Reparation of aged lettuce (*Lactuca sativa*) seeds by osmotic priming and *Azospirillum brasilense* inoculation

Liliana E. Carrozzi, Cecilia M. Creus, Carlos A. Barassi, Gloria Monterubbianesi, and Adalberto Di Benedetto

Abstract: Although *Azospirillum* spp. are considered to be important plant growth promoting bacteria, their possible effects on germination and vigor of aged lettuce seeds has not been previously evaluated. In fact, there is a paucity of published data about inoculation effects on seed germination. The aim of this work was to evaluate seed quality of one-year aged lettuce (*Lactuca sativa* L. cv. Crimor INTA) seeds after *Azospirillum brasilense* Sp245 inoculation with or without an osmopriming pretreatment. Fresh lettuce seeds were stored in the dark in a dry chamber for one year and then subjected to inoculation with *A. brasilense* Sp245, to osmotic priming with 0.37 mol/L MgSO₄, or to both combined treatments. Seed germination, seed vigor, and seedlings emergence percentages were determined, and the abnormal seedling fraction was characterized. *Azospirillum brasilense* inoculation without a previous osmopriming enhanced seed vigor and seedling emergence percentages and decreased the fraction of abnormal seedlings. There was an additional effect of osmopriming as a previous treatment on germination percentage. We concluded that the use of *A. brasilense* inoculation alone or after an osmopriming treatment would contribute to overcome the negative effects of ageing on lettuce cv. Crimor INTA seeds.

Key words: *Azospirillum*, lettuce, osmotic priming, seed aging, germination, seed vigor.

Introduction

The period from harvesting to sowing seeds may vary from a few months to several years. It is during this time that the quality of seeds, defined as the ability to germinate, may decline (Wien 1997; Bradford 2006). The changes occurring in metabolic activities associated with ageing of the seeds are complex and poorly understood (Kalpana and Rao 1997; Bailly et al. 2001; Bradford 2006). Physiological parameters of deterioration such as decreased germinability and seedling growth were found to be associated with a low rate of metabolism in embryos of aged seeds. The process of ageing in seeds involves deterioration of many systems within the tissues (Tarquis and Bradford 1992; Clerkx et al. 2003). An important component of seed vigor is the rate of germination following imbibition. Various prehydration or priming treatments have been developed to increase the speed and synchrony of germination (Heydecker and Coolbear 1977; Bradford 1986; McDonald 2000). The technique of osmotic conditioning or osmopriming is a process in which seeds are allowed to imbibe...
sufficient water to enable the early events in the germination process to occur, but not enough to permit radicle emergence through the seed coat. Usually seeds are pre-imbibed in solutions of polyethylene glycol or inorganic salts, and this technique is proposed to invigorate and improve the rate and uniformity of germination of vegetable and cereal seeds (Burgass and Powell 1984; Hill et al. 2007; Ghiyasi et al. 2008; Salehzade et al. 2009).

Storage appears to have highly deleterious effects on the development of seedlings grown from primed seeds, even though effects on germination percentage are absent. After apparently normal germination of lettuce seeds, subsequent seedling development is, in many cases, abnormal, showing short or stunted roots (Tarquis and Bradford 1992). The incidence and severity of that abnormality is increased with storage time (McDonald 2000). The difference between germination and normal seedling percentages emphasizes the necessity of evaluating not only seeds but also seedlings in studies in which any form of treatment is applied. Although effects of priming on longevity of seeds in storage have been investigated in a number of species (Probert et al. 1991; Hill et al. 2007), there is a paucity of published data on seedling development after those treatments (Alvarado and Bradford 1988; van Pijlen et al. 1996). As seedling evaluation is a tedious and time-consuming operation, its absence from most reports on priming or, indeed, any other seed treatment is very common even though it is an integral part of the official germination test prescribed by International Seed Testing Association (ISTA) (1999).

As seed deteriorates, a cascade of disorganization ensues, ultimately leading to complete lack of function and cell death. Studies examining seed deterioration invariably find physical and physiological effects. The current model of seed deterioration accepts lipid peroxidation as a central theme causing cellular degeneration through free-radical attack on important cellular molecules and structures (Galleschi et al. 2002; Walters et al. 2005). These events are first manifested in the radicle tip (McDonald 1999; Basra et al. 2000).

Plant growth promoting bacteria (PGPB) are beneficial bacteria that colonize plant roots and enhance plant growth and yield of several species, including vegetable crops. Among them, the species of Azospirillum have gained the reputation of being the most studied plant-associative bacterium (Bashan et al. 2004). Azospirillum is a remarkably versatile bacterium, not only able to fix atmospheric N₂ (Döbereiner and Day 1976), but also able to mineralize nutrients from the soil, to sequester Fe, to survive harsh environmental conditions, and to favor beneficial mycorrhizal–plant associations (Bashan et al. 2004; Fibach-Paldi et al. 2012). In addition, Azospirillum can help plants minimize the negative effects of abiotic stresses (Casanovas et al. 2003; Creus et al. 2004; Barassi et al. 2006; Pereyra et al. 2006; Creus et al. 2010). Several mechanisms have been proposed to explain how PGPB enhance growth and development of plants. Recently, the multiple mechanisms theory was proposed by Bashan and de-Bashan (2010) based on the assumption that a combination of a few or many small mechanisms, operating at the same time or consecutively, create a larger final effect on the plant. Moreover, the production of plant growth regulators by the genera Azospirillum has often been regarded as the key factor responsible for the promotion of plant growth (Dobbelaeare et al. 2003; Spaepen et al. 2007). In fact, a variety of auxins such as indole-3-acetic acid (IAA) (Martínez-Morales et al. 2003; Tsavkelova et al. 2007), cytokinins (Tsavkelova et al. 2006), and gibberellins (Bottini et al. 1989, 2004) were reported to be produced by Azospirillum spp. in pure cultures. These phytohormones alter metabolism and the morphology of roots, leading to better absorption of minerals and water, consequently producing larger and healthier plants. In addition, the capability of A. brasilense to produce nitric oxide is recognized as a key factor in the enhanced branching of inoculated roots (Creus et al. 2005; Molina Favero et al. 2008). In spite of the large amount of literature published on Azospirillum effects on plants, few papers have reported on its involvement in seed germination (Zahir et al. 2004; Barassi et al. 2006; Cassán et al. 2009; Mishra et al. 2010; Villegas-Espinoza et al. 2010). Considering the ability of Azospirillum to produce and (or) modify plant growth substances, e.g., the deconjugation of gibberellic acid (GA) – glucosyl conjugates (Piccoli et al. 1997) and the 3β-hydroxylation of inactive 3-deoxy-GAs present in roots (Kobayashi et al. 1994; Piccoli et al. 1997; Cassán et al. 2001), the possibility of improving germination in aged vegetable seeds is an interesting hypothesis to be tested. Azospirillum brasilense inoculation could alleviate the negative effects of NaCl on the germination and initial growing stages of cv. Mantecosa lettuce (Barassi et al. 2006). Moreover, plants grown from inoculated seeds and irrigated with saline media displayed higher total fresh and dry weights and biomass partition to the aerial portion than uninoculated controls (Barassi et al. 2006). Considering that aging itself is a kind of physiological stress, it was interesting to test the effects of inoculation with A. brasilense on aged lettuce seeds. The aim of this work was to evaluate the response of one-year aged lettuce cv. Crimor INTA seeds to an osmotic priming treatment, to Azospirillum brasilense Sp245 inoculation, and to the combination of both of them to improve aged seed quality.

Materials and methods

Bacterial culture

Azospirillum brasilense Sp245 isolated from surface-sterilized wheat roots (Baldani et al. 1986) was used. Bacterial cells were kept in agar – Congo red medium (Rodriguez-Cáceres 1982), transferred to OAB liquid medium (Okon et al. 1977) containing 0.1% NH₄Cl and incubated at 32 °C with orbital agitation (100 rpm) for 18 h. A 10⁷ cell per seed inoculum was obtained by centrifuging OAB liquid media containing late exponential cells (10 min at 8142g) followed by resuspension in 40 mL of 66 mmol/L phosphate buffer, pH 7.

Seed source and storage

Lactuca sativa L. cv. Crimor INTA seeds were kindly provided by the Organic Production Project at La Consulta, EEA-INTA, Mendoza, Argentina. Freshly harvested lettuce seeds were stored in the dark in a dry chamber at 20 °C for one year to obtain the naturally aged seed lot. For seedling emergence tests, aged and newly harvested seeds of Lactuca sativa L. cv. Crimor INTA were used. Initial germination percentages and moisture content were 85.1% ± 6.3% and 5.0%, respectively, for non-aged seeds and 45.0% ± 3.2% and 5.0%, respectively, for aged seeds.

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Seed treatments

Seeds were surface-disinfected in 1% NaOCl for 1 min, washed thrice with sterile distilled water, and immersed for 2 h at 20 °C in the A. brasilense Sp245 inoculum containing 10^7 bacteria per seed suspended in 66 mmol/L phosphate buffer, pH 7. Afterwards, seeds were dried-back under air flow at 20 °C according to Creus et al. (1996). For osmotic priming treatment, surface-disinfected seeds were then imbibed in 0.37 mol/L MgSO_4 (nominal osmotic potential of –0.9 MPa) at 20 °C for 24 h with aeration by means of an aquarium pump. Primed seeds were immediately dried-back under air flow at 20 °C. The osmotic priming followed by A. brasilense inoculation treatment was accomplished by priming seeds as previously described, but before drying, seeds were immediately inoculated as described above. The seeds were then air-dried at 20 °C. Two different types of control seeds were used as treatments: (i) aged seeds immersed for 2 h at 20 °C in phosphate buffer (bacterial suspension media) and then dried at 20 °C; and (ii) nontreated, aged seeds. The notation for each treatment was as follows: A, A. brasilense Sp245 inoculation; OP, osmotic priming; OP + A, osmotic priming followed by inoculation; B, seeds immersed in phosphate buffer; and C, nontreated, aged seeds.

Colonization assessment

Most probable bacterial number (MPN) per gram fresh weight (FW) aged or fresh seeds was accomplished in all treatments. Briefly, 0.5 g lettuce seeds were homogenized in a sterile mortar with 4.5 mL of 66 mmol/L phosphate buffer, pH 7. Serial dilutions were obtained from each sample. Three replicate aliquots from each serial dilution were cultured in semisolid nitrogen-free medium (Döbereiner and Day 1976), and MPN per gram FW was estimated according to Postgate (1969). When indicated, a similar protocol was used to determine bacterial MPN in seedling roots 7 days after sowing.

Germination test

Four replications of 100 seeds from each treatment were uniformly distributed on a single sheet of filter paper (grade 37/N; Munktell Filter, Grycsbo, Sweden) adequately wetted with 40 mL of distilled water in a tray wrapped with transparent polyethylene film and incubated in a germination chamber with an 8 h photoperiod, 1000 Lux, at 20 °C. Germination, defined as the protrusion of the radicle, was determined 7 days after sowing according to the standard germination test for lettuce (ISTA 2004), and the percentage of germinated seeds was calculated accordingly. The percentage of abnormal seedlings produced was registered after 7 days, and the seedlings were classified in the following categories: R, seedlings with only radicle protrusion; RSH, seedlings with radicle protrusion and short hypocotyls; and SH, seedlings without radicle but with short hypocotyls. To obtain the initial germination percentage of the seed lot, the germination test as described above was also performed on nontreated lettuce seeds before storage.

Vigor test

Prior to the treatments evaluated in this work, seed vigor was determined after subjecting seeds to controlled accelerating ageing according to ISTA (2004) rules. Briefly, about 0.5 g of one-year naturally aged seeds were placed in Petri dishes in a humid chamber at 40 °C ± 2 °C for 48 h. Afterwards, the same seed treatments as described above were applied with four replicates per treatment. The germination percentage was calculated and considered as a measure of the vigor of the seeds. The abnormal seedling fraction was also determined as described above.

Seedling emergence test

Non-aged and naturally aged seeds from each treatment were sown in 128-cell plastic trays filled with a mixture of soil–vermicompost–perlite–vermiculite (1:1:1:1, by volume). Two seeds were sown in each cell, and then the trays were covered with a fine layer of vermiculite. Trays were accommodated inside a greenhouse in a complete randomized block design with four replications per treatment. Irrigation was done with a micro-aspersion system each day for 30 min to ensure attaining the full water holding capacity of the soil. At 10 days after sowing, emergence of seedlings was registered, and the percentage was calculated.

Experimental design and statistical analyses

All of the experiments performed in the germination chamber were designed in a complete randomized treatment of 100 seeds arranged with four replications per treatment. Response variables defined as percentages were transformed for statistical analyses (Probit transformation), but pure data without transformations are presented in the tables and figures. Data were analyzed for variance by ANOVA using the R package (R Development Core Team 2004). Means were compared by the least significant difference (LSD) test (P < 0.05). The greenhouse experiment was a factorial combination of seed treatments (five levels: A, OP, OP + A, B, and C) with seed state (two levels: non-aged, and aged one year) in complete randomized blocks, with four replicates and 256 seeds sown in each tray. Seedling emergence percentage was analyzed by ANOVA, studying interaction between factors and the mean compared with LSD test (P < 0.05).

Results

While bacterial MPN per gram FW in aged or fresh seeds was determined 24 h after inoculation, roots collected 7 days after sowing each type of seed showed the highest MPN values, thus confirming a good colonization (Table 1). No significant differences were observed between the MPN per gram FW of fresh or aged seeds (Table 1).

The germination percentage of the non-aged lot of seeds was 85.1% ± 6.3%. After one year of storage, it declined to 66.1% ± 2.0% (C in Fig. 1a). This result corroborates the known effect of ageing on lettuce seeds (Rao et al. 1987). Excepting OP, all the treatments applied to aged seeds increased germination over nontreated control seeds (Fig. 1a). The combination of OP and A. brasilense inoculation produced the highest value of germination percentage (Fig. 1a). The percentage of total abnormal seedlings produced after OP treatment was equal to that produced by both types of controls B and C (Fig. 1b). Azospirillum brasilense inoculation alone or after osmotic priming reduced the abnormal seedling fraction (Fig. 1b). Table 2 shows the percentages of the different types of abnormal seedlings. No seedlings of R category (only radicle protrusion) were produced by any treatment (Table 2). On the other hand, the percentage of RSH seedlings was significantly decreased by A. brasilense inoculation (Table 2). This reduction agrees with the increase in the germination

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Table 1. Bacterial colonization of seeds or roots of one-year naturally aged and fresh seeds of *Lactuca sativa* L. cv. Crimor INTA.

<table>
<thead>
<tr>
<th>Seed treatment</th>
<th>Bacteria (MPN/g FW)</th>
<th>Fresh</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Seeds</td>
<td>Roots</td>
</tr>
<tr>
<td>A</td>
<td>4.5×10^4±9.0×10^3</td>
<td>1.2×10^7±9.0×10^6</td>
</tr>
<tr>
<td>OP</td>
<td>&lt;100</td>
<td>&lt;100</td>
</tr>
<tr>
<td>OP + A</td>
<td>1.5×10^5±6.0×10^4</td>
<td>9.5×10^6±1.2×10^6</td>
</tr>
<tr>
<td>B</td>
<td>&lt;100</td>
<td>&lt;100</td>
</tr>
<tr>
<td>C</td>
<td>&lt;100</td>
<td>&lt;100</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Seed treatment</th>
<th>Seeds</th>
<th>Roots</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>2.5×10^4±1.0×10^3</td>
<td>&lt;100</td>
</tr>
<tr>
<td>OP</td>
<td>&lt;100</td>
<td>&lt;100</td>
</tr>
<tr>
<td>OP + A</td>
<td>7.5×10^5±1.0×10^3</td>
<td>4.5×10^6±4.0×10^3</td>
</tr>
<tr>
<td>B</td>
<td>&lt;100</td>
<td>&lt;100</td>
</tr>
<tr>
<td>C</td>
<td>&lt;100</td>
<td>&lt;100</td>
</tr>
</tbody>
</table>

**Note:** Results are represented by the mean ± SE of three independent replications per treatment. MPN, most probable number; FW, fresh weight. Treatments applied to aged or fresh seeds are as follows: A, inoculated with 10^7 *A. brasilense* cells per seed; OP, osmotic priming in 0.37 mol/L MgSO_4; OP + A, osmotic priming in 0.37 mol/L MgSO_4 prior to *A. brasilense* inoculation; B, 66 mmol/L phosphate buffer; and C, nontreated control seeds.

Fig. 1. (a) Germination and (b) total abnormal seedling percentages after different treatments applied to one-year naturally aged lettuce (*Lactuca sativa* L. cv. Crimor INTA) seeds. Treatments were as follows: A, inoculated with 10^7 *A. brasilense* cells per seed; OP, osmotic priming in 0.37 mol/L MgSO_4; OP + A, osmotic priming in 0.37 mol/L MgSO_4 prior to *A. brasilense* inoculation; B, phosphate buffer control; and C, nontreated control seeds. Bars are means of four experimental replications with 100 seeds for each treatment and replication, and standard errors are indicated. Different lowercase letters indicate statistically significant differences (P ≤ 0.05) among treatments.

The vigor of aged seeds was significantly increased by *A. brasilense* inoculation alone or after osmotic priming compared with the nontreated aged control seeds (Fig. 2a). Osmotic priming enhanced vigor compared with control seeds, but although significant, its effect was less pronounced than OP + A treatment (Fig. 2a). Additionally, inoculation treatments with or without osmotic priming showed lower percentages of total abnormal seedlings (Fig. 2b). These results were associated with lower numbers of seedlings in the R and RSH categories (Table 3).

Statistical analysis of seedling emergence percentages showed interaction between factors, so the means were compared within each type of seed. There were no significant differences in the emergence percentages among treatments when seeds were non-aged (Fig. 3a). However, after one year of storage, A and OP + A treatments significantly increased the percentage of seedling emergence (Figs. 3a, 3b). The use of *A. brasilense* inoculation alone or after previous osmopriming attained similar emergence values to non-aged seeds.

**Discussion**

Lettuce production in Argentina was estimated to be 38 000 tonnes in 2002 (Instituto Nacional de Estadística y Censos de la República Argentina 2002). The majority of seeds used in lettuce production are imported from the US and the European Community, which increases the cost of production. Consequently, small farm growers usually store the remaining seeds for the next year. This practice leads to problems in seed germinability and seedlings emergence. In our study, the germination percentage of one-year aged seeds without any treatment (C in Fig. 1) was lower than the value obtained from non-aged seeds (85.1% ± 6.3%; not shown). This emphasizes the striking effect of one year of storage on the germination capacity of cv. Crimor INTA lettuce seeds, in agreement with Rao et al. (1987).
Table 2. Abnormal seedling percentages after the germination process of one-year naturally aged lettuce (Lactuca sativa L. cv. Crimor INTA) seeds exposed to different treatments.

<table>
<thead>
<tr>
<th>Seedling type*</th>
<th>A</th>
<th>OP</th>
<th>OP + A</th>
<th>B</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td>R (with only radicle protrusion)</td>
<td>0a</td>
<td>0a</td>
<td>0a</td>
<td>0a</td>
<td>0a</td>
</tr>
<tr>
<td>RSH (with radicle protrusion and short hypocotyls)</td>
<td>3.3 ± 1.2b</td>
<td>10.0 ± 2.6a</td>
<td>1.3 ± 0.9b</td>
<td>7.0 ± 0.6ab</td>
<td>9.3 ± 2.3a</td>
</tr>
<tr>
<td>SH (no radicle and with short hypocotyls)</td>
<td>3.7 ± 0.9a</td>
<td>4.0 ± 1.0a</td>
<td>0.7 ± 0.07c</td>
<td>3.0 ± 0.1ab</td>
<td>3.7 ± 0.7a</td>
</tr>
</tbody>
</table>

Note: Results are represented by the mean ± SE of three independent replications per treatment. Different lowercase letters indicate statistically significant differences (P ≤ 0.05) among seed treatments for each abnormal seedling type. Treatments applied to seeds are as follows: A, inoculated with 10^7 A. brasilense cells per seed; OP, osmotic priming in 0.37 mol/L MgSO_4; OP + A, osmotic priming in 0.37 mol/L MgSO_4 prior to A. brasilense inoculation; B, buffer phosphate control; and C, nontreated control seeds. *The abnormal fraction of seedlings was classified in the different types according to ISTA (2004) rules.

Fig. 2. (a) Germination and (b) total abnormal seedling fraction percentages after vigour test for aged lettuce (Lactuca sativa L. cv. Crimor INTA) seeds. Treatments were as follows: A, inoculated with 10^7 A. brasilense cells per seed; OP, osmotic priming in 0.37 mol/L MgSO_4; OP + A, osmotic priming in 0.37 mol/L MgSO_4 prior to A. brasilense inoculation; B, buffer phosphate control; and C, nontreated control seeds. Bars are means of four experimental replications with 100 seeds for each treatment, and standard errors are indicated. Different lowercase letters indicate statistically significant differences (P ≤ 0.05) among treatments.

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Table 3. Abnormal seedling percentages after the vigor test of one-year naturally aged lettuce (Lactuca sativa L. cv. Crimor INTA) seeds exposed to different treatments.

<table>
<thead>
<tr>
<th>Seedling type*</th>
<th>A</th>
<th>OP</th>
<th>OP + A</th>
<th>B</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td>R (with only radicle protrusion)</td>
<td>4.0±0.1bc</td>
<td>5.0±1.5bc</td>
<td>3.0±1.1c</td>
<td>9.0±2.3ab</td>
<td>16.3±2.7a</td>
</tr>
<tr>
<td>RSH (with radicle protrusion and short hypocotyls)</td>
<td>14.7±1.7c</td>
<td>30.3±3.3a</td>
<td>17.3±1.4c</td>
<td>19.3±3.1b</td>
<td>27.3±2.0ab</td>
</tr>
<tr>
<td>SH (no radicle and with short hypocotyls)</td>
<td>3.0±0.6c</td>
<td>2.3±0.2c</td>
<td>4.3±1.2bc</td>
<td>15.7±2.1ab</td>
<td>17.0±1.6a</td>
</tr>
</tbody>
</table>

Note: Results are the mean ± SE of three independent replications per treatment. Different lowercase letters indicate statistically significant differences (P ≤ 0.05) among seed treatments for each abnormal seedling type. Treatments applied to seeds are as follows: A, inoculated with 10^7 A. brasilense cells per seed; OP, osmotic priming in 0.37 mol/L MgSO_4; OP + A, osmotic priming in 0.37 mol/L MgSO_4 prior to A. brasilense inoculation; B, phosphate buffer control; and C, nontreated control seeds.

*The abnormal fraction of seedlings was classified in different types according to ISTA (2004) rules.

Fig. 3. (a) Lettuce (Lactuca sativa L. cv. Crimor INTA) seedling emergence percentage and (b) representative photograph of plug trays with emerged seedlings after different treatments applied to one-year naturally aged or non-aged seeds. Treatments are as follows: A, inoculated with 10^7 A. brasilense cells per seed; OP, osmotic priming in 0.37 mol/L MgSO_4; OP + A, osmotic priming in 0.37 mol/L MgSO_4 prior to A. brasilense inoculation; B, phosphate buffer control; and C, nontreated control seeds. Bars are means of four experimental replications with 256 seeds in each replication for each treatment, and standard errors are indicated. Different uppercase letters indicate statistically significant differences (P ≤ 0.05) among treatments for non-aged seeds. Different lowercase letters indicate statistically significant differences (P ≤ 0.05) among treatments for one-year aged seeds.

There is a close association between the percentage of germination in aged seeds and the repair of deterioration during the lag period between the start of imbibition and the emergence of the radicle (Matthews et al. 2010). Hosseini et
al. (2010) showed that aged seeds significantly increase the incidence of abnormal seedlings; longer lag periods may be needed for older seeds to repair deterioration before physiological germination can be achieved. This result suggests that an increase in abnormal seedling fraction produced from old seeds is the result of incomplete repair. The abnormal seedling was usually grossly deficient in the normality of morphological structures, leading to severely impaired physiological processes (Kapoor et al. 2010; Rastegar et al. 2011). The seedlings with retarded growth eventually died and thus had no field plantable value (Ehiagbonare et al. 2008). The heterotrophic seedling growth can be considered as the product of three components: (i) initial seed weight; (ii) the fraction of seed reserves that are mobilized; and (iii) the conversion efficiency of mobilized seed reserves to seedling tissues. Although the effect of priming on lettuce seeds and their sensitivity to environmental stress have been confirmed recently by Schwemer and Bradford (2011), it is not clear which of these component(s) is affected by seed deterioration. Nik et al. (2011) indicated that seed deterioration results in decreased percentage and rate of germination and decreased percentage of normal seedlings. Seedling growth and the fraction of seed reserve mobilization indicated a significant decrease with the advance of deterioration. However, the effect of seed deterioration on the conversion efficiency of mobilized reserves to seedling tissues was not significant. Siadat et al. (2011) showed that 400 mg·L−1 gibberellin had a positive effect on germination of aged corn seeds. Using seed enhancement treatments such as seed priming or application of phytohormone could improve aged and non-aged seed performance, especially for older seeds. The beneficial effect of Azospirillum spp. of a decrease in abnormal seedling population has not been documented previously but probably could be related to the effect of the gibberellin production by the bacteria (Cassán et al. 2001) on the seed reserve mobilization (Groot and Karssen 1987).

The fact that seedling emergence was improved by A and OP + A when seeds were aged but not when they were newly harvested (Fig. 3) indicated that the physiological state of the seed would be the determinant factor affecting the response to seed treatments. Koenig et al. (2002) found that inoculation of heat-aged soybean seeds with the PGPB Methylobacterium sp. increased germination percentage, but the effect was null when seeds were not aged. Similarly, Korkmaz (2003) reported that the rate and synchrony of germination were increased in low-vigor lettuce seeds by osmotic priming but not in high-quality seeds. Moreover, Burgess and Powell (1984) and Pandy (1988) considered osmotic pretreatment of seeds as a suitable technique to alleviate aging effects but not appropriate when seeds were fresh or had an initial high germination percentage. They also stated that the effect of osmopriming on non-aged seeds could be negative on seedling vigor.

In conclusion, the use of A. brasilense inoculation alone or after an osmopriming treatment would contribute to overcoming the negative effects of ageing on lettuce cv. Crimor INTA seeds. Azospirillum brasilense inoculation pretreatment has high commercial possibilities, as this report, the first relating A. brasilense and seed separation after storage, has demonstrated. The precise mechanisms resulting in the improved performance of aged seeds (enzyme activation, reserve mobilization, membrane repair, etc.) deserve more study in future investigations.

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References


