Activation of the Cell Cycle in Tomato (Lycopersicon esculentum Mill.)
Seeds during Osmoconditioning as Related to Temperature and Oxygen

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INTRODUCTION
Osmoconditioning consists of imbibing seeds in an osmotic solution at low water potential in order to control their water uptake and prevent radicle protrusion. Such treatment improves the germination of seeds of numerous species (Heydecker, Higgins and Turner, 1975; Guedes and Corbineau, Picard and Co., 1993; Bray, 1995). Osmoconditioning is associated with an inhibition of DNA replication and subsequent germination in the presence of high concentrations of NaCl. Osmoconditioning has also been shown to improve germination of seeds transferred onto water at 15 °C but had no effect on DNA replication. For temperatures during priming up to 25 °C, a positive linear correlation existed between the efficiency of the treatment, evaluated by the reciprocal of time to obtain 50% germination, and the frequency of 4C nuclei or the 4C/2C values. Such a correlation did not exist when priming was performed at higher temperatures. At least 5% oxygen in the atmosphere was required during priming for the induction of DNA replication and for the enhancement of subsequent germination. In the presence of 5 x 10^-4 M and 10^-3 M NaN3 during priming, most of the cells were maintained with 2C DNA levels and the treatment had no stimulatory effect on germination. The results show a positive linear relationship between the frequency of 4C DNA nuclei or the 4C/2C ratio and the improving effect of priming. However, in suboptimal conditions of priming (−20 MPa or temperatures higher than 25 °C), the improvement of seed germination was not associated with the onset of DNA replication.

Key words: Cell cycle, germination, osmoconditioning, oxygen, temperature, Lycopersicon esculentum, tomato.

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Using flow cytometric analyses of the nuclear DNA content, we studied the effects of various conditions of osmoconditioning on the activation of the cell cycle in embryo root tips of tomato (Lycopersicon esculentum 'Elko') seeds. In dry untreated seeds, 90% of the nuclei revealed 2C signals. Priming of seeds in polyethylene glycol-8000 (PEG) improved the germination rate of seeds transferred onto water at 15 °C. This was associated with an increase in 4C signals when priming was carried out at −10 and −1.5 MPa. Priming at −20 MPa enhanced subsequent germination but had no effect on DNA replication. For temperatures during priming up to 25 °C, a positive linear correlation existed between the efficiency of the treatment, evaluated by the reciprocal of time to obtain 50% germination and the frequency of 4C nuclei or the 4C/2C values. Such a correlation did not exist when priming was performed at higher temperatures. At least 5% oxygen in the atmosphere was required during priming for the induction of DNA replication and for the enhancement of subsequent germination. In the presence of 5 x 10^-4 M and 10^-3 M NaN3 during priming, most of the cells were maintained with 2C DNA levels and the treatment had no stimulatory effect on germination. The results show a positive linear relationship between the frequency of 4C DNA nuclei or the 4C/2C ratio and the improving effect of priming. However, in suboptimal conditions of priming (−20 MPa or temperatures higher than 25 °C), the improvement of seed germination was not associated with the onset of DNA replication.

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Studies on the effects of osmoconditioning on DNA replication have given inconsistent results. Only low DNA synthesis was found during priming in leek (Allium porrum L.) (Bray et al., 1989; Ashraf and Bray, 1993) and tomato (Lycopersicon esculentum Mill) (Coolbear and Grierson, 1999) seeds. In contrast, studies using flow cytometry have demonstrated that DNA replication was initiated in the radicle tip cells during osmoconditioning of seeds of tomato (Bino et al., 1992; Lanteri et al., 1994) and pepper (Capsicum annuum L.) (Lanteri et al., 1993, 1994; Saracco et al., 1995). An increase in 4C DNA content was also observed in sugarbeet (Beta vulgaris L.) seeds (Redfern and Osborne, 1997). Induction of DNA replication has also been associated with an accumulation of β-tubulin (De Castro et al., 1992).

In pepper and tomato seeds, a positive correlation was found between the induction of DNA replication, measured as the increase in 4C nuclei, and the efficiency of the osmotic treatment (Lanteri et al., 1994). The percentage of 4C nuclei started to increase after 3 d of priming in the radicle tips of pepper embryos (Bino et al., 1992) and after 10 d in pepper embryos (Lanteri et al., 1993). A reduction of the polyethylene glycol concentration used for the priming treatment resulted in a greater proportion of cells with 4C DNA content (Lanteri et al., 1994; Saracco et al., 1995). Nevertheless, Saracco et al. (1995) found that priming could improve germination of pepper seeds without an increase in 4C nuclei.

The aims of the present work were: (1) to investigate...
effects of conditions of priming (water potential, oxygen and temperature) on tomato seed germination and DNA replication; and (2) to determine whether the beneficial effect of priming is correlated with an increase in 4C nuclei.

### MATERIALS AND METHODS

#### Plant material

Experiments were carried out with seeds of tomato (*Lycopersicon esculentum Mill.*, 'Elko') supplied by Clause Semences (France). Seeds were stored dry in the open air at 20 °C and 55% relative humidity before the experiments started.

#### Osmopriming treatments

Seeds were primed for 7 d on a layer of cotton wool imbibed with polyethylene glycol-8000 (PEG) solutions at −1.0, −1.5 and −2.0 MPa. The treatments were carried out at temperatures ranging from 10 to 35 °C (±0.5 °C). Concentrations of PEG solutions were calculated according to Michel and Kaufmann (1973).

Priming of seeds in various oxygen tensions (mixtures of nitrogen and oxygen) was performed for 7 d at 25 °C—the optimal temperature for priming of tomato seeds (Ozbingöl, Corbineau and Côme, 1998)—according to the procedure developed by Côme and Tissatour (1968). Gas mixtures were obtained through capillary tubes by mixing compressed air and pure nitrogen. The atmospheres thus obtained were passed continuously through germination chambers at a constant flow rate (4 l h⁻¹). They contained 0 (pure nitrogen), 1, 3, 5, 10 and 21% oxygen (air).

In order to study whether a particular level of respiratory activity was necessary to achieve priming, seeds were soaked for 7 d at 25 °C in polyethylene glycol solutions at −1.0 MPa containing various concentrations of the respiratory inhibitor NaN₃ ranging from 10⁻⁵ M to 10⁻³ M.

After the osmotic treatments, seeds were rinsed for 30 s with distilled water and were dried for 3 d in the open air at 20 °C and 55% relative humidity. After redrying, the moisture content of primed seeds was similar (8.9% d. wt basis) to that of control untreated seeds. Dry weight was obtained by oven drying seeds at 105 °C for 3 d.

#### Germination assays

Germination ability was tested in samples of 100 seeds placed in 9 cm diameter Petri dishes (25 seeds per dish, four replicates) on a layer of cotton wool moistened with distilled water. Germination assays were carried out at 15 °C in darkness. A seed was regarded as germinated when the radicle protruded through the seed coat. Germination counts were made daily up to 15 d. Results were expressed as the time to reach 50% germination (T₅₀) or the reciprocal of T₅₀ (1/T₅₀). Results presented are the means of the results obtained in four replicates ± s.d.

#### Flow cytometry

Amounts of nuclear DNA were quantified using radicle tips of embryos isolated from control unprimed seeds and from seeds primed for 7 d in various conditions as specified earlier. Radicle tips (1 mm) were dissected from seeds, dried for 2 d at 20 °C in the presence of silica gel and stored at −30 °C for later use. Embryo radicle tips were then chopped with a razor blade in 500 μl of nuclear-isolation buffer containing 0.2 M mannitol, 10 mM 2-(N-morpholino)ethanesulphonic acid, 10 mM NaCl, 10 mM KCl, 10 mM spermine tetrahydrochloride, 2.5 mM ethylenediamine-tetraacetic acid, 2.5 mM dithiothreitol, 0.05% v/v triton X-100 and 0.05% NaN₃ at pH 5.8 as previously described by Bino et al. (1993). The mixture and additional 500 μl nuclear-isolation buffer were sieved through 88 μm nylon mesh into the flow-cytometer test tubes. Twenty μl of 1 mg ml⁻¹ propidium iodide solution (Molecular Probes, Eugene, OR, USA) were then added to allow measurement of amounts of nuclear DNA by fluorescence. For each sample, five radicle tips, representing about 5000 to 10000 nuclei, were used and

<table>
<thead>
<tr>
<th>Conditions of priming</th>
<th>Duration (d)</th>
<th>Water potential (MPa)</th>
<th>Moisture content (% d. wt)</th>
<th>T₅₀ (h)</th>
<th>2C (%)</th>
<th>4C (%)</th>
<th>(4C/2C) × 100</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (0)</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>94±0.4</td>
<td>130±10</td>
<td>90.7±0.4</td>
<td>70±2.0</td>
</tr>
<tr>
<td>3</td>
<td>−1.0</td>
<td>77.3±4.2</td>
<td>50±1</td>
<td>772±10</td>
<td>226±8</td>
<td>29.3±1.3</td>
<td>130±0.5</td>
</tr>
<tr>
<td></td>
<td>−1.5</td>
<td>70.2±4.3</td>
<td>52±2</td>
<td>869±0.6</td>
<td>130±0.5</td>
<td>62±0.5</td>
<td>66±0.6</td>
</tr>
<tr>
<td></td>
<td>−2.0</td>
<td>64.8±8.2</td>
<td>70±5</td>
<td>93.8±0.3</td>
<td>62±0.5</td>
<td>66±0.6</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>−1.0</td>
<td>76.0±2.4</td>
<td>19±1</td>
<td>63.6±3.8</td>
<td>28±1</td>
<td>45.3±4</td>
<td>82±4</td>
</tr>
<tr>
<td></td>
<td>−1.5</td>
<td>68.8±1.7</td>
<td>40±2</td>
<td>89.0±0.2</td>
<td>10±0.5</td>
<td>122±0.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>−2.0</td>
<td>67.6±3.8</td>
<td>48±4</td>
<td>93.0±0.9</td>
<td>68±0.2</td>
<td>7.3±0.3</td>
<td></td>
</tr>
</tbody>
</table>

Control seeds were not primed. Data are means of three (flow cytometry and moisture content) or four measurements (T₅₀) ± s.d.

### Table 1. Effects of 3 or 7 d of priming at 25 °C with PEG solutions at −1.0, −1.5 or −2.0 MPa on the seed moisture content (dry weight basis) before redrying, the subsequent germination rate at 15 °C, expressed as the time to obtain 50% germination (T₅₀), the percentages of 2C and 4C nuclei of the radicle tips, and the 4C/2C ratio.
flow-cytometric determinations were made at least in duplicate.

The DNA content of isolated nuclei was measured about 10 min after sample staining, using a flow cytometer (Coulter Corp., Miami, FL, USA, Epics XL-MCL model). Excitation of DNA-bound propidium iodide was performed by a 488 nm Argon Ion Laser, and fluorescence was detected over the range 605–635 nm. MultiCycle for Windows Cell Cycle Analysis software version 3.0 (Phoenix, Flow System Inc., San Diego, CA, USA) was used for curve fitting of fluorescence frequency distributions. The DNA amount is proportional to the fluorescent signal and is expressed as an arbitrary C value in which the 1C value represents the DNA content of the unreplicated haploid chromosome complement.

Results presented are the means of the percentages of nuclei with 2C DNA levels and those with 4C DNA levels.

RESULTS

Effects of priming at various water potentials

Effects on seed germination of priming for 3 and 7 d at 25 °C with PEG solutions at −1.0, −1.5 and −2.0 MPa are presented in Table 1. The moisture content of the seeds increased with higher water potentials. No seeds germinated during the osmotic treatments (data not shown). In all cases, primed seeds germinated faster and the time to obtain 50% germination ($T_{50}$) was decreased compared to that of unprimed seeds. The improvement of germination was higher after 7 d of priming than after 3 d, and a water potential of −1.0 MPa was the most efficient. For this reason, in all the following experiments seeds were primed for 7 d with a PEG solution at −1.0 MPa.

Table 1 also shows the effects of priming on nuclear DNA content of radicle tip cells. In dry unprimed seeds, 90.7% of the nuclei gave 2C signals, indicating that the majority of cells were arrested at the G1 phase of the cell cycle. Priming with PEG at −1.5 and −1.0 MPa resulted in an increase of 4C signals, which was more marked with the highest water potential. However, priming with PEG at −2.0 MPa had no effect on DNA replication. The 4C/2C ratio increased up to about 29 and 45% after 3 and 7 d of priming at

![Fig. 1. Effects of temperature during priming for 7 d with a PEG solution at −1.0 MPa on the subsequent germination rate at 15 °C (expressed as the time to obtain 50% germination: $T_{50}$) (A) and on the percentage of 4C nuclei (○) and the 4C/2C ratio (■) (B). Means of three (flow cytometry) or four measurements ($T_{50}$). Vertical bars denote the largest s.d. Correlation coefficients of the regression lines in B were between 0.90 and 0.98. The values of $T_{50}$, 4C and 4C/2C for control unprimed seeds were 130 ± 10 h, 7.0 ± 2.0% and 7.7 ± 1.8%, respectively (Table 1).](image1)

![Fig. 2. Relationships between the percentages of 4C nuclei (○) or the values of the 4C/2C ratio (■) and the subsequent germination rate at 15 °C, expressed as the reciprocal of time to obtain 50% germination ($T_{50}$). Seeds were primed for 7 d at 10, 15, 20, 25, 30 and 35 °C with a PEG solution at −1.0 MPa. Values correspond to the mean values given in Fig. 1.](image2)
Effects of temperature during priming

The stimulatory effect of priming (7 d of treatment at −1.0 MPa PEG) on germination and on DNA replication depended on the temperature during treatment (Fig. 1). A higher temperature during priming (up to 25–30 °C) reduced $T_{50}$ (Fig. 1A). $T_{50}$ was 19 h when priming was carried out at 25 or 30 °C compared to 130 h for control, unprimed seeds (Table 1). Priming became less efficient at 35 °C.

Figure 1B shows that there was a positive linear relationship between the temperature during priming up to 25 °C and the percentage of 4C nuclei and the 4C/2C ratio, and a negative linear relationship between 25 and 35 °C. Maximum rates of DNA replication were seen at 25 °C; at this temperature the amount of 4C nuclei and the 4C/2C ratio reached about 29 and 45%, respectively, after 7 d of priming at −1.0 MPa. At 10 °C only 16% of the nuclei gave 4C signals and the 4C/2C ratio was only about 20%. At 35 °C the percentage of 4C nuclei (8%) and the 4C/2C ratio (8.5%) remained similar to those measured in unprimed dry seeds (7% and 7.7%, respectively; Table 1).

For temperatures during priming up to 25 °C, a positive correlation existed between the efficiency of the treatment, evaluated by the reciprocal of time to obtain 50% germination ($1/T_{50}$) at 15 °C, and the frequency of 4C nuclei or the 4C/2C values (Fig. 2). Such a correlation did not exist when priming was performed at higher temperatures. Thus, priming at 35 °C had almost no effect on DNA synthesis while it enhanced the subsequent germination at 15 °C (Fig. 1A).

Effects of oxygen tension during priming

Priming for 7 d at 25 °C in the complete absence of oxygen did not enhance subsequent germination (Fig. 3A);
**TABLE 2.** Effects of NaN\textsubscript{3} concentration during priming for 7 d at 25 °C with a PEG solution at −10 MPa on the subsequent germination rate at 15 °C, expressed as the time to obtain 50% germination (T\textsubscript{50}), the percentages of 2C and 4C nuclei of the radicle tips, and the 4C/2C ratio

<table>
<thead>
<tr>
<th>NaN\textsubscript{3} (m)</th>
<th>T\textsubscript{50} (h)</th>
<th>2C (%)</th>
<th>4C (%)</th>
<th>(4C/2C) × 100</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>19±4</td>
<td>63.6±3.8</td>
<td>28.8±1.3</td>
<td>45.3±4.8</td>
</tr>
<tr>
<td>10\textsuperscript{–3}</td>
<td>21±4</td>
<td>61.9±1.7</td>
<td>29.6±1.0</td>
<td>47.8±0.6</td>
</tr>
<tr>
<td>10\textsuperscript{–4}</td>
<td>27±5</td>
<td>71.6±6.3</td>
<td>21.0±5.0</td>
<td>29.3±9.0</td>
</tr>
<tr>
<td>2×10\textsuperscript{–4}</td>
<td>39±6</td>
<td>82.0±3.2</td>
<td>12.7±18</td>
<td>15.5±2.7</td>
</tr>
<tr>
<td>5×10\textsuperscript{–4}</td>
<td>235±15</td>
<td>90.5±2.2</td>
<td>4.5±0.4</td>
<td>5.0±0.6</td>
</tr>
<tr>
<td>10\textsuperscript{–3}</td>
<td>285±20</td>
<td>92.9±2.2</td>
<td>6.4±1.0</td>
<td>6.9±4.7</td>
</tr>
</tbody>
</table>

Data are means of three (flow cytometry) or four measurements (T\textsubscript{50}) ± s.d. Values of T\textsubscript{50}, 4C and 4C/2C for control unprimed seeds were 130±10 h, 70±20% and 77±18%, respectively (Table 1).

The mean value of T\textsubscript{50} (134 h) was similar to that obtained with unprimed seeds (130 h; Table 1). Priming required more than 3–5% oxygen to be effective. Its improving effect increased over the range of oxygen concentrations used in this investigation and was maximal at 21% oxygen. Levels of oxygen between 21% were not included in the study, but may have had similar/enhanced effects on T\textsubscript{50} values.

DNA replication was induced during priming only when the treatment was carried out in atmospheres containing more than 5% oxygen, and it was higher in the air than in 10% oxygen (Fig. 3B). Moreover, a linear relationship was observed between the frequency of 4C nuclei or the 4C/2C ratio and the rate of germination at 15 °C (expressed as 1/T\textsubscript{50}) with seeds primed in various oxygen tensions (Fig. 4).

Similar results were obtained with seeds primed at 15 °C in the same oxygen concentrations (data not shown).

**Fig. 5.** Relationships between the percentages of 4C nuclei (○) or the values of 4C/2C ratio (●) and the subsequent germination rate at 15 °C expressed as the reciprocal of time to obtain 50% germination (1/T\textsubscript{50}). Seeds were primed for 7 d at 25 °C with solutions of PEG at −10 MPa containing 0, 10\textsuperscript{–5}, 10\textsuperscript{–4}, 2×10\textsuperscript{–4}, 5×10\textsuperscript{–4} and 10\textsuperscript{–3} m NaN\textsubscript{3}. Values correspond to the mean values given in Table 2. Correlation coefficients of the regression lines were 0.97 (○) and 0.98 (●).

**Effects of priming in the presence of NaN\textsubscript{3}**

Table 2 shows the results obtained with seeds primed for 7 d at 25 °C in −10 MPa PEG containing NaN\textsubscript{3} in concentrations ranging from 10\textsuperscript{–5} to 10\textsuperscript{–3} m. At concentrations of 10\textsuperscript{–4} and 2×10\textsuperscript{–4} m, NaN\textsubscript{3} negatively affected the stimulatory effect of priming on subsequent germination at 15 °C and also DNA replication. In the presence of high concentrations of NaN\textsubscript{3} (5×10\textsuperscript{–4} and 10\textsuperscript{–3} m), most nuclei of the radicle tips were maintained in the cell cycle with 2C DNA levels and a low frequency with 4C DNA levels (Liu et al., 1997), in contrast to that shown in Fig. 4 concerning the effects of oxygen concentrations during priming.

**DISCUSSION AND CONCLUSION**

As previously shown by Bino et al. (1993) and Lanteri et al. (1994), the majority of the nuclei (90% of the radicle tips of dry mature tomato seeds are arrested in the cell cycle with 2C DNA levels and a low frequency with 4C DNA levels (Table 1). Arrest of some of the nuclei with 4C DNA levels and the rate of germination at 15 °C increased over the range of oxygen concentrations used in this investigation and was maximal at 21% oxygen. Levels of oxygen between 21% were not included in the study, but may have had similar/enhanced effects on T\textsubscript{50} values.

As shown in this study, the arrest of cells in the tomato radicle tip occurs between 30 and 45 d after pollination.

In the present study, with ‘Elko’ tomato seeds, flow
cytometric determination of nuclear DNA content showed that osmopriming resulted in replication of DNA in the radicle tip. The amounts of 4C nuclei reached 22.6 and 28.8% after priming at 25°C and −10 MPa for 3 and 7 d, respectively (Table 1). A comparable induction of DNA replication has also been observed during osmopriming of seeds of other tomato cultivars (‘Agata’ and ‘San Marzano’) and of pepper (Lanteri et al., 1993, 1994) and during presowing imbibition of sugarbeet seeds (Redlearn and Osborne, 1997). In osmoprimed leek seeds, DNA synthesis would result from both DNA mitochondrial synthesis and repair type synthesis (Asraf and Bray, 1993).

Induction of DNA replication during priming depends on the species, the cultivar and the seed batch (Lanteri et al., 1994), and on the conditions of the treatment. Our results showed that the advancement in DNA synthesis was inversely related to the water potential of the osmoticum (Table 1). In particular, the percentage of 4C nuclei remained close to the initial value measured in dry unprimed seeds (7%) during 7 d of priming carried out at 25°C and −20 MPa, i.e. when the seed moisture content was less than about 68% (d. wt basis). Osmotic inhibition of DNA replication has also been observed with pepper seeds, when primed at 20°C and −1.5 MPa (Lanteri et al., 1994). In tomato seeds, the efficiency of priming was strongly influenced by the temperature of the treatment and was optimal at 25–30°C (Fig. 1A). In addition, a positive linear relationship existed between temperature of priming up to 25°C and the 4C signals or the 4C/2C ratio, and a negative linear relationship at higher temperatures (Fig. 1B). Similar relationships were demonstrated between the rate of germination (expressed as the reciprocal of time to obtain 50% germination: 1/T$_{50}$) of unprimed tomato seeds and temperature of germination (Özbingöl et al., 1998); and (2) the rate of germination (also expressed as 1/T$_{50}$) of primed seeds and the temperature of the treatment (Özbingöl et al., 1998).

To affect germination of tomato seeds, the priming treatment required at least 5% oxygen in the atmosphere (Fig. 3A), indicating that respiration was necessary for processes associated with priming (Bray, 1995). Similar oxygen concentrations were also required for DNA replication during priming (Fig. 3B) and for germination of unprimed seeds (Özbingöl et al., 1998). The requirement for energy metabolism during priming was also demonstrated by the treatments with NaN$_3$ (Table 2). Inefficiency of priming in low oxygen tensions or in the presence of high concentrations of NaN$_3$ (5×10$^{-4}$ M and 10$^{-3}$ M) was associated in both cases with a reduced energy charge (data not shown).

As in pepper seeds (Laner et al., 1993, 1994), a close positive relationship was observed between the percentage of 4C nuclei in the radicle tip after priming, or the 4C/2C ratio and the beneficial effect of the osmotic treatment in tomato seeds (Figs 2, 4 and 5). However, there were some limits to this relationship, since osmopriming at too high a temperature (35°C) (Fig. 1) or with a solution of PEG at a low water potential (−20 MPa) (Table 1) improved subsequent germination without generating an increase in 4C signals. These results, which agree with those obtained by Saracco et al. (1995) with pepper seeds primed for 6 d at 20°C with a solution of PEG at −1.5 MPa, show that improvement of seed germinability by priming is not always associated with the onset of DNA replication.

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**Literature Cited**


