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<https://doi.org/10.5109/23846>

出版情報 : 九州大学大学院農学研究院紀要. 31 (3), pp.227-234, 1987-03-10. Kyushu University
バージョン :
権利関係 :

Studies on the Vigour of Soybean Seeds
II. Varietal Differences in Seed Coat Quality and Swelling
Components of Seed during Moisture Imbibition

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(Received July 31, 1986)

Laboratory experiment was conducted to elucidate the physiological factor determining the association of seed size and seed coat quality with varietal differences in seed vigour, and to pursue an alternative on the mechanism of varietal differences in seed resistance to field weathering. Results of this study revealed that seed polymer change (seed volume change minus seed weight change during moisture imbibition) was the physiological factor determining the association of seed size and seed coat quality with varietal differences in seed vigour. The mechanism of varietal differences in seed resistance to field weathering, therefore, seems to be mainly controlled by seed coat quality (seed coat-seed weight ratio and seed coat colour) through controlling the magnitude of seed polymer change during moisture absorption at postphysiological maturity, which in turn determine the degree of membrane damage in the seed.

INTRODUCTION

Leopold (1983) reported species differences, including soybean, in volumetric components of seed during imbibition and considered if swelling components relate to limitation on seed viability and vigour. However, although there was a margin between seed weight and seed volume increments during imbibition, he did not use this phenomenon as a parameter of swelling components, instead, he used weight quotient, volume quotient and swelling coefficient as the parameters. No report since then has related varietal differences in seed vigour of soybean to swelling component during seed imbibition.

Among the genotypical factors in question, Mugnisjah *et al.* (1987) found the association of seed size, the occurrence of defective seed and seed coat colour with varietal differences in seed vigour. On the other side, Yaklich and Cregan (1981) reported varietal differences in water uptake by the pod and seed of soybeans, whereas Calero *et al.* (1981) found a negative correlation between seed size and either seed coat-seed dry weight ratio or percentage of water uptake.

It should be taken into consideration that seed colour, the occurrence of defective seed and seed coat-seed dry weight ratio are components of seed coat quality. No report, however, has related the association of water uptake with reference to its swelling components to varietal differences in seed vigour. Such study may be important in understanding the mechanism of field weathering since the seed is usually

harvested at postphysiological maturity. In relation to this, Mugnisjah *et al.*'s (1987) study implied varietal differences in seed resistance to field weathering since the seed were harvested at full maturity stage according to Fehr and Caviness's (1977) criterium. They found the association of reproductive growth period and accumulative daily mean temperature and accumulative rainfall during this period.

The objectives of this experiment were (1) to elucidate the physiological factor determining the association of seed size and seed coat quality with varietal differences in seed vigour, and (2) to pursue the mechanism of seed resistance to field weathering.

MATERIALS AND METHODS

1. Materials

Twenty-three late maturing cultivars, including small-(less than or equal to 11.00 g/100 seeds) and large-(more than 11.00 g/100 seeds) seeded cultivars, produced in 1983 (Mugnisjah *et al.*, 1987) were used after omitting the seeds with visible coat defect. These materials, consisting of cultivars with black and light seed coat colour, had undergone storage in 10°C until June 1985, when this study was conducted.

2. Measurement of seed coat quality and seed volume and weight changes during imbibition

Three replicates of 5 g seeds were soaked in 25 ml deionized water in 50 ml messycylinder followed by measuring their volume and weight increments after 0, 2, 4.5, 7 and 24 hours (V_t and W_t , respectively). Deionized water was renewed after each measurement. Seed coat-seed weight ratio was determined after 24 hours of soaking on dry weight basis (24 hours at 105°C). Changes in seed volume and weight during imbibition were determined using the following parameters : Speed of seed volume increment (SV, ml/h), speed of seed weight increment (SW, g/h), speed of seed polymer change (SP, g/h) and swelling coefficient of seed (k). Except swelling coefficient of seed which was computed using Pacheles' equation (Leopold, 1983), the equations for SV, SW and SP were devised by the present authors following Agrawal's (1980) equation for speed of germination,

$$SV = \frac{V_2 - V_0}{2} + \frac{V_{4.5} - V_2}{4.5} + \frac{V_7 - V_{4.5}}{7} + \frac{V_{24} - V_7}{24}$$

$$SW = \frac{W_2 - W_0}{2} + \frac{W_{4.5} - W_2}{4.5} + \frac{W_7 - W_{4.5}}{7} + \frac{W_{24} - W_7}{24}$$

$$SP = \frac{(V_2 - V_0) - (W_2 - W_0)}{2} + \frac{(V_{4.5} - V_2) - (W_{4.5} - W_2)}{4.5} + \frac{(V_7 - V_{4.5}) - (W_7 - W_{4.5})}{7} + \frac{(V_{24} - V_7) - (W_{24} - W_7)}{24}$$

$$kt = -\log_e \frac{a_{\max} - a}{a}$$

In this study, V_{24} and V_t referred to a_{\max} (volume at 24 hours, full hydration), and a (volume at time t), respectively. Then a straight line of slope was established as $-k$ after plotting the calculated

$$\log_e \frac{a_{\max} - a}{a} \text{ to } t.$$

RESULTS AND DISCUSSION

Table 1 shows varietal differences in seed size, seed coat-seed weight ratio and swelling components of seeds during imbibition in terms of speed of seed weight change, speed of seed volume change, speed of seed polymer change and swelling coefficient.

Except Burdete-19 characterized with large size seed, the cultivars with black seed coat colour had greater seed coat-seed weight ratio than the others. Among the cultivars with light seed coat colour, L4 has the highest seed coat-seed weight ratio, but lower than those of small-seeded cultivars with black seed coat colour. This value in large-seeded cultivars, however, was lower regardless of seed coat colour. Speed of seed weight change (SW) was lower in cultivars with black seed coat colour regardless of seed size. In cultivars with light seed coat colour SW tended to be greater when the seed size was small, though some large-seeded cultivars (Hood, Lee and Kent) with greater SW were also occurred. Cultivars with black seed coat colour also showed slower speed of seed volume change (SV) than those of light seed coat colour. SV seemed to be not different among the latters. Speed of seed polymer change (SP) was, in general, slower in cultivars with black seed coat colour regardless of seed size than

Table 1. Seed size (100-seed weight), seed coat-seed weight ratio (Coat), speed of seed weight (SW), volume (SV) and polymer (SP) changes, and swelling coefficient of seed (k) during imbibition.

Cultivar	Seed size (g)	Coat (%)	SW (g/h)	SV (ml/h)	SP (g/h)	k
<i>Black seed coat colour</i>						
Southern Prolific	5.68	14.4	1.62	1.63	0.01	0.319
L-12/9	5.77	13.4	1.86	1.74	-0.12	0.300
PK-8-53	7.45	13.8	1.51	1.59	0.08	0.291
Burdete-19	16.54	7.9	1.51	1.53	0.02	0.210
<i>Light seed coat colour</i>						
L-4	5.59	11.4	2.02	2.00	-0.02	0.486
Nganjuk I	9.35	7.5	2.03	2.21	0.18	0.385
Longgepak	7.16	8.0	2.15	2.14	-0.01	0.399
Empyek	8.48	8.2	2.05	2.26	0.21	0.392
Logel	7.68	8.0	2.15	2.32	0.17	0.440
Mojosari	8.53	7.8	1.94	2.09	0.15	0.371
Presi Ulung	9.02	7.2	2.04	2.25	0.21	0.351
Presi Ulung I	10.31	7.6	1.88	2.06	0.18	0.363
Pagak I/Z	8.93	8.1	2.10	2.16	0.13	0.361
Pagak I /3	9.34	7.7	1.96	2.08	0.12	0.356
H-32	13.45	6.7	1.78	2.06	0.28	0.381
Jackson	16.33	7.1	1.84	2.07	0.23	0.314
Hood	16.79	7.5	2.00	2.08	0.08	0.436
Lee	16.06	6.9	2.00	2.36	0.36	0.409
Kent	18.23	7.1	2.02	2.40	0.38	0.364
Hougyoku	23.82	6.3	1.68	1.89	0.21	0.347
Tamahomare	25.33	5.8	1.85	2.09	0.24	0.358
Dwarf Hyuga	25.98	6.1	1.87	2.17	0.30	0.386
Fukuyutaka	28.28	5.3	1.89	2.06	0.17	0.360

those of light seed coat colour, except L4 and Longgepak of small-seeded cultivars and Hood of large-seeded ones. Except for Jackson with small value, cultivars with light seed coat colour, regardless of seed size, had higher swelling coefficient (k) than those with black seed coat colour.

Table 2 shows average value and its standard deviation of seed characteristics presented in preceding table with respect to seed coat colour and seed size. This table simplifies the relation of seed size and, especially, seed coat colour to the characteristics measured.

Lower speed of seed weight increment in the genotype with black seed coat colour regardless of their seed size was comparable to Tully *et al.*'s (1981) finding. Higher speed of seed weight increment in small-seeded genotypes with light seed coat colour than those of large-seeded ones fitted the results of electron microscopic observation reported by Calero *et al.* (1981). They found that nearly all pores in seed coat of small size seeds appeared to be open and therefore functional, whereas in large size seeds pore were often distorted due to stretching over a large surface area and some were plugged. Larger surfaced area per weight unit in small-seeded cultivars (Tao, 1978) might be also the reason for higher speed of seed weight increment from those cultivars than those from large-seeded ones.

Statistically, intercorrelation among the aforesaid seed characteristics is presented in Table 3. This table demonstrates a consistent negative correlation between seed coat-seed weight ratio and seed size, regardless of the presence of cultivars with black seed coat colour in samples. This result was in accordance to those of Calero *et al.* (1981) who found larger seed coat-seed weight ratio in small-seeded cultivars, and this ratio was negatively correlative with seed dry weight but positively with seed weight after 8 days of imbibition at saturated condition.

Regardless of the presence of cultivars with black seed coat colour, a consistent

Table 2. Relationship among seed coat colour, seed size (100-seed weight), seed coat-seed weight ratio (Coat), speed of seed weight (SW), seed volume (SV) and seed polymer (SP) changes, and swelling coefficient of seed (k) during imbibition¹.

Seed colour	n ²⁾	Seed size (g)	Coat (%)	SW (g/h)	SV (ml/h)	SP (g/h)	k
Black	3	6.30 (0.81) ³⁾	13.86 (0.41)	1.66 (0.15)	1.65 (0.06)	-0.01 (0.08)	0.303 (0.010)
Black	1	16.54	7.90	1.51	1.53	0.02	0.210
Mean (black)	4	8.86 (4.49)	12.37 (2.61)	1.63 (0.14)	1.62 (0.08)	-0.01 (0.00)	0.280 (0.042)
Light colour	10	8.44 (1.27)	8.15 (1.12)	2.03 (0.08)	2.16 (0.10)	0.13 (0.08)	0.390 (0.041)
Light colour	9	20.47 (5.06)	6.53 (0.67)	1.88 (0.11)	2.13 (0.15)	0.25 (0.09)	0.373 (0.034)
Mean (light colour)	19	14.14 (7.01)	7.38 (1.24)	1.96 (0.12)	2.14 (0.13)	0.19 (0.10)	0.382 (0.040)

1) Computed from Table 1 (as averages)

2) Numbers of cultivars

3) Data in parenthesis denote standard deviations

Table 3. Intercorrelation among seed size (100-seed weight), seed coat-seed weight ratio (Coat), speed of seed weight (SW), volume (SV) and polymer (SP) changes, and swelling coefficient of seed (k) during imbibition.

Factor	r value with"				
	Seed size (g)	Coat (%)	SW (g/h)	SV (ml/h)	SP (g/h)
<i>Including black seeds (n= 23)</i>					
Seed size (g)					
Coat (%)	-0.672**				
SW (g/h)	-0.201	-0.349			
SV (ml/h)	0.129	-0.626**	0.856**		
SP (g/h)	0.536**	-0.685**	0.179	0.663**	
k	-0.089	-0.217	0.751**	0.675**	0.195
<i>Excluding black seeds (n= 19)</i>					
Seed size (g)					
Coat (%)	-0.791**				
SW (g/h)	-0.636**	0.518*			
SV (ml/h)	-0.198	-0.014	0.650**		
SP (g/h)	0.491*	-0.616**	-0.356	0.479*	
k	-0.357	0.645**	0.506*	0.347	-0.426

1) Levels of significance were 5 % (*) and 1 % (**).

relationship was also occurred between SP and seed size (positive correlation), seed coat-seed weight ratio (negative correlation) and SV (positive correlation) (Table 3). Swelling coefficient (k), however, gave a consistent positive correlation with SW only, and did not with those variables correlated to SP. Interestingly, k was consistently not correlative with SP. These results, therefore, indicated that SP and k as swelling component parameters had a different nature during imbibition. On the other hand, the same consistent trend of correlation of both SV and k to SW indicated that both parameters were resemble to each other. However, this was not surprising since either SV or k were derived from seed volume change during imbibition. Thus, the results suggested that the SP should be evaluated rather than k of Leopold (1983), as a physiological factor determining the association of seed size and seed coat quality with varietal differences in seed vigour.

Time courses for volume and weight gains during imbibition of different soybean cultivars are presented in Fig. 1. The cultivars used in this figure were as same as those presented in Table 2. It was evident that the large-seeded cultivars showed more enormous swelling up to 4.5 hours of imbibition, resulting from a remarkable increase in seed volume outstripping seed weight increase. Small-seeded cultivars with black seed coat colour showed a negative swelling since the first 2 hours of imbibition. Large-seeded cultivars with black seed coat colour begun to follow the swelling trend of cultivars with light seed coat colour at 4.5 hours of imbibition, but was much lower than that achieved by cultivars with light seed coat colour.

In studying the mechanism of chilling injury, Tully *et al.* (1981) hypothesized that different sensitivity of soybean seeds to chilling could relate to the rate of rehydration rather than to compositional differences in seed membrane. More recently, Vertucci and Leopold (1983) speculated that damage to soybean seeds during imbibition may be

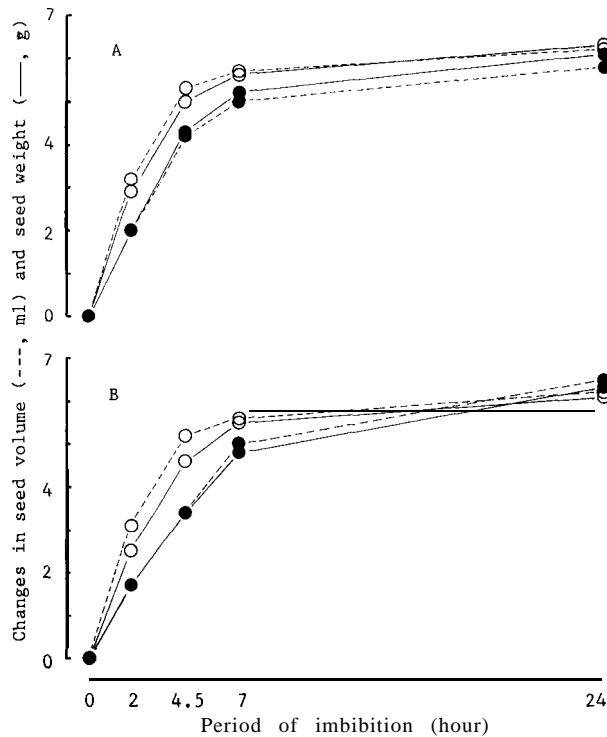


Fig. 1. Time course of changes in seed volume and weight during imbibition (●black seed coat colour with 6.30f0.81 g/100 seeds in A and 16.54 g/100 seeds in B ; ○light seed coat colour with 8.44k1.27 g/100 seeds in A and 20.47f5.06 g/100 seeds in B).

particularly related to initial wetting reaction rather than to longer-term imbibitional rate. Using the analogy to these opinions, it was the first 4.5 hours of imbibition (Fig. 1) that more damage occurred in large-seeded genotypes as they experienced more seed polymer change than the small-seeded ones. The least damage, if any, would occur in small-seeded genotypes with black seed coat colour, since they hardly experienced seed polymer change during imbibition.

Using 35 genotypes, Dassou and Kueneman (1984) have reported that nearly all large-seeded genotypes were highly susceptible to field weathering, whereas the small-seeded ones, though not all, were resistance. Thus, it seemed accordingly that the phenomenon of seed polymer change might have occurred to soybean seeds in the field at postphysiological maturity, causing large-seeded genotypes more susceptible to field weathering, and therefore lower seed vigour, than small-seeded genotypes. An analogy could be derived for higher resistance to field weathering of genotypes with black seed coat colour. Higher r value of seed size to SP ($r=0.536^{**}$) when the cultivars with black seed coat colour were included in analysis, rather when they were excluded ($r=0.491^*$), indicated that the seed coat quality would be more important in controlling seed resistance to field weathering than seed size. Therefore, it should be taken into consideration that the less seed coat defect might not be the cause of superior vigour

of small-seeded genotypes, but the less damage in seed polymer resulting from alternate wetting and drying in the field. The seed coat defect itself might be considered result of seed coat weakness to response against such process.

Leopold (1983) used many species with variable seed characteristics including proteinous seed, oil seed and starch seed according to the criteria of Justice and Bass (1979). Supposed that seed polymer change during imbibition is especially respected to seed protein, as Leopold (1983) postulated, the species differences in such phenomenon would also be especially respected to different protein content among them. Since merely soybean cultivars were used in the present study, it is easily to imagine that range of seed protein content among soybean cultivars would be very smaller than that that would be among species of different seed compositional classes. This means that the magnitude of any change in seed polymer during moisture absorption by soybean seeds would be especially controlled by its seed coat quality rather than by its seed protein content. Therefore, it seemed that seed resistance to field weathering was particularly controlled by seed coat quality through governing the magnitude of seed polymer change during moisture absorption at postphysiological maturity which in turn determining the degree of membrane damage in the seed. This was supported by the consistent negative correlation between SP and seed coat-seed weight ratio, and that its r value was higher ($r = -0.685^{**}$) when cultivars with black colour seeds were included in the analysis rather than when they were excluded ($r = -0.616^{**}$).

In conclusion, our data revealed that seed polymer change during imbibition was the physiological factor determining the association of seed size and seed coat quality with varietal differences in seed vigour. The mechanism of varietal differences in seed resistance to field weathering, therefore, seems to be particularly controlled by seed coat quality through controlling the magnitude of seed polymer change during moisture absorption at postphysiological maturity which in turn determine the degree of membrane damage in the seed.

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