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Relationship of Seed Germination and Lipoxygenase Activity in Soybean

Beom Young Son*, Yeong Ho Lee*, Hong Suk Lee, and Suk Ha Lee**†**

**National Crop Experiment Station, RDA, Suwon 441-857, Korea*
***School of Plant Science, Seoul National University, Suwon 441-744, Korea*

ABSTRACT : Lipoxygenase might be associated with seed deterioration by catalyzing the incorporation of molecular oxygen into fatty acids and generating free radicals. This study was performed to determine whether seed lipoxygenase activity would alter soybean seed longevity. In this study, germination percentage of lipoxygenase-lacking cultivar Jinpumkong2 (*lx1lx1lx2lx2lx3lx3*) was lower than that of Taekwangkong (*Lx1Lx1Lx2Lx2Lx3Lx3*). Segregation ratio for the three lipoxygenase isozymes of the F2-derived from the cross between Taekwangkong and Jinpumkong2 was fitted to 9 (*Lx1Lx2Lx3*) : 3 (*Lx1Lx2lx3*) : 3 (*lx1lx2Lx3*) : 1 (*lx1lx2lx3*), suggesting the tight linkage between the *Lx1* and *Lx2* loci. Germination percentages varied widely but not differed among lipoxygenase isozyme types of F_3 seeds before and after accelerated aging. Seed coat of Jinpumkong2 was damaged severely following accelerated aging, whereas that of Taekwangkong was not. Thus, seed of lipoxygenase-lacking soybean cultivar, Jinpumkong2 showed greater deterioration compared with that of the normal Taekwangkong. However, the presence or absence of lipoxygenase activity had no effect on soybean germination.

Keywords **:** soybean, lipoxygenase, germination, accelerated aging

Soybean seeds continue to deteriorate after harvest in the storage environment. Loss of germination ability is one of important characters associated with seed during seed deterioration. Lipoxygenase might be associated with seed deterioration (Bewley, 1986). This may be due to the fact that lipoxygenase catalyzes the incorporation of molecular oxygen into fatty acids and generates free radicals (Hatanaka *et al.*, 1987; Vick & Zimmerman, 1987). Oxidation of fatty acids in membrane-bound and storage lipids by lipoxygenase might contribute to seed deterioration by disrupting membrane integrity.

Soybean seeds contain three lipoxygenase isozymes, called lipoxygenase-1 (L-1), lipoxygenase-2 (L-2), and lipoxygenase (L-3) (Axelrod *et al.*, 1981). Genetic analysis indicated that the absence of L-1, L-2, and L-3 from the seeds is due to single recessive alleles, *lx1*, *lx2*, and *lx3* respectively, and that *lx3* locus is independent of the *lx1* and *lx2* loci that are tightly linked (Kitamura *et al.*, 1985).

Lipoygenase-lacking soybean seeds might exhibit increased seed longevity if lipoxygenase contributes to seed deterioration. L-2 and L-3 lacking seeds were more sensitive to the accelerated aging treatment than normal seeds (Wang *et al.*, 1990). They suggested that L-2 isozyme may play some important physiological roles in maintaining viability rather than promoting senescence of soybean seeds. Kim (1996) reported that accelerated aging did not affect germination percentage of normal Hwangkeumkong, but lowered that of Jinpumkong (*Lx1Lx1lx2lx2lx3lx3*) and Jinpumkong2 (*lx1lx1lx2lx2lx3lx3*). Relationship between germination and seed lipoxygenase has not been clearly indicated.

This study was carried out to investigate the role of lipoxygenase in seed deterioration using L-1, L-2, and L-3 less Jinpumkong2 and normal Taekwangkong. In a segregating population derived from the cross between Taekwangkong and Jinpumkong 2, association of seed lipoxygenase activity with seed germination capacity was evaluated.

MATERIALS AND METHODS

Taekwangkong (*Lx1Lx1Lx2Lx2Lx3Lx3*), Jinpumkong2 (*lx1lx1lx2lx2lx3lx3*), and F3-derived lines from the cross between Taekwangkong and Jinpumkong2 were used. Two soybean cultivars, Taekwangkong and Jinpumkong2, were planted in the field of Gyeongnam Agricultural Research and Extension Services, Jinju, Korea on May 15, June 8, and June 26 in 1997. The cross between Taekwangkong and Jinpumkong2 was done in the greenhouse in the spring of 1997, and F_2 seeds were obtained from selfed F_1 plants grown in the same year.

F_3 seeds were obtained from each F_2 individual planted on May 18 in 1998. Soybean seeds were subjected to accelerated aging at 40°C and 90% relative humidity for 4 days. After this treatment, seeds were air-dried at room temperature to retain their initial weight. Germination was considered to have sprouts when hypocotyl was emerged about 3 mm from radical cap of the seed. Electric conductivity was

†Corresponding author: (Phone) +82-31-290-2305 (E-mail) sukhalee@snu.ac.kr <Received May 20, 2002>

measured on ten seeds in each replication with three replications, as was described in AOSA Seed Vigor Testing Handbook (AOSA, 1983). Electron micrographs of seed coat were obtained with Scanning Electron Microscope (SEM), LEO-440. Genotyping of F_2 seeds on the presence or absence of seed lipoxygenase isozymes were evaluated by carotene bleaching test as described by Kikuchi & Kitamura (1987).

RESULTS AND DISCUSSION

Seed quality

Germination percentage of Jinpumkong2 was 78.2% which was lower than that of Taekwangkong (94.2%) (Table 1). Bewley (1986) reported that seed lipoxygenase activity might be associated with seed deterioration, as seed lipoxygenase catalyzes the incorporation of molecular oxygen into fatty acids and generates free radicals (Hatanaka *et al.*, 1987; Vick & Zimmerman, 1987). Lipoygenase-lacking soybean cultivar, Jinpumkong2 might exhibit good seed quality if lipoxygenase contributes to seed deterioration. However, Jinpumkong2 had lower germination percentage compared with Taekwangkong. These results were similar to the previous report that germination percentage of Jinpumkong2 was lower than that of Hwangkeumkong (Kim, 1996). We postulated that lower germination of Jinpumkong2 would be caused by poor seed quality rather than the presence or absence of lipoxygenase isozymes.

As planting date was delayed, germination percentage of two soybean cultivars, Jinpumkong2 and Taekwangkong was higher. Earlier studies have shown that seed germination was improved in harvested seeds after delayed planting in soybean (Green *et al.*, 1965; Chin *et al.*, 1993).

Genetic study

In the F_2-derived population from the cross between Taekwangkong (*Lx1Lx1Lx2Lx2Lx3Lx3*) and Jinpumkong2 (*lx1lx1lx2lx2lx3lx3*), genetic segregation ratio was observed to be 9 (*Lx1Lx2Lx3*) : 3 (*Lx1Lx2lx3*) : 3 (*lx1lx2Lx3*) : 1

Table 1. Germination percentage of Taekwangkong and Jinpumkong2 as affected by planting date.

Cultivar	Planting date			
	May 15	June 8	June 26	Mean
Taekwangkong	91.3	96.0	95.3	94.2a*
Jinpumkong 2	66.7	84.0	84.0	78.2b
Mean	79.0b	90.0a	89.7a	

*Means within the same row or within the same column not followed by the same letter are significantly different at P = 0.05 based on LSD.

Table 2. Segregation of F_2 seeds derived from the cross between Taekwangkong and lipoxygenase lacking Jinpumkong2 for the presence or absence of L-1, L-2 and L-3.

Phenotype			No. of seeds		Chi-square		Probability
L-1	L-2	L-3*	Observed	Expected	(9 : 3 : 3 : 1)		
+	+	+	40	41.06	0.027		
+	+	–	21	13.69	3.903	6.071	0.1<P<0.5
–	–	+	9	13.69	1.607		
–	–	–	3	4.56	0.534		
	Total		73				

*+ + + : Lx1Lx2Lx3, + + – : Lx1Lx2lx3, – – + : lx1lx2Lx3, – – – : lx1lx2lx3

(*lx1lx2lx3*) (Table 2), suggesting that tight linkage was found between *Lx1* and *Lx2* loci. This result was consistent with previous reports (Kitamura *et al.*, 1985, Davies & Nielson, 1986; Son *et al.*, 2000).

Germination characteristics of lipoxygenase isozymes

Based on the lipoxygenase isozyme types of F_3 seeds, germination percentages were ranged to be 34.5~94.8% (*Lx1Lx2Lx3*), 46.4~97.8% (*Lx1Lx2lx3*), 46.1~91.4% (*lx1lx2Lx3*), 49.2~88.4% (*lx1lx2lx3*), and their mean germination percentages of them were 68.4%, 69.7%, 72.6%, 68.2%, respectively (Table 3). Single analysis of variance revealed that germination percentages showed wide ranges but not differed among lipoxygenase isozyme types of F_3 seeds derived from the

Table 3. Germination percentage for F_3 seeds of lipoxygenase isozyme types derived from F_2 populations of Taekwangkong/Jinpumkong2 before and after accelerated aging.

Phenotype			No. of F_2 lines	Germination (%)	
L-1	L-2	L-3		Range	Mean
Standard					
+	+	+	40	34.5~94.8	68.4a*
+	+	–	21	46.4~97.8	69.7a
–	–	+	9	46.1~91.4	72.6a
–	–	–	3	49.2~88.4	68.2a
	Total		73		
Accelerated aging					
+	+	+	32	3.1~55.0	20.7a
+	+	–	15	3.7~53.5	22.5a
–	–	+	8	2.5~46.0	17.1a
–	–	–	3	13.0~47.2	30.1a
	Total		58		

*Means within the same column not followed by the same letter are significantly different at P = 0.05 based on LSD.

cross between Taekwangkong and Jinpumkong2. Following accelerated aging, germination percentages also showed wide ranges but not differed among lipoxygenase isozyme types of F_3 seeds derived from the cross between them. If lipoxygenase contributes to seed deterioration, lipoygenase-lacking soybean lines might exhibit increased seed longevity. However, it indicated that loss of one to three of the three lipoxygenase isozymes had no effect on soybean seed deterioration. This is in a good agreement with our previous report from the different population of Pureunkong (*Lx1Lx1Lx2Lx2Lx3Lx3*) × Jinpumkong2 (*lx1lx1lx2lx2lx3lx3*) (Son *et al.*, 2000).

Wang *et al.* (1990) reported that L-2 and L-3 deficient seeds were more sensitive to the accelerated aging treatment than normal seeds, and L-1 and L-3 deficient seeds, suggesting that L-2 isozyme may play some important physiological role in maintaining viability rather than promoting senescence of soybean seeds. However, Trawatha *et al.* (1995) reported that Century which contained all three lipoxygenase isozymes and the lipoxygenase-lacking genotypes had high initial seed quality and the loss of one or two of the three lipoxygenase isozymes had no effect on soybean seed deterioration. Our study is in a good agreement study by Trawatha *et al.* (1995) rather than Wang *et al.* (1990). This study also revealed that lipoxygenase activity may not be associated with seed deterioration since the lack of isozymes had no effect on seed quality.

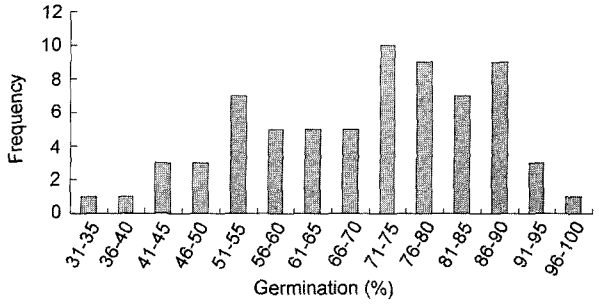

Fig. 1. Frequency distribution of germination percentage for F_3 seeds derived from a F_2 population of Taekwangkong/ Jinpumkong2.

Frequency distribution of germination percentage is shown in Fig. 1. The normal distribution of germination percentage indicated that germination percentage is not involved in a major factor such as lipoxygenase activity but a variety of factors such as hardness of seed, susceptibility to seed damage, and seed chemical composition.

Changes of seed coat structure

Before and after accelerated aging, structures of seed coat for soybean cultivars, Taekwangkong and Jinpumkong2 are shown in Fig. 2. Seed coat of Jinpumkong2 was damaged severely after accelerated aging but not that of Taekwangkong. Thus, seeds of Jinpumkong2 showed greater deterioration

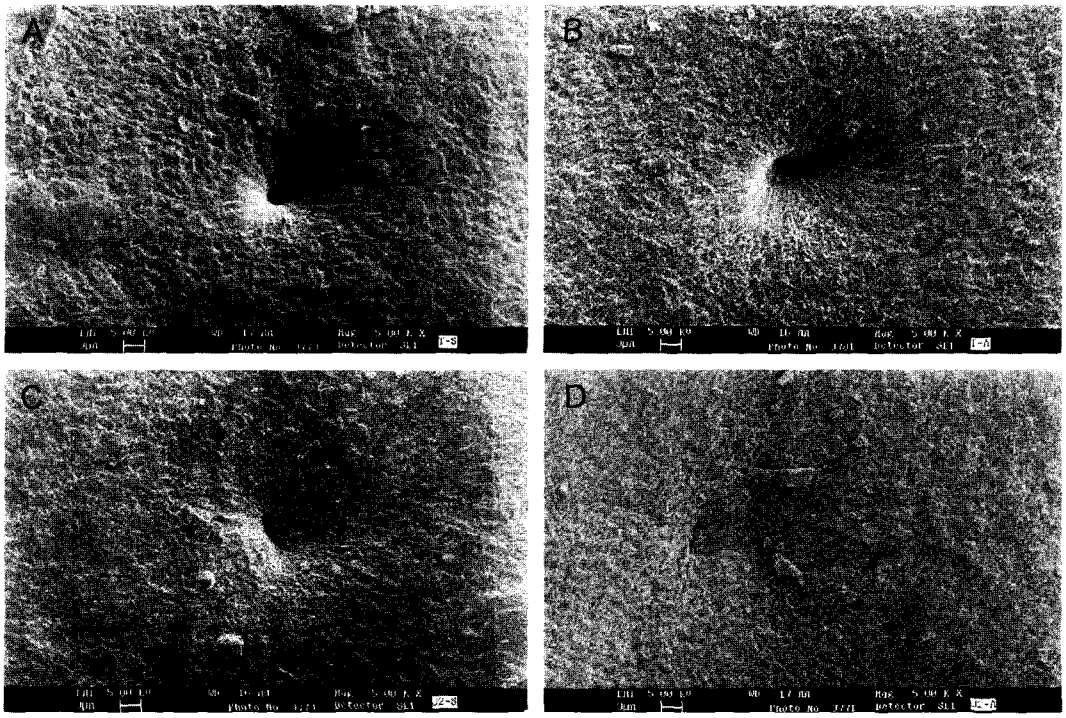

Fig. 2. Seed coat of Taekwangkong, and Jinpumkong2 before and after accelerated aging. A : Taekwangkong (standard), B : Taekwangkong (accelerated aging), C : Jinpumkong2 (standard), D : Jinpumkong2 (accelerated aging)

compared with Taekwangkong. It could be summarized that seed coat of Jinpumkong2 was easily damaged in response to accelerated aging. Related to that, it can be concluded that lower germination of Jinpumkong2 would be caused by poor seed quality rather than the presence or absence of lipoxygenase isozymes.

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