Madhava Rao. 1997. Protein metabolism of seeds of pigeonpea (*Cajanus cajan* (L.) Mill sp.) cultivars during accelerated ageing. Seed Sci. Tech. 25: 271-279.

Lee, P. C. and A. G. Taylor. 1995. Accuracy of sinapine leakage in *Brassica* as a method to detect seed germinability. Plant Varieties and Seeds. 8: 17-28.

Leopold, A. C. 1980. Temperature effects on soybean imbibition and leakage. Plant Physiol. 65: 1096-1098.

Lin, S. S. and R. S. Pearce. 1990. Changes in lipids of bean seeds (*Phaseolus vulgaris*) and corn caryopses (*Zea mays*) aged in contrasting environments. Ann. Bot. 65: 451-456.

Matthews, S. 1980. Controlled deterioration: a new vigor test for crop seeds. In Seed Production. p. 647-662. Butterworths, London.

McKerisie, B. D. and R. H. Stinson. 1980. Effect of dehydration on leakage and membrane structure in *Lotus corniculatus* L. seeds. Plant Physiol. 66: 316-320.

Powell, A. A. and S. Matthews. 1981. A physical explanation for solute leakage from dry pea embryos during imbibition. Ibid. 32: 1045-1050.

Pukacka, S. and P. J. C. Kuiper. 1988. Phospholipid composition and fatty acid peroxidation during ageing of *Acer plantanoides* seeds. Physiol. Plant. 72: 89-93.

Rosen, H. 1957. A modified ninhydrin colorimetric analysis for amino acid. Arch. Biochem. Biophys. 67: 10-15.

Samad, I. M. A. and R. S. Pearce. 1978. Leaching of ions, organic molecules and enzymes from seeds of peanut (*Arachis hypogea* L.) imbibing without testa or with intact testa. J. Exp. Bot. 29: 1471-1478.

Sreeramulu, N. 1983. Germination and food reserves in bambarra groundnut seeds (*Voandzeia subterranean* Thouars) after different periods of storage. J. Exp. Bot. 34: 27-33.

Takayanagi, K. and K. Murakami. 1969. New method of seed viability test with exudates from seed. Proceedings ISTA 34: 243-252.

Tang, Z. J. and M. Song. 1999. Physiolgical and biochemical analysis of artificially aged Chinese cabbage (*Brassica campestris* L. ssp. Pekinensis (Lour.) Olsson). Acta Hortic. Sinica. 26(5): 319-323.

Taylor, A. G., X. L. Huang, and H. J. Hill. 1988. Sinapine leakage from non-viable cabbage seeds. J. Exp. Bot. 39: 1433-1438.

Tzagoloff, A. 1963. Metabolism of sinapine in mustard plants. I. Degradation of sinapine into sinapin acid and choline. Plant Physiol. 38: 202-206.

Vieira, R. D., J. A. Paiva-Aguero, D. Perecin, and S. R. M. Bittencourt. 1999. Correlation of electrical conductivity and other vigor tests with field emergence of soybean seedlings. Seed Sci. Tech. 27: 67-75.

Woodstock, L. W. and D. F. Grabe. 1967. Relationships between seed respiration during imbibition and subsequent seedling growth in *Zea mays* L. Plant Physiol. 42: 1071-1076.

'秋王'甘藍種子之活力檢測之方法

汪 芝 穎[1)] 宋 妤[2)]

關鍵字：甘藍種子、活力檢測、滲漏物、發芽

摘要：在活力檢測試驗中，'秋王'種子隨著老化時間延長顯著降低其發芽率。老化種子的導電度、可溶性碳水化合物、氨基酸和鉀離子顯著較對照組提高一倍以上，老化 36 與 48 小時後種子之二氧化碳產生量較未老化者高出 1.2 及 2.7 倍。未老化種子的脫氫酶活性顯著較老化種子高，經 Tetrazolium chloride 染色後，種子染色情形分為五群。對照組種子染色較完全，種子活力亦較高。利用 UV 光可測定各處理浸潤種子之 sinapine 螢光反應，其方法則是最簡單及非破壞性之甘藍種子活力檢測法。

1) 國立中興大學園藝系碩士班研究生。

2) 國立中興大學園藝系教授，通訊作者。