PLANT BREEDING AND SEED SCIENCE
Volume 70 2014

DOI: 10.1515/plass-2015-0013

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SOYBEAN SEED GERMINATION AND SEEDLING GROWTH IN RESPONSE TO DETERIORATION AND PRIMING: EFFECT OF SEED SIZE

ABSTRACT

A laboratory experiment was conducted to determine if separation of soybean [*Glycine max* (L.) Merr.] seeds by size might be effective in germinability of aged and primed seeds and subsequent seedling vigour. A known soybean seed lot was separated into four size classes using round-hole screens. The seed lots were deteriorated by rapid aging and invigorated by hydro- and halo-priming. These pre-treated seeds were planted in rolled paper towels and the results were evaluated according to ISTA rules. The small soybean seeds had higher speed of germination than the other size classes. The seedlings produced from large and medium seeds were longer and heavier than those from other size classes. Our results indicated that the large seeds had less sensitivity to short-term aging condition owing to the number of normal seedlings, while the deterioration more increased the germination time of large and medium seeds, compared to small ones. The alleviatory effects of halo-priming on deterioration of seeds are greater compared with hydro-priming. Although there are some debates, the present data further indicate that larger soybean seeds are susceptible to aging condition.

Key words: aging, germination, priming, seed size, soybean

INTRODUCTION

Soybean (*Glycine max* (L.) Merr.) is one of the most valued oilseed crops in the world (Singh and Shivakumar, 2010). In general, proper germination of seeds and seedling establishment are critical processes in the survival and growth cycle of field crops (Ellis and Roberts, 1981; Roberts and Osei-Bonsu, 1988; Basra *et al.*, 2003; Hadas, 2004). This is especially true in soybean cultivation, since these processes determine uniformity and density of crop stand, and the efficient use of the nutrients and water resources available to theindi-

Communicated by Grzegorz Żurek

vidual plants and ultimately affect the yield and quality of this important oilseed crop (Singh *et al.*, 2010). Seed deterioration is a major problem in crop production, which leads to the loss of seed and seedling vigour (Douglas, 1975; McDonald, 1976; Perry, 1980; Dourado and Roberts, 1984). Seed deterioration can be defined as deteriorative changes occurring with time that increase the seed's vulnerability to external challenges and decrease the ability of the seed to survive (Powell *et al.*, 1984; Priestley, 1986; McDonald, 2004).

Seed deterioration is inexorable, and the best that can be done is to control its rate. In general, it is accepted that repair of seeds influenced by deteriorative events occurs by priming (McDonald, 2004). Seed priming is a commercially used technique for improving seed germination and vigour (Khan *et al.*, 1978; Kuc, 1978). It involves imbibition of seeds in water under controlled conditions to initiate early events of germination, followed by drying the seed back to its initial moisture content. Its benefits include rapid, uniform and increased germination, improved seedling vigour and growth under a broad range of environments resulting in better stand establishment (Beckers and Conrath, 2007; Varier *et al.*, 2010). Dell'Aquila and Taranto (1986) demonstrated that primed embryos of aged wheat seeds have a faster resumption of cell division and DNA synthesis on subsequent imbibition. Rao *et al.* (1987) reported a reversal of chromosomal damage (induced during seed deterioration) with the partial hydration of lettuce seeds by osmo-priming. However, it is not clear whether seed size can influence both the deleterious effects of deterioration and the beneficial effects of priming on soybean germination characteristics and subsequent seedling growth.

Large seed size is widely thought to improve the chances for crop emergence under a wide range of environments. It is also generally considered that, within a seed lot, seeds with a greater seed weight have greater storage reserves and thereby have increased seed vigour (Powell, 1988; Lopez-Castaneda *et al.*, 1996). The effect of seed size on early seedling growth has been addressed in many studies (Salim *et al.*, 1985). But, there are some debates about the effect of seed size on germination characteristics in soybean. Singh *et al.* (1972) reported that there is no difference in the performance of different sized soybean seeds, while Aguiar (1974) found that medium sized soybean seeds are more vigourous than larger and smaller ones. In another study, Barkke and Gradner (1987) declared that small soybean seeds, in comparison with large ones, are more efficient in the mobilization of seed reserves. On the other hand, Morrison and Xue (2007) argued that large-seeded soybean, compared to small-seeded one, are superior in germination and vigour. As well, Harnowo (2004) has reported the same trend in soybean and suggested that the deterioration rate of larger seeds is higher and their storability is lower, compared to smaller ones. The main aim of this research was to determine whether the seed size is consistently associated with the effects of deterioration and priming on soybean seed germination and seedling development.

MATERIALS AND METHODS

Seeds of soybean lot (cv. L_{17}; provided by Iran Agriculture Organization) were separated into three size classes (large, medium and small) based on the seed diameter using round-hole screens. A part of the ungraded seed lot was regarded as the control mixed lot. Seeds with 15% moisture content from all four sub-samples were artificially deteriorated at 40˚C through the rapid aging test (Roberts and Osei-Bonsu, 1988). A sub-sample was kept as control (non-deteriorated seeds) and the two other sub-samples were obtained after 3 and 4 days of aging, respectively. Distilled water and 75 mM NaCl solution was used to pre-treat each of 12 sub-samples (combinations of seed size and deterioration level) for 18 and 12 hours, respectively. The times of aging and hydro- and halo -priming and the proper concentration of the salt were determined by the pre-tests.

Laboratory tests were carried out as completely randomized design in the Seed Technology Laboratory of the University of Mohaghegh Ardabili, Iran. Four replicates of 50 seeds were placed between moist paper towels and germinated in an incubator at 15 °C for 14 days. Germination (protrusion of radicle by 2 mm) was recorded in daily intervals. The germination rate for each treatment was calculated according to Ellis and Roberts (1981). Four replicates of 50 seeds were used in standard germination test (temperature of 25 °C and incubation time of 8 days). At the end of germination test, lengths of normal seedlings were measured and then they dried in an oven at 80 °C for 24 hours. The dried seedlings were weighed and the mean seedling length and dry weight for each treatment at each replicate was determined. Vigour index was calculated as the product of germination percentage by seedling length. Total soluble carbohydrate of seedlings was determined using anthrone reagent according to Roe (1995). The data were analysed by MSTATC and the means were compared using Duncan's multiple range test at $P \leq 0.05$. Excel software was used to draw the figures.

RESULTS

Significant differences in the percentage of viable seeds were found among seed deterioration levels and priming techniques. The viability of seeds was significantly decreased with increasing the deterioration of seed lots (Table 1). The seeds deteriorated for 3 and 4 days show about 7 and 13 % reduction in viability respectively, compared to non-deteriorated ones. The two priming techniques also significantly but negligibly increased the percentage of viability. The interaction of seed deterioration and priming for this trait was insignificant.

Table 1

The percentage of viable seeds affected by deterioration and priming of soybean seeds

Parameter	Lot	Percentage of viable seeds [%]
Seed deterioration (SD)	SD1	97.6a
	SD2	90.8b
	SD3	85.3c
Priming techniques	control	90.3c
	hydro-priming	91.1b
	halo-priming	92.3a

Different letters indicating a significant difference at p≤ 0.05

SD_2 and SD_3 were the seed lots with 3 and 4 days aging respectively, compared to SD_1 as non-aged control.

Seedlings produced from large and medium seeds were significantly longer, heavier and vigourous than those from other two seed lots (Table 2). As expected, seed deterioration reduced the vigour index of seedlings. In contrast, pre -treatments of seeds especially halo-priming improved the vigour index in comparison with non-priming control. The length and the weight of seedlings from deteriorated and pre-treated seed lots were statistically the same.

Table 2

Seedling length, dry weight and vigour index affected by deterioration and priming of different sized soybean seeds

Parameter	Lot	Seedling length [cm per seedling]	Seedling dry weight [mg per seedling]	Vigour index
Seed size	mixed lot (control)	23.9c	50.1b	16.8b
	large	26.0a	54.3a	19.5a
	medium	25.6ab	53.5a	18.9a
	small	24.2bc	50.7b	16.6b
Seed deterioration (SD)	SD1 (control)	-		29.8a
	SD2	-		18.8b
	SD3	-		7.1c
Priming techniques	non-priming (control)	-		16.7c
	hydro-priming	-		17.4b
	halo-priming	-		19.7a

Different letters indicating a significant difference at p≤ 0.05.

The standard germination percentage of the most vigourous seed lot (SD_1 or control) was not influenced by varying the size of the seeds (Fig. 1). While small seeds and seeds of mixed lot produced less normal seedlings than larger ones, when exposed to deterioration. As observed in small seeds under four days of aging, the normal seedlings percentage were decreased more than two-fold compared with non-aged seed lots.

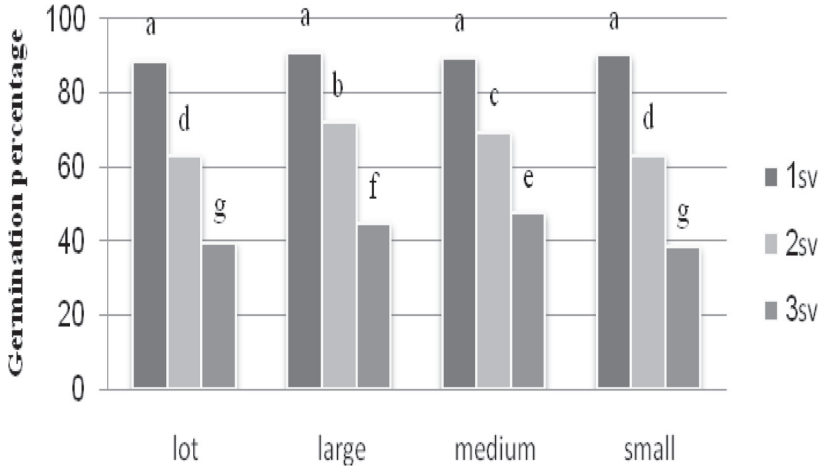

Fig. 1. The standard germination percentage of soybean affected by seed size and deterioration

As indicated in Fig. 2, hydro- and halo-priming had no significant promotive effects on the standard germination percentage of non-deteriorated seeds, compared to non-priming (control). While, the percentage of normal seedlings from aged seeds were significantly influenced by the two priming techniques and especially increased by approximately 8% when the most deteriorated soybean seeds (SD_3, four days of aging) were pre-treated with NaCl solution, in comparison with the control.

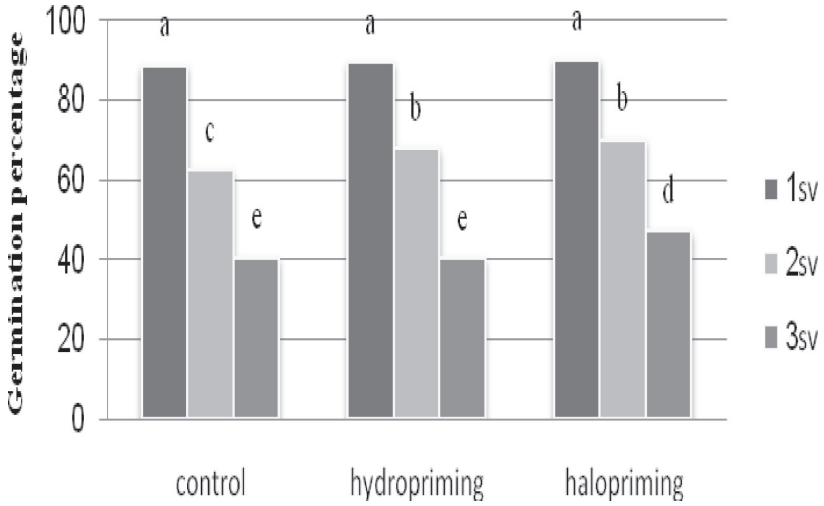

Fig. 2. The standard germination percentage of soybean affected by seed deterioration and priming

Overall, the small soybean seeds germinated faster than other seed size classes with or without aging treatment (Fig. 3). On the other hand, the germination rate of large seed lot was significantly reduced compared to other lots, especially the most reduction was observed when these large seeds were deteriorated for four days using the rapid aging test.

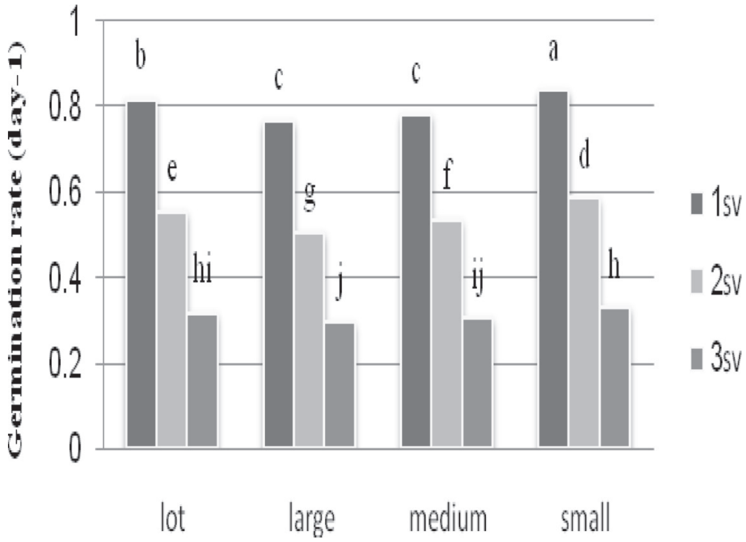

Fig. 3. The germination rate of soybean affected by seed size and deterioration.

Pre-treatment of both aged and non-aged soybean seeds was led to increase the velocity of germination, significantly (Fig. 4). Undoubtedly, there was no considerable significant difference between the two priming techniques in reducing the time of the germination.

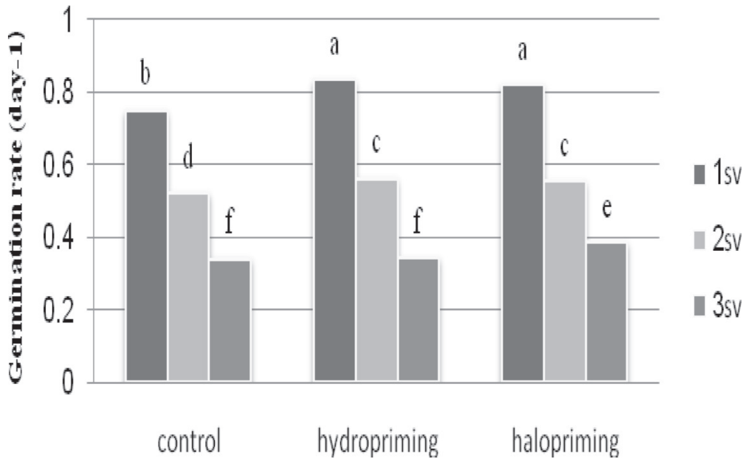

Fig. 4. The germination rate of soybean affected by seed deterioration and priming.

Hydro- and halo-priming could increase the length and the dry weight of soybean seedlings produced from the seeds of all three vigour levels, except those from SD_3 when pre-treated with distilled water (Fig. 5 and 6).

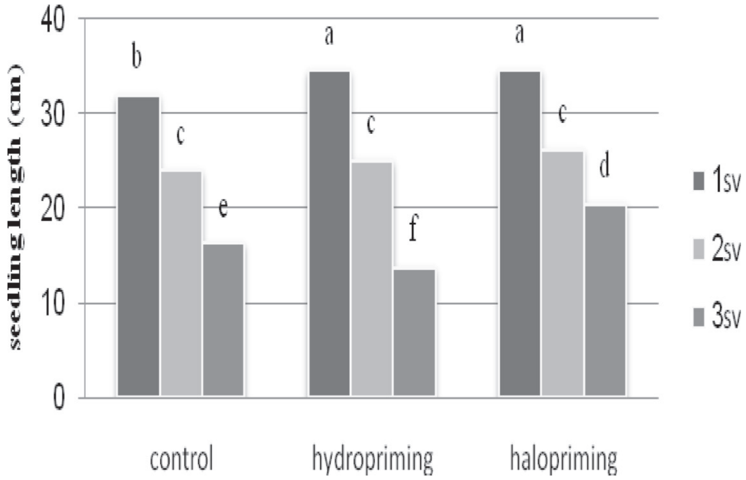

Fig. 5. The length of soybean seedling affected by seed deterioration and priming.

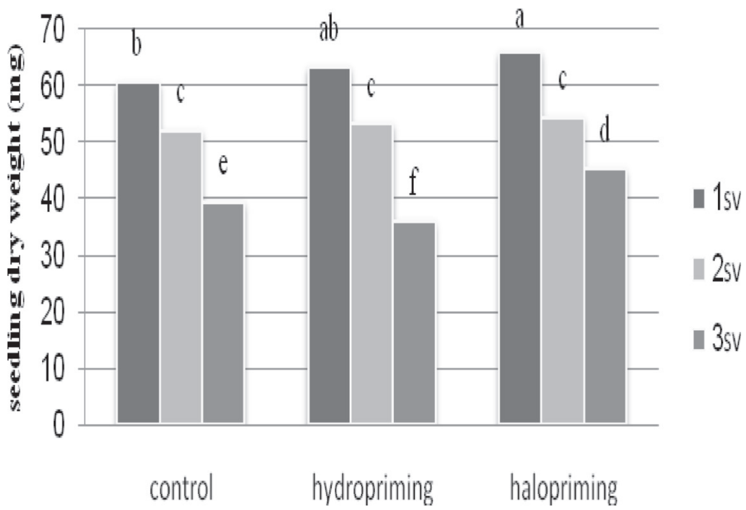

Fig. 6. The dry weight of soybean seedling affected by seed deterioration and priming.

The seedlings from the large seeds were significantly heavier than those from the other seed size classes (Fig. 7). However, the deteriorating conditions could decrease the seedling dry weight in all four seed lots, especially in small soybean seeds.

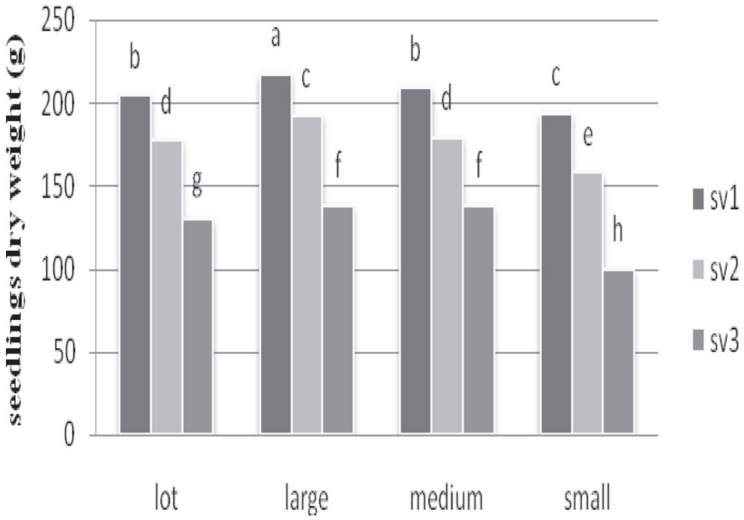

Fig. 7. The dry weight of soybean seedling affected by seed size and deterioration.

In spite of diminishing soluble carbohydrates in soybean seedlings produced from deteriorated seeds, pre-treatment of seeds especially halo-priming enhanced the sugar content of seedlings, significantly (Fig. 8). On the other hand, increase of artificially aging duration slightly caused to reduce the more relative advantage of seed pre-treatment with salt compared to non-primed control.

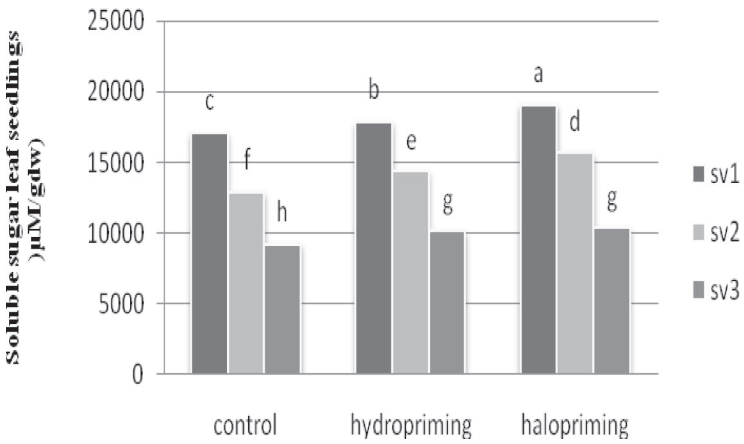

Fig. 8. The soluble carbohydrate content in soybean seedling affected by seed deterioration and priming.

DISCUSSION

Generally, our results indicated that the small soybean seeds had higher speed of germination than the other size classes (Fig. 3). Correspondingly, the superiority of small and medium soybean seeds in rate of germination and emergence was cited in some literatures (Edwards and Hartwig, 1971; Aguiar, 1974; Hoy and Gamble, 1987). There are few data in the literature relating seed size to emergence rate as most studies relate seed size to total emergence. But, there are some disagreements on this respect: The investigations of Ahmad and Bano (1992) on sunflower and Hoy and Gamble (1985) and Sung (1992) on soybean showed that seed size has a direct effect on seed germination and seedling vigour and that small seeds have a lower germination rate. In contrast, others believe that small seeds germinate faster and radicle protrudes sooner in them (McDonald, 1999; Tomes *et al.*, 1988). The general trend toward faster germination rates from small seeds observed in this research might be partially explained by the reduced mechanical resistance encountered during seedling emergence by the small seeds (Burris *et al.*, 1973) and the low water imbibition time (Singh *et al.*, 2009). On the other hand, Gardner *et al.* (2003) concluded that despite the rapid germination of small seeds than large ones, the difference in subsequent seedling growth is observable only in the early stages of development and after a while it will disappear.

The seedlings produced from large and medium seeds were longer and heavier than those from other two (mixed lot and small) size classes (Table 2). Although it has been reported that smaller soybean seeds are more efficient than large ones regarding reserves remobilization (Edje and Burris, 1971; Barkke and Gradner, 1987), higher germination percentage (Larsen and Andreasen, 2004) and longer and heavier seedlings from large seeds are due to their high reserves (Helm and Spilde, 1990) which may lead to increase yield of some crops (Lowe and Rise, 1973). Subsequent seedling growth after seed germination directly depends on the quantity and efficient mobilization of seed reserves (Westoby *et al.*, 1992; Soltani *et al.*, 2006).

Investigating the effect of aging on seed and seedling vigour, our results are in agreement with those of Jain *et al.* (2006) and Saha and Sultana (2008). Failure of aged seeds to germinate might be due to lipid peroxidation, mitochondrial dysfunction and less adenylate energy charge (McDonald, 1999; Basra *et al.*, 2003). Previously, Saha and Sultana (2008) indicated that deterioration reduces percentage and velocity of germination of soybean seeds. On the other hand, the extent to which seed size may influence exhaustive and invigourative effects of different pre-treatments (aging and priming) of seeds on subsequent seedling development and field establishment was proposed to be as one of the major questions in soybean production. As obtained from the present study, the large seeds had less sensitivity to short-term aging condition owing to the number of normal seedlings (Fig. 1), while the deterioration more increased the germination time of large and medium seeds, compared to small ones (Fig. 3). Accordingly, Harnowo (2004) has found that the deterioration of larger soybean seeds is greater than that of smaller ones. On the contrary, the deterioration reduced the dry weight of soybean seedlings produced from small-sized seed lot more than those from larger seeds (Fig. 7).

Our results indicated that halo-priming of soybean seeds significantly increased the seed viability, the germination percentage, the rate of germination, the length and the dry weight of seedlings and the total soluble carbohydrates in 8-day old seedlings, especially in seed lots that were imposed to the rapid aging (Tables 1 and 2 and Fig. 2, 4, 5, 6 and 8). McDonald (2004) discusses the ability of priming to overcome the low vigour effects that result from seed deterioration in aging and to improve the germination of aged seeds. In sunflower seeds, recovery of germinability of aged seeds by priming was associated with a lower rate of lipid peroxidation (Bailly *et al.*, 2000). Priming also appears to increase germination metabolism in aged embryonic axes more than those that are not aged (McDonald, 1999). Yaklich (1985) reported that soluble sugars decreased with aging of soybean seeds. Bearing in mind too that priming of a given species may be conducted using a range of hydration and drying procedures and a spectrum of water potentials and durations, so it is perhaps not surprising that metabolic and cellular events have been found to differ (McDonald, 2004). Reversal of seed deterioration by priming generally occurs in the meristematic axis or the radicle tip, e.g., peanut (Fu *et al.*, 1988). As we also observed in soybean seeds, Sivritepe and Dourado (1994) found that controlled humidification of aged pea seeds to 16.3 to 18.1 percent just prior to sowing decreases chromosomal aberrations, reduces imbibitional injury, and improves seed viability. In fact, the efficacy of osmopriming of low-vigour orthodox seeds is because repair processes occur while the seeds are held at water potentials that allow this metabolism but preclude germination (Bray, 1995).

CONCLUSIONS

In conclusion, the results described here demonstrate that both hydro- and halo-priming especially the later improve the physiological quality of soybean seeds. The alleviatory effects of halo-priming on deterioration of seeds are greater compared with hydro-priming. The present data further indicate that larger soybean seeds are susceptible to aging condition. Further researches in soybean and other crop plant species are necessary to clarify whether the benefits of priming are absolutely influenced by seed size or vigour level, or both. In general, the interactive effects of seed size×aging and aging×priming on seed germination capability and seedling development indicate that the potential for large scale vigour improvement in soybean seed lots through processing or conditioning is not limited.

ACKNOWLEDGMENT

Authors are sincerely thankful to Seed Physiology and Technology laboratory of University of Mohaghegh Ardabili for providing lab facilities for the present research project.

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