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The Use of Multiple Tests in Predicting the Vigor of Soybean Seeds

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大豆 種子 發芽勢 檢定方法 比較

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ABSTRACT

Five soybean varieties were used to measure seed vigor according to the artificially different deterioration. A vigor index derived from the product of percent germination x hypocotyl length was used as the parameter in comparison of other tests for seed vigor. There were the varietal differences in initial vigor. The warm germination test was the best measurement for seed vigor at the advanced stages of seed deterioration. All other vigor measurements, except ATP and GADA measurements, showed highly significant correlations with the vigor index. Hypocotyl length, conductivity index and cold germination measurements for seed vigor were appropriate for predicting seed quality.

INTRODUCTION

Seed vigor measurements are of interest for assessing seed quality in a wide range of crops, largely because the warm germination test predicts field performance by varying levels[5,18]. Although many tests for measuring seed quality[10,11,21], have been proposed, most researchers believe that seed vigor can not be possibly measured by single test both viable seed for labeling purposes and still measure the more complex expression of vigor. Thus, scientists have continued to respond to the challenge of addressing the vigor issue and have suggested combining physiological and biochemical test indices for improving accuracy in predicting field performance[2,9,20].

In attempting to develop the best single vigor measurement, Bishnoi and Delouche[6] reported that assays which simulated adverse field conditions such as the cold test and accelerated aging test were adequate for predicting deterioration levels and field performance of cotton. Using six soybean varieties that had been naturally and artificially aged, Burris et al[7]. found that 4-day germination count and seedling growth rate made the best estimates of soybean vigor. Tekrony and Elgi[17] found that the 4-day germination, standard towel germination and the accelerated aging tests were all significantly correlated with field

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emergence. Other studies[1,3] have indicated that measurement of embryonic axis rather than whole seeds gave a better indication of vigor when using physiological or biochemical type tests[1,3].

Abdul-baki and Anderson[2] proposed multiple criteria approach for evaluating soybean seed vigor by measuring parameters such as CO_2 uptake, leaching of metabolites, O_2 production, upake of labeled glucose or leucine, and conversion of isotopes into $^{14}CO_2$ and polysaccharides or proteins. Yaklich and Kulik[23] reported that different combinations index and three of germination, seedling vigor classification, germination index and several tetrazolium tests could accurately predict field performance of soybean. Don et al.[8] concluded that several test methods were needed for a full evaluation of wheat seed quality. Tekrony[16] proposed a combination of the standard germination test with one or more vigor tests to provide a broad evaluation of soybean seed vigor.

This study was carried out to investigate the effects of artificial aging on seed vigor of several soybean cultivars, and to determine the best vigor test or combination of tests predicting seed and seedling vigor accurately.

MATERIALS AND METHODS

Seed materials

Seeds of five soybean(*Glycine max*(L).) cultivars obtained from the Michigan Crop Improvement Association grown in 1985 were artificially aged for 0(control), 1, 2, 3 and 4 day periods using the "Wire-mesh" tray procedure developed by McDonald[12]. Plastic box containing a 10.0cm x 10.0cm x 3.0cm copper wire mesh tray held 2.0 cm above the bottom of the box was used for each treatment. Forty ml of water were added to each box and 400 seeds were placed on the wire tray. Each box sealed with tape, and then incubated at 41.0 ± 1.0℃ and near 100% relative humidity for 1 to 4 days. Seeds were then allowed to dry in a single seed layer at room temperature to have a 12−14% moisture content.

Methods

Warm germination test

This test was conducted using standard procedures as described in the "Rules for Testing Seeds" of the Association of Official Seed Analysts[4]. Four × 100−seed replications were germinated on rolled towel paper at 25℃ for 7 days, then classified into the normal, abnormal seedlings, and dead seeds. Only the percent normal seedlings were recorded for this study. Hypocotyl length of the seedlings was measured for 20 normal seedlings, and seedling dry weight was determinated by the Seedling Growth Rate Test in the AOSA "Seed Vigor Testing Handbook" [22].

Vigor index

Vigor levels were calculated by multiplying percent normal germination by millimeters of hypocotyl length[23]. This index was used for comparison with all other vigor tests.

Cold test

A modified cold test procedure was conducted by exposing the seeds to low temperature for 3 days and then germinating them for 7 days under warm germination conditions. The cold test was performed by planting 400 seeds (eight× 50−seed replicates) in a soil medium composed of equal parts of peat and sand. A 2cm thick layer of soil was placed at the bottom of each plastic box (29.5cm×8.5cm) on which 50 seeds were placed and covered uniformly with the same amount of soil. After replacing the lids, the plastic boxes were incubated for 3 days at 5℃ and then transferred to 25℃ for 7 days for seedling emergence. Normal seedlings were evaluated after seven days and separated into vigor categories based on hypocotyl lengths of less 5cm, 5−13cm, and longer than 13cm,

indexed as 1, 2, and 3, respectively. The total combined index of eight boxes(replicates) was categorized into high, medium, or low vigor on the basic of the following : less 200, low : 200−399, medium : 400−600, high.

Conductivity test

A modification of the electrical conductivity test was performed using the ASA−610 conductivity machine developed by Agro Sciences Inc., Ann Arbor, Michgan. On the basis of preliminary studies, a 20 hour soak at 25℃ was used, and the germination percentage was predicted by the ASA−610 machine using a partition value of 90 microamps. This value was chosen by comparing the actual germination to the predicted germination using a number of partition values according to the root mean square equation :

$$m=\frac{[E(p-a)^2]^{1/2}}{n}$$

Where p=predicted germination, a=actual germination, and n=number of sample tested. The partition value showed the smallest m-value was 90, which was chosen for this experiment. The average microamp per seed was calculated by dividing the sum of all 100 cells in microamps and dividing by 100 seeds. The condctivity vigor index was calculated by partitioning the microamp reading from 0 to 90 into 5 microamps intervals, and assigning a number from 1 to 18 to each interval. The number of seeds in each interval was then divided by the assigned number and results from all intervals were added to obtain a conductivity index.

Tetrazolium vigor test

This test was conducted on the Standard procedures of the AOSA "Tetrazolium Testing Handbook for Agricultural Seeds" [19]. Tetrazolium vigor was categorized as high, medium, and low and the total number of seeds in each category was calculated to obtain a cumulative index of vigor. Vigor determinations were made according to the following criteria as described by Moore[13] as high, medium, low, and dead(nonviable). Vigor classifications were assigned into classes as : less 200, low ; 200−399, medium ; and 400−600 ; high. Extra seeds were reserved in case of seed loss or uncertainly due to artifacts of the slicing process.

Adenosine triphosphate level test

ATP level was determined by the luciferin-luciferase method in boiling water extract[14] of seeds imbibed at 25℃ for 4 hours, and its concentration in the extract was calculated according to the formula of St. John.[14]

Glutamic acid decarboxylase activity test

The glutamic acid decarboxylase activity test was measured 5.0 grams of homogenized seed sample and a substrate solution containing 35.0 ml of 0.1 M L-glutamic acid in 0.50M sodium phosphate buffer, pH5.2. The mixture was placed in a 250ml Erlenmeyer flask, and shaken in a water bath for 8 minutes at 30℃. After 8 minutes, 5cc of air were removed by a syringe and the concentration(ppm) of CO_2 was determined by an ADC Model 225-MK3 Nondispersive Infrared Gas Analyzer.

Metabolite leaching test

This test was conducted according to the method described by Abdul-Baki and Anderson[2]. For replicates of 50 seeds from each cultivar were surface disinfected by soaking them for 5 minutes in 1% sodium hypochlorite and rinsed 5 times in sterile water. The seeds were then imbibed for 3 hours at 25℃ in 100ml of sterile water containing 50 μg/ml of penicillin-G and a streptomycin sulfate. The embryonic axes were then excised and held at 25℃ in 5cm petri dishes containing 3ml of the same imbibition medium until ready for use. To measure leaching of metabolites, 35 axes per

replicate were rinsed with sterile water, blotted on filter paper and transferred into 25ml Erlenmeyer flasks containing 3ml of incubation media. The incubation media were made up of 0.5 mM glucose containing 5wug/ml of penicillin-G and strepomycin sulfate and a specific activity of 0.6 uci D-Glucose-UL-14 Carbon(Sigma Chemical Co.) per μmole. The flasks were incubated in a shaking 25℃ water bath for 1 hour, and the axes were then washed for 20 seconds in a large volume of water, drained and placed in 100ml beakers containing 15ml of water at 3℃. After 1 hour of intermittent shaking to allow uniform distribution of the leached isotope in the water, a 1-ml aliquot of the water containing the leachate was used to determine the radioactivity that leached out of the axes, using a Liquid Scintillation Counter.

Statistical analysis : Results were analyzed as a factoral experiment according to the method described by Steel and Torrie[15] with 4 replications and aging treatments and varieties as the two factors. Regression and correlation analysis were performed on all the data. Stepwise multiple regression was employed to determine the most effective vigor tests.

RESULTS AND DISCUSSION

Five soybean cultivars were used to measure seed vigor with increasing time of artificial aging and to compare for any cultivar differences to the aging treatments. The vigor index (percent germination x hypocotyl length) was used as the performance parameter in comparison with other tests. Table 1 indicated that only one cultivar, Century, differed in initial vigor which was significantly lower than that of the other 4 cultivars. Since germination initially showed no significant difference between the cultivars, the source of the difference in vigor were primarily based on the hypocotyl length which was significantly less for Century than the other cultivars. Such results would indicate that two cultivars differing in hypocotyl length could give a different vigor index. Caution must be observed in vigor index according to the different cultivars, especially when a test component includes growth measurements. Clearly, further studies should be performed to find out whether the observed differences in hypocotyl length were inherent in the cultivars tested or were just due to differences between seed lots.

Figure 1 demonstrates the relationship between germination percent, hypocotyl length and the resultant vigor index of the five cultivars at different aging days. The vigor index was more influenced by hypocotyl length than by germination percent. Dividing each plot into three parts, 0-1, 1-3, and 3-4 aging days, there showed non-significant differences in germination in the first part. Although there was little variation in the vigor index, it tended to follow the similar trend to that of hypocotyl length. The second part : (1-3 days) indicated an almost parallel relationship between the

Table 1. Initial mean germination, hypocotyl length and vigor index of the 5 soybean cultivars with no aging treatment.

Cultivar	Warm Germination (%)	Hypocotyl Lenght (mm)	Vigor Index
Corsoy 79	95	162ab*	15330a
Hodgson 78	89	173a	15315a
Hardin	91	166a	14998a
Elgin	92	151b	13878a
Century	85$^{n.s.}$	105c	8873b

*Column means followed by the same letter were not significantly different at the 5 percent level according to DMRT.

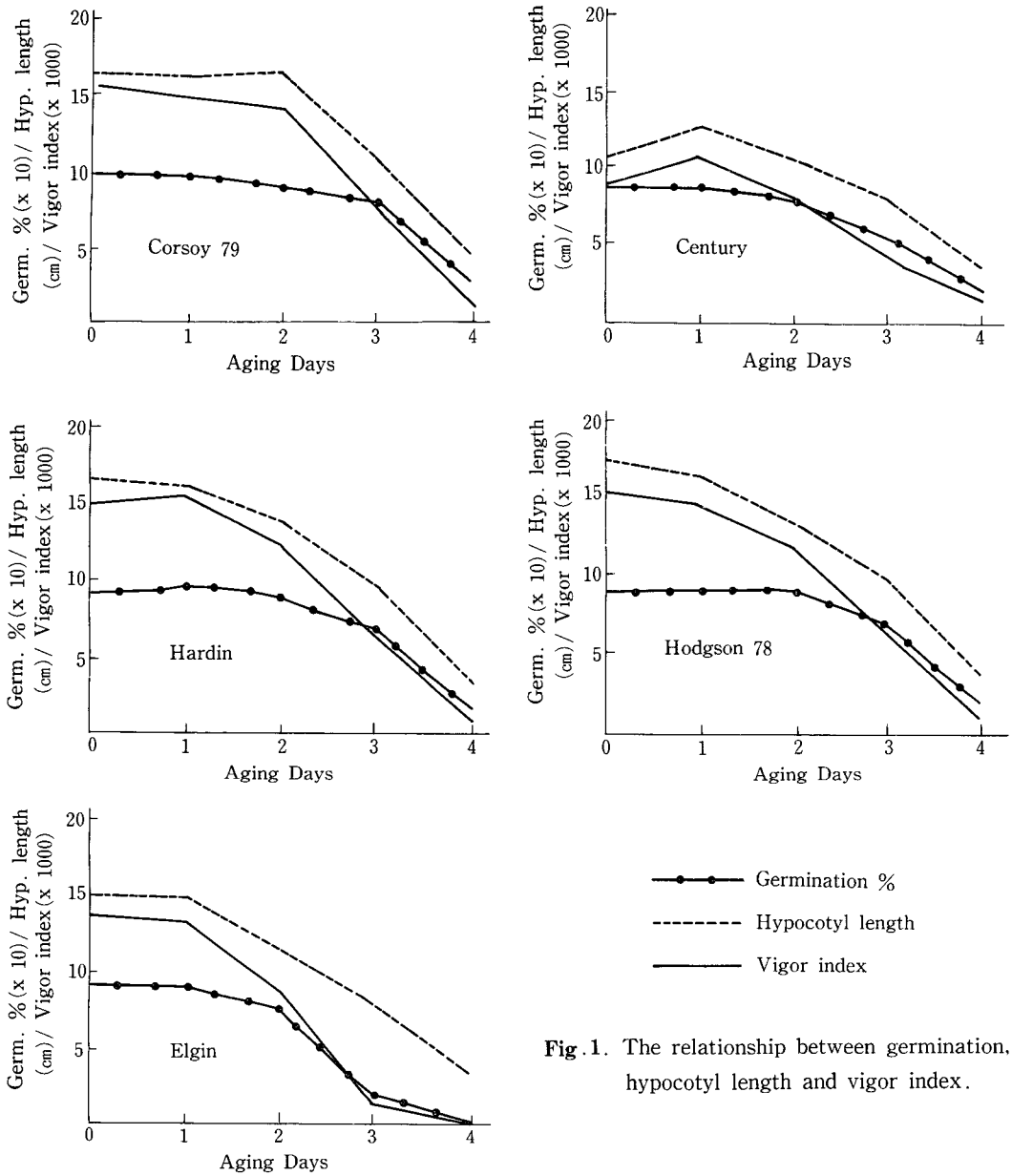

Fig. 1. The relationship between germination, hypocotyl length and vigor index.

hypocotyl length and vigor index. These two characters showed a significant decrease, but germination percent of all cultivars (except Elgin) showed no such decrease. The last part : (3−4 days) at advanced stages of deterioration showed the sharpest drop in germination, showing the similar trends with the hypocotyl length and vigor index. These results indicated the insensitivity of the germination test as a vigor test, and showed that tests in which germination is the final measure of vigor may not indicate any change in the status of seeds until advanced stages of deterioration were reached or if the test had some stress factor incorporated in it.

Based on the above results, several vigor index was performed to determine a sensitive indicator of seed vigor and deterioration (Table

Table 2. Results of seed vigor index and several vigor tests on 5 soybean cultivars aged for different lengths of time.

Cultivar	Aging days	Vigor index	Hypocotyl length. mm	% Cold germ.	Metab. leach. dpm/ 30axes	% Warm germ.	Dry wt. mg/seedling	%ASA610 germ. pred.	uA/ seed	Cond. index	Vigor of TZ test	% of TZ germ. pred	ATP nmoles/1	GADA, ppm CO_2/ min/5g
Corsoy 79	0	15330a*	162a−c	85a−c	55gh	95a	1.27f−h	96a	60ij	8.4a	544a	96a	1.83a−c	1004b
	1	14739a	158b−d	90ab	95gh	94a	1.28f−h	89a−c	68hi	7.1b	505a−c	94ab	1.26c−f	902d−f
	2	13863a	159b−d	35h	128fg	88ab	1.28f−h	83cd	72f−h	6.3b−c	373d	81de	1.17c−f	985b−d
	3	7195ef	101i	37h	151fg	72bc	1.26f−h	78de	78fg	5.6de	305e	74e	0.72f	846f
	4	870h	36k	0j	483b	23e	0.43jk	19i	111c	0.9i−k	143g	27h	0.94d−f	913c−f
Hodgson 78	0	15315a	173a	78c−e	−	89ab	1.36d−f	89a−c	67hi	7.1b	−	−	2.37a	993bc
	1	14370a	161bc	87ab	−	89ab	1.35d−f	88a−c	69hi	7.0b	−	−	1.21c−f	857f
	2	11399c	131ef	64f	−	87ab	1.38d−f	75e	76fg	5.5de	−	−	1.53b−e	913c−f
	3	5785f	94i	47g	−	61cd	1.31e−g	73e	81f	5.2e	−	−	0.93d−f	886ef
	4	447h	27k	0j	−	15ef	0.38jk	9j	124b	0.6jk	−	−	0.77ef	893ef
Hardin	0	14998a	166ab	92a	14h	91ab	1.22g−i	96a	54j	9.2a	551a	93a−c	2.19ab	1143a
	1	15306a	160b−d	92a	136fg	96a	1.16hi	94ab	58j	6.7a	514ab	92a−c	1.12c−f	954b−e
	2	12038bc	136e	57f	360c	89ab	1.13i	87cd	70f−h	6.9bc	403d	87b−d	1.02d−f	985b−d
	3	5909f	95i	37h	334cd	62cd	1.13i	84cd	76f−h	6.0c−e	204f	61f	0.76f	914c−f
	4	479h	28k	0j	509b	15ef	0.37k	30h	102de	2.0gh	109gh	27h	0.65f	895ef
Eigin	0	13876a	151cd	83b−d	57gh	92ab	1.54c	95ab	56j	9.0a	483bc	94ab	1.55b−d	764g
	1	13407ab	148d	73e	69gh	91ab	1.42de	90a−c	66hi	7.2b	398d	92a−c	1.09d−f	727g
	2	8838de	114gh	20i	224ef	77a−c	1.38d−f	44g	99c	3.3f	392d	85cd	0.86d−f	729g
	3	1649gh	78i	0j	251de	21e	1.45cd	26h	112c	1.8g−i	268e	77e	0.85d−f	683g
	4	0h	35k	0j	352cd	0f	0.50j	0k	150a	0.0k	111gh	24h	0.71f	712g
Century	0	8873e	105hi	65f	275c−e	85ab	1.84ab	84cd	73f−h	6.4b−d	474bc	90a−c	1.98ab	730g
	1	10551cd	124fg	75de	336cd	85ab	1.84ab	71e	81f	5.2be	456c	86a−d	0.82o−f	733g
	2	8016e	106hi	23i	352cd	75a−c	1.85a	52f	94e	2.7fg	256e	64f	0.64f	723g
	3	3616g	77j	15i	342cd	47d	1.73b	24hi	110cd	1.5h−j	206f	47g	0.76ef	680g
	4	684h	30k	0j	1223a	16ef	0.42jk	1k	147a	0.1k	78h	25h	0.69f	690g

* column means followed by the same letter were not significantly different at the 5% level according to DMRT.

2). In general, measurements for some biochemical parameter were less sensitive than those with a broader scope. In comparing hypocotyl length to dry weight content generally showed significant decreases at later stages of aging, while hypocotyl length differed significantly with aging treatment, especially after one day of aging. This might indicate that cell enlargement is hindered before cell division is affected. All characters except ATP and GADA highly correlated with the vigor index as shown in Table 3. Both ATP and GADA showed lower correlations than those of any other vigor test (Table 3 and Fig. 2) as shown in non -significant with vigor index. There were two reasons for the overall low correlation in GADA

Table 3. Simple correlation coefficients of 12 variables (seeds vigor tests) on 5 soybean varieties aged for different lengths of time.

Index	Dry wt. mg/seedling	% cold germ.	Cold vigor	%ASA−610 germ. pred.	Average uA/seed	Cond. index	Vigor of TZ test	% of TZ germ. pred.	ATP moles/1	GADA, ppm CO_2, min., 5g	Metab. leachate, dpm, 35 axes
Vigor index	0.519*	0.870**	0.906**	0.872**	−0.846**	0.883**	0.852**	0.833**	0.487*	0.410*	−0.655**
Corsoy 79	0.833**	0.843**	0.834**	0.919**	−0.929**	0.922**	0.963**	0.997**	0.426	0.395	−0.907**
Hardin	0.831**	0.954**	0.963**	0.874**	−0.932**	0.917**	0.960**	0.921**	0.602**	0.467*	−0.760**
Hodgson 78	0.807**	0.935**	0.951**	0.866**	−0.878**	0.898**	−	−	0.531*	0.329	−
Eigin	0.673**	0.917**	0.908**	0.953**	−0.931**	0.940**	0.983**	0.942**	0.469*	0.716**	−0.872**
Century	0.795**	0.845**	0.890**	0.893**	−0.866**	0.819**	0.921**	0.960**	0.277	0.340	−0.743**

*=significant at the 5% level.
**=significant at the 1% level.

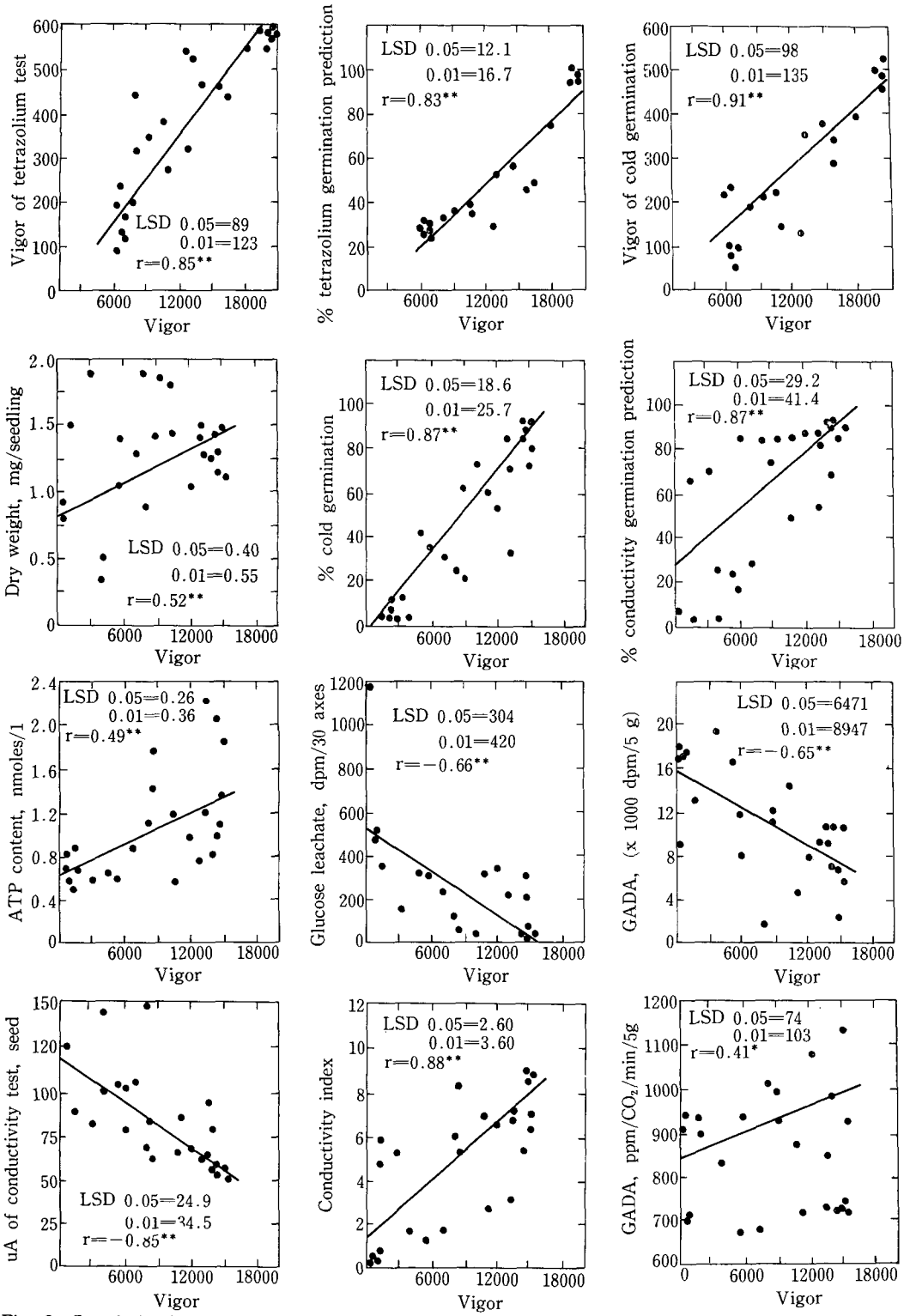

Fig. 2. Correlation between vigor and multiple seed vigor tests on 5 soybean(*Glycine max*. L.) varieties aged for different length of time.

Table 4. Mean squares of the effects of cultivars, treatments and their interaction on results of 13 vigor tests.

Variance	d.f.	Vigor index	Hypocotyl length	% cold germ.	Metab. leach.	% warm germ.	Dry wt.	% ASA−610 germ.pred.	Average uA/seed	Cond. index	Vigor of TZ test	% of TZ germ. pred.	ATP nmoles/l	GADA, ppm Co_2/min. 5g
Cultivar	4	57,031,739**	3,784**	2,062**	4,024**	1,029**	0.83^{NS}	3,834**	3,077**	33**	2,346**	2,130**	0.46NS	308,755**
Aging		681,773,261**	51,688**	25,352**	9,230**	22,060**	3.93**	20,251**	12,993**	163**	10,274**	19,474**	4.93**	47,893**
Treatment	4													
Cultivar x Treatment	16	7,472,976**	587**	415**	274**	277*	0.05^{NS}	523**	311**	3^{NS}	.141**	312**	0.14NS	5,968*

*=significant at the 5% level.
**=significant at the 1% level.

and ATP. The first was due to cultivar specificness since the two tests were not significantly correlated with all cultivars, and the second was that correlation with specific cultivars, when significant, was lower than for other tests. Such results do not necessarily mean that those tests could not adequately measure ATP or GADA activity but rather that using a single biochemical parameter may not be a sensitive enough indicator of seed vigor.

Since different biochemical parameters are affected at different rates and times during the process of deterioration, a better indicator would be a measure of overall changes rather than specific ones. Our data (Table 3) indicated that tests measured overall performance of the seed under stress like the cold treatment and reflected the overall cellular condition (conductivity) were the best estimate of seed vigor.

By using stepwise regression analysis the hypocotyl length and conductivity index gave the best prediction for vigor index. When hypocotyl length was removed, conductivity index, cold germination and cold vigor were chosen. Table 4 shows the mean squares of cultivars, treatments, and their interaction on different vigor tests. Aging treatment was highly significant in mean squares in all vigor tests.

Since we used only one seed lot a cultivar, these results cannot be fully verified. Establishment of the existence of true cultivar differences for these tests require the use of a larger number of seed lots. Until that is accomplished, it cannot be confidently concluded whether difference observed were due to cultivar effects or to differences in initial quality of the seed lots.

These results indicated that, for a good evaluation of vigor, several tests or a single test that measures the sum of many physical and biochemical processes were required. In this study the vigor index might be used as a parameter in comparison with other tests. Obviously, more comparisons should be made with the actual field performance of the different seed lots to determine the applicability of vigor testing in predicting field performance.

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摘　　要

大豆種子의 退化過程에서 일어나는 生理·化學的인 變化를 탐색하여 種子勢의 差異를 比較하고 種子勢 豫測에 適合한 方法을 究明하기 위하여 大豆 5品種의 種子를 人爲老化處理시켰다.

大豆種子勢의 指標로서 發芽率×胚軸의 길이로 나타낸 값을 매개변수로 하여 各種 種子勢 檢定成積과 比較檢討하였다.

種子勢檢定의 가장 적합한 방법으로는 發芽試驗에 의한 방법임을 알 수 있었다. ATP와 GADA 檢定法을 除外하고는 모든 檢定法이 種子勢 指標와 高度의 有意性이 있었다. 大豆種子勢 檢定을 위하여는 胚軸의 길이, 電氣傳導度에 의한 檢定 및 低溫發芽檢定法이 適合한 方法임을 알 수 있었다.

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