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Early Germination Response of Soybean Seed to Accelerated Aging and Low Dose Gamma Irradiation

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ABSTRACT: The responses of soybean seeds were evaluated to accelerated aging and gamma irradiation with regard to germination, seed leakage, seed leachate component and dry weight of hypocotyl and primary root of the germinating seed. Accelerated aging significantly reduced the final germination rate while gamma irradiation increased the final germination rate. Furthermore, the interactive effects occurred that the final germination rate of 5-day aged seeds increased considerably in response to 4 Gy of gamma irradiation. The extent to which the electrolyte was leaked from the seeds (conductivity) was significantly affected by accelerated aging and showed a close negative correlation with the germination rate. Gamma irradiation, however, did not significantly affect the electrical conductivity of seed leachate. The accelerated aging significantly increased the concentrations of the particular electrolytes leaked from the seeds while the gamma irradiation did not affect those concentrations. Of the electrolytes leaked from the seeds, Ca and Mg showed relatively lower concentrations while K showed greater concentrations than others. Moreover, N and P showed similar responses to aging treatment. Aging treatment significantly affected dry weight (DW) of hypocotyls and primary root. Also, gamma irradiation decreased DW of hypocotyls and primary root, particularly for 8 Gy associated with 5 days aging treatment. The data were discussed in terms of the relationships of seed vigor with aging treatment and gamma irradiation.

***Keywords*:** seed aging, gamma irradiation, germination, electrolyte leakage, leachate concentration, hypocotyls, primary root, soybean

Storage of seeds under adverse conditions results in the production of aged seeds with a variety of symptoms ranging from reduced viability or germinability to more or less full viability but with abnormal development of seedling (Bewley & Black, 1994). The symptoms loss of seed vigor or viability resulting from due to prolonged storage or accelerated aging are commonly associated with is evidenced by a range of biochemical and metabolic alterations; the loss of enzymatic activities, impaired membrane integrity (Sung & Chiu, 1995), reduced ATP production, declined respiratory activity and impaired RNA and protein synthesis (Bewley & Black, 1994), and increase in free fatty acid content (Copeland & McDonald, 1995), all of which led contributed to a reduced germination percentage, seedling emergence, growth and ultimately imposed deleterious effects on crop establishment (Priestly, 1986). The exact mechanism through which the seed deterioration occurred has not been well established yet, some possible causes have been proposed such as membrane lipid peroxidation by free radicals inducing increased leakage of the solutes from aged seeds (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 (Petruzelli & Taranto, 1985; Bernal-Lugo *et al.*, 1999).

It has been proposed that the gamma irradiation produced positive effects on seed germination as well as early seedling growth, particularly at low doses (Sharon & Muralidharan, 1978; Sheppard & Evenden 1986), although the extent of crop responses to a low dose gamma irradiation varied with species. This stimulating effect of the gamma irradiation at low doses was termed as "radiation hormesis" (Luckey, 1980). There are many reports supporting the occurrence and explaining its mechanisms of radiation hormesis in plants, amongst which the 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).

This preliminary study is to address 1) whether or not the gamma irradiation improves the germinability of aged soybean seeds, and 2) to provide some insights for the possible mechanisms underlying germination response to ionizing radiation. The germination of aged seeds, electrolyte leakage, concentrations of particular minerals in the seed leachate and growth of hypocotyl + primary root of the seed were examined after gamma irradiation with low doses.

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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 6.8% on a dry weight basis. Accelerated seed aging and seed moisture adjustments were described in detail in a previous study (Hwangbo *et al.*, 2003). 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 assay and measurement of electrical conductivity of the seed leachate were referred to a previous study (Hwangbo *et al.*, 2003).

After measurement of electrolyte leakage with EC meter, concentrations of electrolytes in the solution were determined including nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), and magnesium (Mg). N concentration was determined with Kjeldahl Digestion and Distillation system (BDD unit B-324, Japan). P concentration was measured with a method of Chapman & Pratt (1961). Concentrations of K, Ca, and Mg were determined with an atomic absorption/flame emission spectrophotometer (AA-680, Shimadzu, Japan). Hypocotyls and primary roots were removed from the germinating seeds at 5 days after seeding (DAS) and oven-dried at 70°C for 3 days. The early growth of the soybean seedling was determined by measuring the dry weight of hypocotyl and primary root. The data was analyzed by the two-way ANOVA (General Linear Model, GLM) and Tukeys HSD test (SPSS 10.0, USA).

RESULTS AND DISCUSSION

Germination and electrolyte leakage

Accelerated aging significantly reduced the final germination rate compared to the non-aged control ($F_{1,2}$=399.5, P<0.001, Fig. 1a), while gamma irradiation increased the final germination rate considerably ($F_{1,2}$=4.438, P<0.05, Fig. 1a). Furthermore, the interactive effects occurred between both factors ($F_{1,4}$=3.165, P<0.05); the final germination rate of 5-day aged seeds increased considerably in response to 4 Gy of gamma irradiation (Fig. 1a). This type of germination response induced by low dose gamma irradiation was also noted in a study (Hwangbo *et al.*, 2003), in which a positive interaction was encountered for 3-day aging treatment in association with 4 Gy.

It has been unknown yet how the germination of the aged seeds is stimulated by gamma irradiation, but it could be possible that the gamma irradiation might facilitate germinability of the aged seeds through partly alleviating negative impacts of aging on seed germination. There are a number of causal mechanisms responsible for the reduced germination of the aged seeds; the 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 the loss of membrane integrity or membrane deterioration (Zacheo *et al.*, 1998).

Of those mentioned above, the loss of membrane integrity, mediated by lipid peroxidation by lipoxygenase or reactive oxygen species generated in the aged seeds (Zacheo *et al.*, 1998; Bailly *et al.*, 1996), could be ameliorated by gamma irradiation. 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 partly suppress free radical-induced deterioration in aged seed membranes and possibly allow a lesser amount of electrolytes to be exuded. The extent to which the electrolyte was leaked from the seeds (conductivity) was significantly affected by accelerated aging ($F_{1,2}$=33.2, P<0.001, Fig. 1b), and showed a close negative correlation with the final germination rate as shown in other studies (Fig. 2(a), Loomis & Smith, 1980; Hwangbo *et al.*, 2003). Gamma irradiation, however, did not significantly affect the electrical conductivity of seed leachate, irrespective of the dose of gamma irra-

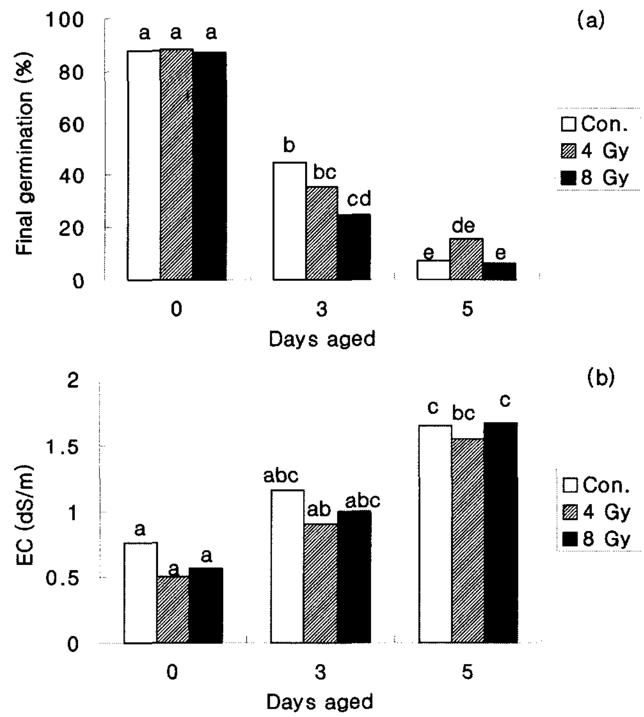

Fig. 1. Effects of accelerated aging and gamma irradiation on final germination (a) and electrical conductivity of seed leachate (b) of soybean. Bars with same letters are not significantly different at 0.05 level of Tukeys test.

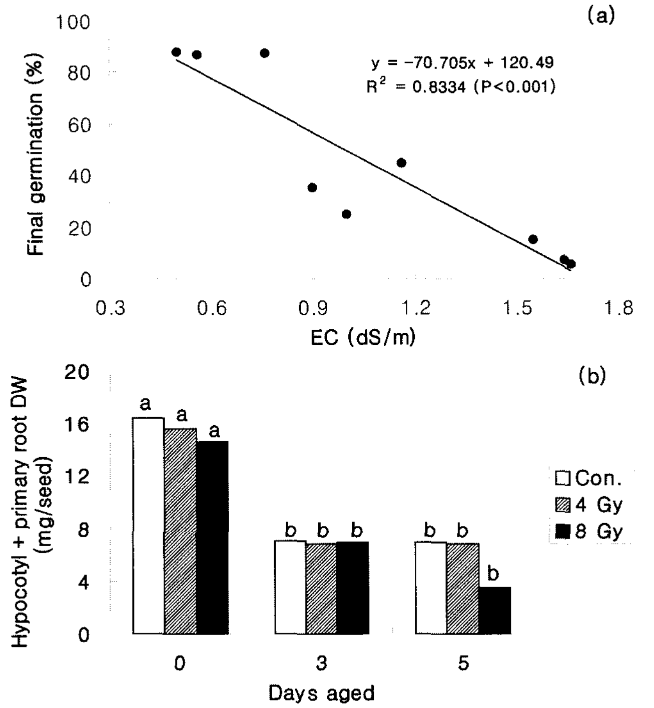

Fig. 2. Relationships between final germination and electrical conductivity (a) and effects of accelerated aging and gamma irradiation on dry weight of hypocotyl+primary root of germinating seeds. Bars with same letters are not significantly different at 0.05 level of Tukeys test.

diation ($F_{1,2}$=1.35, P=0.27, Fig. 1(b)), reflecting that the gamma irradiation did not alleviate the membrane deterioration of and/or reduce the extent of electrolytes leaked from the aged seed.

Hypocotyl and primary root of germinating seeds

The early growth of the seedling was evaluated based on dry weight (DW) of hypocotyl and primary root of the seed at 5 days after seeding. Aging treatment significantly affected DW of hypocotyls and primary root ($F_{1,2}$=148.29, P<0.001, Fig. 2(b)), which is commonly found in other studies (Parrish & Leopold, 1978; Guy & Black, 1998; Hwangbo *et al.*, 2003). Also, gamma irradiation decreased DW of hypocotyls and primary root, particularly for 8 Gy associated with 5 days aging treatment ($F_{1,2}$=4.704, P<0.05, Fig. 2(b)). However, there was no interactive effects of both factors on DW of hypocotyls and primary root ($F_{1,4}$ =1.584, P=0.201).

Concentrations of particular electrolytes

Since the measurement of electrical conductivity of seed leachate is very simple but non-specific test for seed vigor (Loomis & Smith, 1980), the determination of concentrations of particular ionic species in the leachate may provide an insight into studying the cause-effect relationship between electrolyte leakage and germinability of aged seeds.

Irrespective of the type of electrolyte measured, the accelerated aging significantly increased the concentrations of the particular electrolytes leaked from the seeds while the gamma irradiation did not affect those concentrations (Table 1). It seems to be a general phenomenon that the loss of electrolytes from the seeds occurred on early stage of imbibition, after which the seed membrane became selectively permeable and the loss of electrolytes was considerably reduced (Bewley & Black, 1994). However, as the length of

Table 1. Electrolyte concentrations in the solution of soybean seeds. Values with same letters are not significantly different within each column (P<0.05, Tukey test). Analysis of two-way ANOVA is shown by symbols (***P<0.001, n.s.: not significant).

Ageing (days)	Dose (Gy)	Electrolyte concentration (ppm)				
		N	P	K	Ca	Mg
0	0	24.1^{ab}	103.5^{abc}	321.1^{ab}	6.98^{ab}	11.0^{a}
	4	23.3^{ab}	80.3^{ab}	150.4^{a}	6.19^{a}	8.42^{a}
	8	20.6^{a}	58.2^{a}	177.9^{ab}	6.38^{a}	6.86^{a}
3	0	26.9^{abc}	157.5^{bcd}	478.5^{bcd}	10.7^{bcde}	19.0^{a}
	4	35.8^{bcd}	121.5^{abc}	357.0^{abc}	10.4^{bcd}	13.8^{a}
	8	33.6^{abcd}	175.6^{cd}	398.5^{abcd}	9.16^{abc}	19.1^{a}
5	0	42.1^{d}	188.1^{cd}	697.5^{d}	13.2^{de}	43.8^{b}
	4	43.9^{d}	219.4^{d}	648.1^{cd}	11.6^{cde}	38.4^{b}
	8	41.2^{cd}	191.7^{cd}	665.0^{d}	14.7^{e}	38.2^{b}
Source of variation						
Aging (A)		0.000***	0.000***	0.000***	0.000***	0.000***
Irradiation (I)		0.412$^{n.s.}$	0.818$^{n.s.}$	0.100$^{n.s.}$	0.742$^{n.s.}$	0.229$^{n.s.}$
Interaction (A×I)		0.470$^{n.s.}$	0.101$^{n.s.}$	0.895$^{n.s.}$	0.631$^{n.s.}$	0.804$^{n.s.}$

aging treatment increased, so did the loss of electrolytes from the seeds in this study (Table 1). This might be attributed to the inability of the seed to repair age-induced damage to cellular membranes (Berjak & Villiers, 1972).

Of the electrolytes leaked from the seeds, Ca and Mg showed relatively lower concentrations while K, known as a main inorganic ion leached from the seeds during imbibition (Marcos-Filho, 1998), showed greater concentrations than others (Table 1). It is likely that Ca and Mg appeared to be retained by the seeds in an insoluble form during the initial stage of germination (Loomis & Smith, 1980), which may delay their release from the seeds and eventually contribute to lowering their concentrations in the leachate. It was noted that the leachate from aged cabbage seeds showed not only a high concentration of K, but also a high degree of correlation between germination, emergence and K concentration (Loomis & Smith, 1980). In this respect, increased levels of K in the seed leachate could cause lower rates of metabolic activity and subsequently decreased seed vigor. Also the leakage N and P from the seeds showed the patterns similar to those shown by other minerals in response to aging treatment and gamma irradiation (Table 1). The aging-induced leakage of these essential elements utilized otherwise for optimum germination and seedling growth could be partly responsible for lower germination and poor seedling emergence.

In conclusion, aged seeds showed declined germination, increased leakage, lower hypocotyl+primary root growth of the seeds and higher solute concentrations in the seed leachate. The gamma irradiation with 4 Gy produced significantly positive effects on the germination of the seeds aged for 5 days. However, this increased germination after gamma irradiation was not associated with electrical conductivity of the solute leachate and particular ion concentrations of the leachate. Further studies are needed to establish a hypothesis for explaining increased germination rate with a low dose gamma irradiation found in this study.

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