Korean J. Crop Sci. 48(4): 334~338(2003)

Alterations in Seed Vigour and Viability of Soybean Related with Accelerated Seed Aging and Low Dose Gamma Irradiation

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ABSTRACT: The objective of this study was to demonstrate whether or not the deleterious effects of accelerated aging on seed vigour and viability are alleviated by interaction with gamma irradiation. Seeds of soybean (*Glycine max* L.) were artificially aged and subsequently irradiated with 4 and 8 Gy of gamma irradiation. Germination rate was negatively affected by accelerated aging and positively by gamma irradiation, with a positive interaction of a 3 day-seed aging treatment occurring with 4 Gy, possibly suggesting that 4 Gy of gamma irradiation partially offset the adverse effects of seed aging on germination. However, 5-day aged seeds did not gain any benefits from the gamma irradiation. Electrolyte leakage from the seeds increased with the duration in days aged. Irradiation, however, did not impose any effects on the leakage. Respiration rate of the seed with hypocotyl and primary root was significantly low for the aged seeds, but not for the seeds with both irradiation and aging treatments. Accelerated aging decreased the dry weight of the hypocotyl and primary root of the seeds without any measurable effects of irradiation. α-Amylase activity decreased with seed aging and positively responded to gamma irradiation. The data is discussed with regard to the possible roles of gamma irradiation for improving the seed vigour and viability of aged seeds.

***Keywords*:** seed aging, gamma irradiation, seed vigour, electrolyte leakage, respiration, germination, α-amylase

It is widely known that seed deteriorates during storage, leading to the progressive loss of seed vigour and eventually influencing crop productivity at harvest (Tesar, 1984). The loss of seed vigour due to prolonged storage or accelerated aging is manifested by a range of biochemical and metabolic alterations involving the loss of enzymatic activities, impaired membrane integrity (Sung & Chiu, 1995), reduced ATP production and respiratory activity, and increase in free fatty acid content (Copeland & McDonald, 1995), all of which contributed to a reduced germination percentage, seedling emergence, growth and ultimately imposed deleterious effects on crop yield (Priestly, 1986). Although the exact mechanism through which seed deterioration or aging occurred has not been well defined yet, some possible causes have been proposed including lipid peroxidation by free radicals (Wilson & McDonald, 1986), protein degradation and/or inactivation (Tesar, 1984), accumulation of chromosome aberrations and toxicants (Copeland & McDonald, 1995), and the altered responsiveness to growth hormone (Petruzzelli & Taranto, 1985; Bernal-Lugo *et al.*, 1999).

Slow, unreliable germination and seedling emergence arose due to seed aging and therefore caused considerable problems for a successful crop production (Powell *et al.*, 2000). "Seed enhancement" is a term for any type of post-harvest treatment that improves seed performance and seedling growth or facilitates the delivery of seed and other materials required at the time of sowing (Taylor *et al.*, 1998). For instance, pre-sowing hydration, biological seed treatments, seed conditioning and coating have been developed and employed to ameliorate the negative effects of seed deterioration and improve crop productivity (Taylor *et al.*, 1998; Copeland & McDonald, 1995).

It has been revealed that a low dose gamma irradiation improved seed germination as well as early seedling growth (Sharon & Muralidharan, 1978; Sheppard & Evenden 1986), although the extent to which crops responded to a low dose gamma irradiation varied with species. These might be driven by the stimulating effect of low dose ionizing radiation on the germinating seeds and/or subsequent seedling growth, namely, "radiation hormesis" (Luckey, 1980). Debates are still ensuing on the occurrence of radiation hormesis and its mechanism in plants, but free radical-mediated biochemical and physiological effects of ionizing radiation were proposed as a possible candidate for the hormesis mechanisms (Luckey, 1980; Miller & Miller, 1987). The radiation hormesis frequently found in the plants suggests further that a low dose gamma irradiation would be an alternative for seed enhancement technology through improving the seed germination and/or seedling growth developed from the seeds deteriorated during storage.

This preliminary study aims at addressing whether or not the negative impacts of accelerated aging are alleviated by gamma irradiation on seed vigour and viability of soybean via examining the seed germination, electrolyte leakage, α-

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<Received July 18, 2003>

amylase activity, respiration and finally growth of hypocotyl+primary root of the seeds.

MATERIALS AND METHODS

This experiment was carried out with seeds of cv. Hwangkeumkong harvested in 2002. The seeds were stored at room temperature after harvest and its moisture content was 5.12±0.07% on a dry weight basis just before the experiment. Seed moisture content was measured with Infrared Moisture Analyzer (SMO 01, Scaltec, Germany). Accelerated aging was performed by placing the seeds at 41°C in a water bath with 100% relative humidity (RH) for 3 or 5 days as described by Bailly *et al.*, (1996). After each treatment the seeds were dried for 7 days at room temperature, allowing the seed moisture content to be similar to that of the non-aged control seed mentioned above. The seeds (aged and non-aged) were irradiated with a dose of 4 or 8 Gy generated by a gamma irradiator (^{60}Co, *ca.* 150 TBq of capacity, AECL) in Korea Atomic Energy Research Institute (KAERI).

Germination assays were performed in darkness at 25°C in a 14 cm tissue culture dish (60 seeds per dish, 5 replicates), on layers of Advantec filter paper moistened with distilled water. The seeds were considered germinated when the radicle emerged through the seed coat (Copeland & McDonald, 1995). Germinated seeds were counted daily for 3 days. In order to estimate the extent to which solutes were leaked from the seeds, 10 seeds (5 replicates) were soaked in 30 ml of distilled water at 20°C and 100% RH for 5 days and then the electrical conductivity of the medium surrounding the seeds was measured with a conductivity meter (Walklab, Trans Instruments, Singapore) according to Fountain *et al.*, (2002).

The concentration of CO_2 evolved from 10 imbibed seeds with hypocotyls and primary roots (4 replicates) was measured with a gas chromatograph (GC 14-B, Shimadzu, Japan) after the seeds were kept at 20°C for 2 hours. Respiration rates based on the total dry weight of hypocotyls and primary roots of the seeds were estimated by the equation of Park (2003). Hypocotyls and primary roots of the seeds were oven-dried at 70°C for 3 days, and the early growth of the soybean seedling was determined by measuring the dry weight of hypocotyl and primary root only.

For the determination of α-amylase activity, seeds (0.5 g) were homogenized in a pestle and mortar with liquid nitrogen and extracted with a 0.1 M cold sodium phosphate buffer (pH 7.0). An aliquot of the supernatant after centrifugation was used for the analysis following a modified method of Lim (2003). The total protein concentration was estimated by the methods of Zhang & Halling (1990) and Smith *et al.*, (1985). The data was analyzed by the two-way ANOVA (General Linear Model, GLM) and Tukeys HSD test (SPSS 10.0, USA.).

RESULTS AND DISCUSSION

Electrolyte leakage and respiration

Accelerated seed aging considerably increased the extent of electrolyte leakage from the seeds; the longer the duration aged in days, the more the solutes leaked (Fig. 1(a)). According to Parrish and Leopold (1978), increased leakage of electrolytes results from the deteriorations in the membranes of the aged soybean seeds possibly due to lipid peroxidation by lipoxygenase or reactive oxygen species (Zacheo *et al.*, 1998; Bailly *et al.*, 2002). It was reported that gamma irradiation stimulated the activity of free radical-scavenging enzymes such as catalase and peroxidase (Kim *et al.*, 2002), which might ameliorate free radical-induced deterioration in aged seed membranes and allow a lesser amount of electrolytes to be exuded. Gamma irradiation, however, did not significantly affect electrolyte leakage, irrespective of the dose of gamma irradiation (Fig. 1(a)).

Decreases in respiration rate were found in the aged seeds (Fig. 1(b)), as shown in other studies (Tesar, 1984; Copeland & McDonald, 1995). Disorganization of the mitochondria

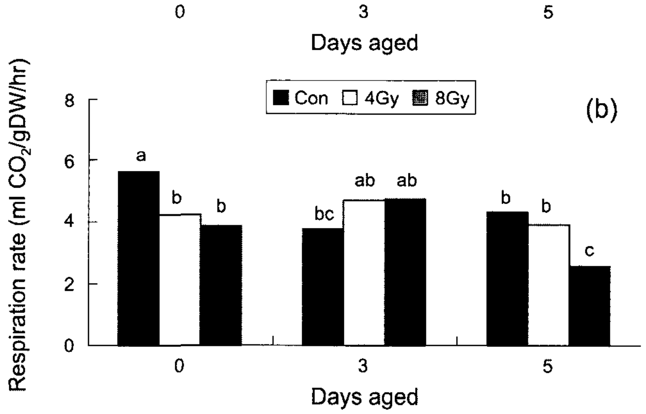

Fig. 1. Effects of accelerated aging and gamma irradiation on electrolyte leakage (a) and respiration rate (b) of soybean seeds with hypocotyls and primary roots. Bars with same letters are not significantly different at 0.05 level of Tukey's test.

and damage to the pea seed membranes were reported to occur after aging treatment (Harman & Granett, 1972). Respiration within the mitochondria is a function of the unit membranes and a loss of the membrane integrity due to aging would presumably alter the functional relationships of the membrane-bound components of the respiratory chain (Parrish & Leopold, 1978). Surprisingly, gamma irradiation, as demonstrated by Sidrak and Suess (1973), alleviated the extent to which aging treatment reduced respiration, particularly for the 3 days aged seeds (Fig. 1(b)). Low dose gamma irradiation frequently induced the hormetic effects on the germination rate and early seedling growth (Sidrak & Suess, 1973; Sheppard & Evenden, 1986), probably through stimulating a fairly nonspecific, increased production of enzymes (Sheppard & Evenden, 1986). It is, therefore, supposed that low dose gamma irradiation might facilitate reorganization of the mitochondria membrane damaged by aging and/or stimulate biochemical activities including the respiratory chain reactions, which might contribute to alleviating the negative impacts of aging on the respiration rates in this study, although the exact mechanisms should be addressed.

Germination

Accelerated aging significantly reduced the germination rate at 2 days after seeding (DAS) compared to the non-aged control (Table 1). Gamma irradiation alone did not impose any considerable effects on germination at 2 DAS, but had a positive interaction with 3 days aging (Table 1). Germination rates of the non-aged seeds measured at 3 DAS were more than 90% and significantly different from those aged (Table 1). Gamma irradiation, 4 Gy in this case, exhibited positive interactions with the aging treatment, particularly for the increased germination rate of 3 DA (Table 1). Artificial seed aging and gamma irradiation at 4 DAS showed effects on the germination rate similar to those found at 3 DAS (Table 1).

One of the major impacts imposed by seed aging is evident as a delayed germination/or reduced germination rate (Bewley & Black, 1994), which is accounted for by a number of causal mechanisms; disruption of the germination-related hormone balance (Koepp & Kramer, 1981), decrease in germination-related protein production (Guy & Black, 1998), degradation or inactivation of the respiratory system (Parrish & Leopold, 1978) and membrane deterioration (Zacheo *et al.*, 1998). It is uncertain how the germination of the aged seeds was stimulated by gamma irradiation, but it could be proposed that activation of the aged respiratory system by gamma irradiation, as shown in Fig. 1(b), could be partly responsible for the increased germination rate. Furthermore, the germination rate showed a close relationship with the respiration rate (Germination rate=6.4076×Respiration rate+51.698, $P<0.05$). However, it could not exclude the possibility that changes in the balance between the germination-related phytohormones, such as gibberellin / abscissic acid ratio, might be induced in favour of the germination rate as suggested by Koepp and Kramer (1981).

α-Amylase activity and Growth

It is β-amylase that was frequently assayed in soybean

Table. 1. Germination rate of soybean according to days after seeding (DAS).

		DAS		
		2	3	4
Ageing period (days)	Gamma Dosage (Gy)	% germination		
0	0	52.9^{ab}	93.3^{ab}	95.6^{a}
	4	59.3^{a}	94.3^{a}	95.0^{a}
	8	47.4^{abc}	96.7^{a}	97.9^{a}
3	0	34.8^{cd}	77.2^{b}	82.2^{ab}
	4	29.4^{de}	83.6^{ab}	88.5^{a}
	8	39.2^{bcd}	59.5^{c}	64.9^{bc}
5	0	17.5^{e}	47.6^{c}	62.7^{c}
	4	19.0^{e}	53.2^{c}	66.3^{bc}
	8	17.5^{e}	44.3^{c}	53.8^{c}
Aging x Gamma dosage		*	*	*

†Values with same letters are not significantly different within each column at 0.05 level of Tukeys test (*: the significant int eraction between aging and gamma irradiation factors at 0.05 level of two-way ANOVA).

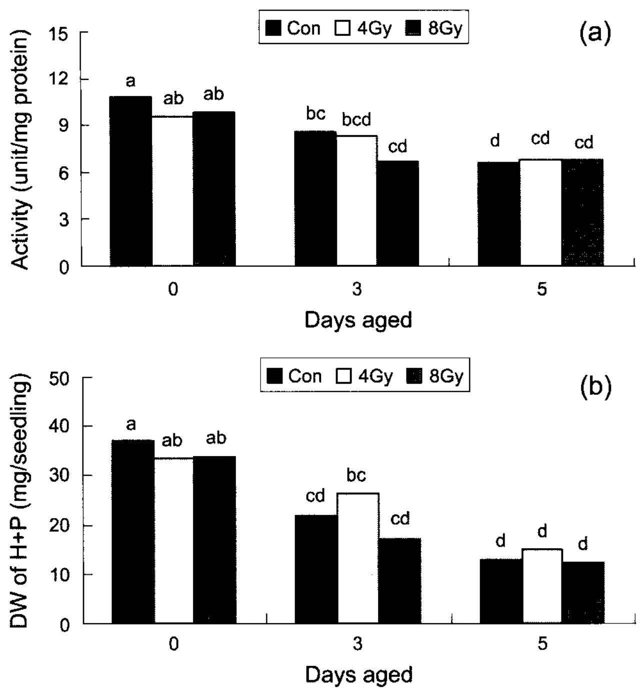

Fig. 2. Effects of accelerated seed aging and gamma irradiation on α-amylase activity (a) and dry weight of hypocotyls and primary roots of soybean (b). Bars with same letters are not significantly different at 0.05 level of Tukey's test.

germination study (Ren *et al.,* 1993), but α-amylase was analysed in this study. As shown in a recent study with artificially aged maize seeds (Seo *et al.*, 2003), accelerated aging negatively affected the amylase activity, with the 5 days aged seeds exhibiting the lowest activity (Fig 2(a)). Although the gamma irradiation did not interact with the aging treatment, it imposed measurable effects on the á-amylase activity (Fig. 2(a)). According to Bernal-Lugo *et al.* (1999), germinating aged seeds produced less α-amylase, which was a consequence of a reduced response to the gibberellic acid. It is, therefore, suggested that the stimulating effects of gamma irradiation on the amylase activity in aged seeds might be mediated by phytohormones.

Dry weight of the hypocotyls+primary root of the seeds showed a negative response to the aging treatment, with a more than 50% reduction occurring in the 5 days aged seeds compared to the control (Fig 2(b)), which is commonly found in other studies (Parrish & Leopold, 1978; Guy & Black, 1998). There were positive effects of irradiation for the dry weights of 3 days aged seed. It is not certain whether positive effects of gamma irradiation on the dry weight resulted from the gradual disappearance of gamma irradiation-induced stimulation during the germination stage or not.

In conclusion, we suggest a possibility in this study that a low dose gamma irradiation may be applied to alleviate the deleterious effects of aging due to long-term storage on seed vigour and viability. Low dose gamma irradiation increased the germination rate of aged soybean seeds, which might be mediated by the increased respiration rate and amylase activity of the aged seeds. Further detailed studies are needed in order to illustrate the possible roles of low dose gamma irradiation for improving the viability and germinability of the aged seeds.

ACKNOWLEDGEMENT

This research was carried out under the Nuclear R&D Program of the Ministry of Science and Technology, Korea.

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