韓作誌(Korean J. Crop Sci.) 32(4) : 417~424

Vigor Determination in Barley Seeds by the Multiple Criteria

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보리 種子勢 檢定方法 比較

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ABSTRACT

The seeds of three barley varieties of which initial seed vigor were different were used to measure seed vigor of accelerated aging techniques. A vigor index derived from the product of percent germination and plumule length was used to estimate seed vigor. The index was compared with the results of other tests. The results of warm germination test well suited to the measurements of seed vigor at the advanced stages of seed deterioration. Other vigor measurements except ATP and GADA values, showed close relationships with the vigor index. The measurements of plumule length in cold test and tetrazolium test were found to be appropriate for predicting seed quality.

INTRODUCTION

The measurement of seed vigor is of interest for scientists in assessing seed quality. The warm germination test was considered to be a suitable method to predict field performance of the seed lot[4,8]. With an attempt to develop the best single vigor measurement, Bishnoi and Delouche[6] reported that assays of seed vigor in simulated adverse field conditions, such as the cold test and accelerated aging test, were adequate for predicting deterioration levels and field performance of cotton seed. Anderson[4] that older barley seeds evolved more CO_2 up to 200% than those of newer seeds. Recent works by Grabe in corn[12], by Kittock and Law in wheat[16], and by Linko and Sogn in wheat[18] have been carried out with an interest in glutamic acid decarboxylase activity (GADA) in relation to viability and seed quality of stored seeds. It was also suggested that the GADA was able to predict seed deterioration and seedling vigor. Although many tests have been proposed for measuring seed quality[13, 19, 26], most researchers came to believe that seed vigor can not be measured by a single test because the vigor is a complex expression of biochemical activities of seeds. Thus, scientists have suggested combined indices to express physiological and biochemical properties of seeds with an accuracy in predicting field performance[3, 11, 26]. Ching et al.[9] found that seed weight, ATP content of 3-day old seedlings, ATP content of hydrated embryo, and dry weight of 7-day old seedlings made the best estimates of barley vigor. Tekrony and Elgi[25] found that the 4-day germination of soybean seeds, standard towel germination and the accelerated aging tests were all significantly

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correlated with field emergence. Yaklich et al.[27] proposed a multiple criteria approach for evaluating barley seed vigor by measuring such parameters as ATP content and conductivity. Don et al.[10] found that a combination of several test methods was required for a full evaluation of wheat seed quality.

This study was undertaken to investigate the effects of artificial aging on seed vigor of three barley cultivars, and to determine the best vigor test of seeds or combination of tests that can predict seed and seedling vigor more accurately.

MATERIALS AND METHODS

Seed materials and aging treatment

Seeds of three barley cultivars (Saesalbori, *Bohobori* and *Doosan* #22) were obtained from Gyeongnam Provincial Rural Development Administration. The seeds were artificially aged for 0 (control), 1 and 8 days using the "Wire-mesh" tray procedure developed by McDonald[20]. Each box sealed with tape was incubated at 41.0±1.0°C and near 100% relative humidity. Seeds were then placed in a single-seed layer at room temperature and dried up to 12-14% moisture content.

Warm germination test

According to the "Rules for Testing Seeds" of the Association of Official Seed Analysts(AOSA)[5], three 100-seed replications of seeds were germinated by top planting method at 20°C for 7 days, then plumule length of each normal seedling was measured and speed of germination was estimated by the following formula:

$$\text{Speed of germination (X)} = \frac{\text{Number of normal seedlings}}{\text{Days of first count}} + \frac{\text{Number of normal seedlings}}{\text{Days of final count}}$$

Seedling growth rate (SGR) was estimated by the following formula:

$$\text{SGR (X)} = \frac{\text{Seedling dry weight}}{\text{Total normal seedlings}}$$

Vigor index

Vigor levels were calculated by multiplying percent normal germination by length of plumule[22]. This index was used as a reference in comparing all other vigor tests.

Cold test

A cold test was conducted by exposing the seeds to 5°C for 3 days, followed by successive 7 days at 20°C under warm conditions. The cold test was performed by planting 300 seeds (three 100-seed replicates) in a soil medium which was composed of equal parts of vermiculite and soil (v/v). A 3-cm thick soil layer was placed on the bottom of each plastic box (9.7cm x 8.4cm x 9.4cm) on which 100 seeds were placed and covered uniformly with the half amount of soil. Seventy percentage of water holding capacity was maintained. After replacing the lids, the plastic boxes were incubated for 3 days at 5°C and then transferred to 20°C for 7 days until seedling emergence. Normal seedlings were evaluated after seven days with the number of emerged seedling above the germination medium, and classified into vigor categories based on plumule lengths of shorter than 2cm, 2-4cm, and longer than 4cm for Saesalbori; 3cm, 3-6cm and 6cm for Buhobori and Doosan #22, of which categories were indiced as 2, 4 and 6, respectively. The total combined index was categorized into high (400-600), medium (200-399), or low vigor (less than 200).

Conductivity test

A conductivity test was performed by using the Suntax SC-17 conductivity tester. The sample seeds were soaked in tube with 30ml deionized water at 20°C for 24 hours. The average microamp per seed was calculated by dividing the sum of all 100 seeds and the values were shown in microsiemens cm/g/seed 30ml.

Tetrazolium vigor test

The AOSA standard procedures described in the "Tetrazolium Testing Handbook for Agricultural Seeds" [21] were adopted to categorize the vigor into high, medium, and low. A cumulative index of vigor was calculated by the information collected. The criteria, as described by Moore[21], were high (400-600), medium (200-399), low (less than 200) and dead (nonviable), which are indiced as 2, 4 and 6, respectively.

Adenosine triphosphate level test

ATP level was determined by the luciferin-luciferase method[7] in boiling water extract: Seeds were soaked for 5 minutes in 1 percent sodium hypochlorite, rinsed 4 times in sterile water, and the imbibed seeds were placed at 20°C for 4 hours on a paper towel. Three replications of each 10 seeds were dropped with 5ml into microcentrifuge tube-boiled-distilled water and extracted for 10 minutes. The extract was cooled and stored in an ice bath. An aliquot of the extract was used to measure ATP with an LKB-1250 ATP Photometer, and the data were transformed according to the formula of St. John[23].

Glutamic acid decarboxylase activity test

The glutamic acid decaboxylase activity test was performed following the method described by Linko[18]. Seed were soaked for 5 minutes in 1 percent sodium hypochlorite, rinsed 4 times in sterile water. Five grams of homogenized seed sample were placed in a substrate solution containing 35.0ml of 0.1M L-gluatmic acid in 0.50M sodium phosphate buffer at pH 5.2. The mixture was transferred into a 250ml Erlenmeyer flask, and was shaken in a water bath for 8 minutes at 30°C. Five cc of the air in the flask was withdrawn with a syringe. The concentration of CO_2 was determined with a Beckman Model 864 Nondispersive Infrared Gas Analyzer.

Statistical analysis: Based on the methods of Steel and Torrie[24] the stepwise multiple regression analysis was made to determine the most effective factors in vigor test.

RESULTS AND DISCUSSION

The vigor index was estimated from percent germination x plumule length (Table 1). The initial index of Saesalbori was significantly lower than that of Buhobori or Doosan #22. Since the initial warm germination percent was not significantly different between the cultivars, the difference in vigor index was primarily due to the difference of the plumule length. Therefore caution must be given to determine vigor index, especially when a test includes one of the growth measurements.

Table 1. Initial mean germination, plumule length and vigor index of the three barley cultivars with no aging treatment (control).*

Cultivar	% warm germinatin	Plumule length in warm germination (mm)	Vigor index
Seasalbori	98[a]	58[b]	5684[b]
Buhobori	99[a]	92[a]	9108[a]
Doosan #22	93[a]	97[a]	9021[a]

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

The characteristics of combined vigor index based on percent germination, length of the plumule and seedling growth rate is shown in Fig. 1. The vigor index in each cultivar was decreased as aging days increased, and the score shown by the multiplication of percent germination by length of plumule seemed to be appropriate to measure seed vigor.

Figure 2 demonstrates the changes of percent germination, plumule length and the combined vigor index in three cultivars at different aging days. It was found that the combined vigor index was influenced largely by the plumule length. Perry[22] suggested that field emergence percent of the seedlings was closely related to the plumule growth particularly under the unfavourable soil conditions. Grain yield was found to be closely related to plumule growth. In this study, the plumule length was found to be closely related to vigor index provided the aging day was extended only to 4th day. The relationship was not maintained when the seed aging was extended more than 4 days.

As shown in Table 2, most of the masurements such as percent germination, speed of germination,

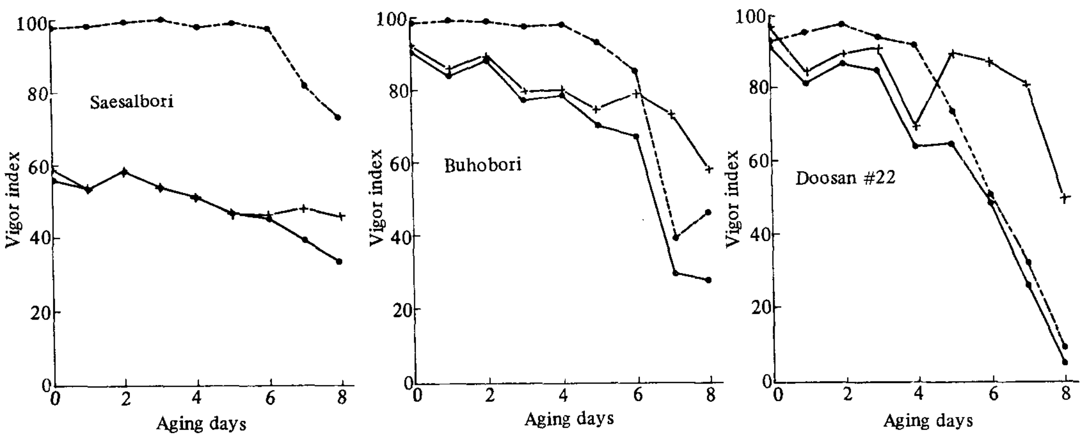

Fig. 1. Characteristics of seed vigor indices measured by the results of three vigor tests for three cultivars under the different levels of aging days. Curves shown are: ●—●, percent warm germination; ●——●, vigor index (x100); +——+, plumule length.

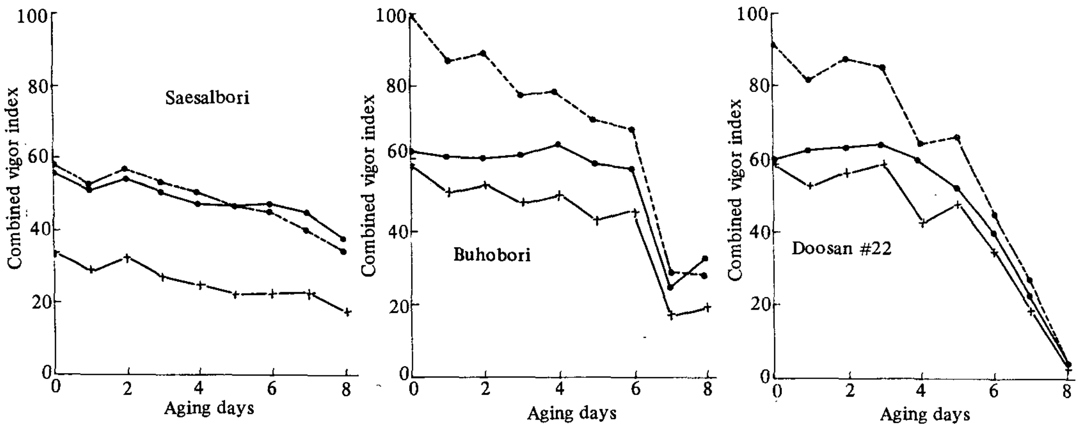

Fig. 2. Characteristics of combined vigor indices of three measurements of seed vigor for three barley cultivars affected by different levels of aging days. Combined vigor index of perceng warm germination x SGR (X10, ●——●); percent warm germination x plumule length (x100, ●---●); and percent warm germination x plumule length x SGR (x1,000, +——+).

length of plumule, percent cold germination, cold vigor, vigor in Tetrazolium Chloride (TTC) reduction and percent of TZ germination prediction were decreased as aging days extended. SGR seemed not to be appropriate to relate to vigor index. No consistent relationships were found betweeen ATP content, conductivity measurements and CO_2 production from GADA. Lamkin et al.[17], however, suggested that activity of the glutamic acid decarboxylase was a useful index to express viability, and the test be employed as supplement test in addition to the currently-used germination tests. The relationship of ATP content of barley seed and vigor was recently challenged by many researchers in large-scale experiments. The relationships between ATP content of barley seed and field emergence, stand, and yield were intensively studied. In this experiment, we aimed to use ATP content as measure of vigor.

The mean squares of cultivars, treatments, and their interaction in different vigor tests were shown in Table 3. As a whole, the effect of aging treatment was recognized in the most of vigor tests except seedling growth rate.

Simple correlation coefficients between seed vigor index and each variable were shown in Table

Table 2. Seed vigor index and several vigor tests of three barley cultivars aged for different length of time.

Cultivars	Aging days	Seed vigor	% warm germ.	Speed of germ.	Plumule length (mm) (WGT)	SGR	% cold germ.	Cold vigor	Plumule length (mm) (CT)	ATP n moles/ liter	Cond., μs cm/g, seed 30ml	Vigor in TZ test	% TZ germ. pred.	GADA, ppm CO_2, min. 5g
Saesalbori (Naked)	0	5739 a*	98 a	235 ab	58 a	5.7 a	99 a	586 a	79 a	3.30 a	299 e	520 a	99 a	1338 a
	1	5295 ab	98 a	226 b	53 ab	5.1 ab	97 ab	570 a	75 a-c	2.94 a	313 de	512 a	96 a	1320 ab
	2	5781 a	99 a	249 a	58 a	5.4 ab	94 a-c	558 a	78 ab	2.93 a	341 cd	520 a	99 a	1307 ab
	3	5333 ab	100 a	198 c	53 ab	5.0 ab	93 bc	549 a	71 bc	2.73 a	351 c	445 b	98 a	1294 ab
	4	5060 ab	98 a	200 c	51 ab	4.8 b	96 ab	564 a	78 ab	1.14 bc	359 bc	344 c	87 b	1305 ab
	5	4621 bc	99 a	182 cd	46 b	4.7 b	78 d	384 c	63 d	1.18 bc	406 a	341 c	82 bc	1286 ab
	6	4491 bc	97 a	177 d	46 b	4.8 b	90 c	472 b	68 cd	0.86 c	396 a	306 c	78 cd	1271 b
	7	3947 cd	81 b	124 e	48 b	5.5 ab	55 e	241 d	52 e	1.84 b	387 ab	304 c	72 de	1284 ab
	8	3360 d	73 c	97 f	46 b	5.1 ab	31 f	116 e	49 e	1.42 bc	232 f	221 d	68 e	1114 c
Mean		4848	94	188	51	5.17	82	449	69	2.04	343	391	87	1280
Baihobori (Covered)	0	9201 a	99 a	172 a	92 a	6.2 bc	97 a	579 a	109 a	5.35 a	213 e	552 a	96 a	1235 ab
	1	8415 ab	99 a	166 ab	85 ab	6.0 c	96 a	573 a	104 ab	5.18 a	216 e	496 b	96 a	1184 b-d
	2	8875 ab	99 a	164 ab	89 ab	5.9 c	96 a	573 a	103 ab	5.16 a	224 de	468 b	96 a	1219 a-c
	3	7722 bc	97 a	156 b	79 bc	6.2 bc	96 a	574 a	100 ab	4.86 a	219 e	308 e	89 ab	1235 ab
	4	7775 bc	98 a	161 ab	79 bc	6.4 a-c	93 a	552 a	99 ab	2.95 b	216 e	398 c	87 a-c	1247 a
	5	7006 cd	93a	139 c	74 cd	6.1 c	74 b	398 b	94 b	2.47 bc	237 cd	386 cd	78 bc	1238 ab
	6	6734 cd	85 b	124 d	79 bc	6.7 ab	56 c	291 c	95 b	2.15 c	254 b	343 de	75 c	1205 a-c
	7	2855 e	38 d	48 e	73 cd	6.1 c	38 d	160 d	74 c	2.91 b	303 a	101 f	29 d	1170 cd
	8	2737 e	46 c	56 e	58 e	6.9 a	22 e	96 d	51 d	2.54 bc	252 bc	146 f	38 d	1125 d
Mean		6602	84	132	77	6.35	75	422	92	3.73	237	356	76	1207
Dusan #22 (Malting)	0	9116 a	93 a	152 a	97 a	6.3 bc	93 a	555 a	112 a	2.36 a	208 b	524 a	96 a	1219 a
	1	8181 a	96 a	151 a	84 b	6.4 bc	95 a	562 a	103 bc	2.15 a	222 ab	440 b	91 a-c	1219 a
	2	8724 a	98 a	152 a	89 ab	6.4 bc	92 a	544 a	101 cd	2.15 a	229 ab	463 ab	92 ab	1228 a
	3	8544 a	94 a	142 ab	91 ab	6.8 ab	91 a	525 a	107 b	1.53 b	234 a	460 ab	90 a-c	1216 a
	4	6372 b	92 a	132 b	69 c	6.5 b	81 b	469 b	105 bc	0.86 c	236 a	409 b	87 bc	1192 a
	5	6587 b	73 b	100 c	89 ab	7.1 ab	58 c	311 c	97 d	1.18 bc	233 a	289 c	68 d	1198 a
	6	4406 c	50 c	66 d	87 ab	7.8 a	33 d	160 d	89 e	1.17 bc	239 a	269 cd	83 c	1022 b
	7	2552 d	31 d	35 e	81 bc	7.1 ab	13 e	65 e	78 f	0.85 c	218 ab	202 d	52 e	941 c
	8	406 e	8 e	8 f	50 d	5.3 c	3 f	16 f	38 g	0.88 c	222 ab	82 e	25 f	865 d
Mean		6099	71	105	82	6.69	62	357	93	1.46	227	349	76	1123
LSD for varietal means (5%)		487	4.1	11.4	4.8	0.20	3.8	21.0	5.1	0.32	7.0	46.8	2.1	37.4

*Column means followed by the same letter in each cultivar were not significantly different at the 5% level based on DMRT.

Table 3. Significance test of variance affected by cultivars, aging treatment and their interactions.

Variance	D.F.	Vigor index	% warm germ.	Speed of germ.	Plumule length (mm) (WGT)	SGR	% cold germ.	Cold vigor	Plumule length (mm) (CT)	ATP n moles/ liter	Cond., μs cm/g, seed 30ml	Vigor in TZ test	% TZ germ. pred.	GADA, ppm CO_2, min., 5g
Cultivar (A)	2	ns	ns	ns	ns	ns	ns	ns	*	ns	ns	ns	ns	ns
Aging treatment (B)	8	**	**	**	**	ns	**	**	**	**	**	**	**	*
A x B	16	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

*Significant at 5% level.
**Significant at 1% level.

Table 4. Simple correlation coefficients between seed vigor index and 10 variables of seed vigor tests in 3 barley cultivars affected by aging time.

Cultivars	% warm germ.	Speed of germ.	Plumule length (mm) (WGT)	SGR	% cold germ.	Cold vigor	Plumule length (mm) (CT)	ATP n moles/ liter	Cond., μs cm/g, seed 30ml	Vigor in TZ test	% TZ germ. pred.	GADA, ppm CO_2, min., 5g
Saesalbori	0.76**	0.84**	0.89**	0.41*	0.79**	0.83**	0.87**	0.03	0.16	0.79**	0.79**	. 0.33
Buhobori	0.92**	0.92**	0.77**	−0.19	0.85**	0.85**	0.87**	−0.65**	−0.75**	0.85**	0.87**	−0.70**
Doosan #22	0.96**	0.96**	0.71**	0.14	0.95**	0.94**	0.90**	−0.65**	0.01	0.91**	0.89**	−0.61**
Overall mean (Varietal pool)	0.74**	0.51**	0.67**	0.26	0.75**	0.76**	0.86**	−0.51**	−0.33	0.75**	0.72**	−0.55**

*Significant at 5% level.
**Significant at 1% level.

4. Among the variables, ATP and conductivity were not related to vigor index for the cultivar such as Saesalbori with comparatively lower ATP and conductivity. However, the seed vigor of Buhobori and Doosan #22, showing high and ATP and conductivity, was negatively related to vigor index.

By using the stepwise regression analysis, the plumule length and cold vigor index were selected to predict for vigor index, and it was found that both percent cold germination and TZ germination measurements were found to be effective in predicting vigor index.

Based on the information obtained, it was confirmed that though many biochemical processes are involved in many vigor tests, the combined vigor index should be used to measure the actual field performance of the given cultivars.

摘　　要

보리種子의 老化過程에서 일어나는 生理化學的인 活性을 比較 檢討하여 種子勢를 豫測하기에 適合한 方法을 究明하기 위하여 새쌀보리, 부호보리 및 두산22호의 3品種을 'Wire-mesh tray' 方法으로 41℃, 相對濕度 100%로 調節하여 1日에서 8日間 人爲老化시켰다. 種子勢의 指標로서 發芽率×幼芽의 길이로 表示한 값과 각종 매개변수와의 關係를 比較하였다. 發芽試驗에 의한 方法이 보리種子의 退化進展을 확실히 反映해 주고 있으며, ATP 檢定과 GADA檢定方法을 除外한 低溫發芽試驗, 電氣傳導度檢定 및 테트라조리움 檢定方法은 보리 種子勢 評價에 適合한 方法임을 알 수 있었다. 특히 低溫發芽試驗에 의한 幼芽長과 테트라 조리움 檢定法이 가장 效果的인 方法임을 알 수 있었다.

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