

# Treatment conditions and biochemical processes influencing seed priming effectiveness

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## Abstract

A review of the scientific literature indicates osmotic priming (osmopriming) as the principal method of seed priming and polyethylene glycol (PEG) as the principal osmotic agent. An analysis of the available data across experiments carried out with different species under varying conditions showed an average 11% increase in percent germination and 36% shorter mean germination time (MGT) in primed vs unprimed seeds. Moreover, in primed seeds MGT was less dependent on temperature, which is consistent with the effects expected from the treatment. Priming effects are mainly influenced by osmotic potential, temperature and time; major biochemical processes (repair of damaged DNA and RNA, preparation for cell division and increased antioxidant activity) are involved in treatment effects to an extent which is not fully ascertained in literature. A reduction of seed storage life is the major disadvantage of priming and the principal constraint to its diffusion, since dehydration to the initial moisture (drying-back) is needed to allow seed storage. Seed behaviour during drying-back, the role of the raffinose family oligosaccharides in cell membrane integrity and the expression of antioxidant enzymes in germinating seeds need to be further elucidated in a sufficient number of species, to promote a more reliable use of this technique.

## Introduction

Rapid and uniform field emergence is a fundamental requisite for a good crop establishment, especially under adverse environmental conditions (Wurr and Fellows, 1983; Subedi and Ma, 2005; Gupta *et al.*, 2008). The time from sowing to plant establishment is a crucial period in crop growth (Bray, 1995), with a direct impact on final yield and quality (Wurr and Fellows, 1983; Gupta *et al.*, 2008).

Techniques enhancing and stabilizing field emergence are the basis of crop success. Among them, seed priming is a pre-sowing method of improving germination, for the purpose of reducing the time from sowing to emergence, while improving emergence uniformity (Brocklehurst and Dearman, 1983). This technique involves seed hydration (usually within 10-20% of full imbibition) (Pill, 1995), sufficient to permit pre-germinative metabolic events to proceed, but insufficient to allow radicle protrusion (Bradford, 1986). Thus, the term *priming* is used to describe a seed treatment devised to enhance the speed and uniformity of germination (Gupta *et al.*, 2008).

There are three principal methods of seed priming: i) water-only treatments (hydropriming); ii) solid matrices hydrated or soaked with osmotic solutions (solid matrix priming or matri-priming) (McDonald, 2000), such as hydrated sand (Hu *et al.*, 2005), peat and vermiculite (Taylor *et al.*, 1998), or cotton soaked with osmotic solutions; iii) osmotic solutions (osmopriming) with polyethylene glycol (PEG) (Taylor *et al.*, 1998; Foti *et al.*, 2002), or inorganic salts (Alvarado *et al.*, 1987; Haigh and Barlow, 1987; Tiryaki and Buyukcingil, 2009). The three methods may be grouped into two categories: non-controlled water uptake (hydropriming) and controlled water uptake (osmopriming, solid matrix priming and drum priming, the last a variant of hydropriming) (Taylor *et al.*, 1998). Additional methods include seed coating with bacteria (biopriming or bio-osmopriming), e.g. *Trichoderma* spp. (Taylor *et al.*, 1994; Pill *et al.*, 2009; Begum *et al.*, 2010) and *Pseudomonas aureofaciens* (Warren and Bennett, 1999). Biopriming has been able to control damping-off of seedlings in sweet corn (*Zea mays* L.) (Callan *et al.*, 1990), cucumber (*Cucumis melo* L.) (Taylor *et al.*, 1994; Pill *et al.*, 2009), pea (*Pisum sativum* L.) (Taylor *et al.*, 1994) and soybean (*Glycine max* (L.) Merr.). More to these, plant hormones may be combined with osmopriming treatments (Tiryaki and Buyukcingil, 2009).

Despite its potential benefits, seed priming has not achieved widespread circulation, as there are critical points undermining its practical use. Seed sorting is seen an interesting alternative to improve seed vigour while overcoming priming constraints (Dell'Aquila, 2009). However, the interest in seed sorting within the scientific community may not be shared by seed producers, as this technique implies selecting and discarding significant amounts of seed.

Therefore, this review is aimed at setting the state of the art on priming, focusing on treatment factors, ambient conditions and biochemical processes, to identify which aspects deserve a deeper insight in future research.

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## Priming effects on seeds and seedlings

The results obtained with seed priming are heterogeneous in literature, according to species, seed quality, procedure parameters, germination conditions, *etc.* To ascertain the effects observed in a variety of species, we have carried out a survey of the literature since the 1980's: data of percent germination (35 cases on 18 species) and/or mean germination time (MGT; 34 cases on 18 species) with and without seed priming are reported in several scientific papers (Table 1). Osmopriming with PEG was the most frequent priming method, inducing an osmotic potential varying between -0.5 MPa (sweet corn, Ghiyasi *et al.*, 2008) and -2 MPa (sunflower, Bailly *et al.*, 2000; sugar beet, Capron *et al.*, 2000). Treatment duration widely varied according to species and experiment, from a minimum of 8 h (sunflower primed in a salt solution, Wahid *et al.*, 2008) to a maximum of 14 days (four ornamental species, Finch-Savage *et al.*, 1991). Treatment temperature varied between 15°C (several cases) and 30°C (rice in hydropriming, Basra *et al.*, 2005; *Rudbeckia fulgida*, Fay *et al.*, 1994); most cases are comprised in the range 15-20°C. The temperatures of seed germination also varied depending on species and aim of each experiment: sub-optimal temperatures were often adopted to better test seed response to priming.

In the 35 surveyed cases, an average germination of  $71.7 \pm 23.4\%$  and  $80.3 \pm 18.2\%$  was observed in unprimed and primed seed, respectively. Despite a difference of almost 10%, data dispersion was too wide as to indicate a statistical difference between the two averages (*t*-test). If only the gramineous species are taken into account (17 cases out of 35), the same pattern repeats:  $78.8 \pm 21.9\%$  and  $87.5 \pm 11.5\%$  germination in unprimed and primed seed, respectively. In both complete and reduced data set, priming restrained the variation of the cases below the average, as the lower standard deviations indicate. This in turn determined slightly higher averages.

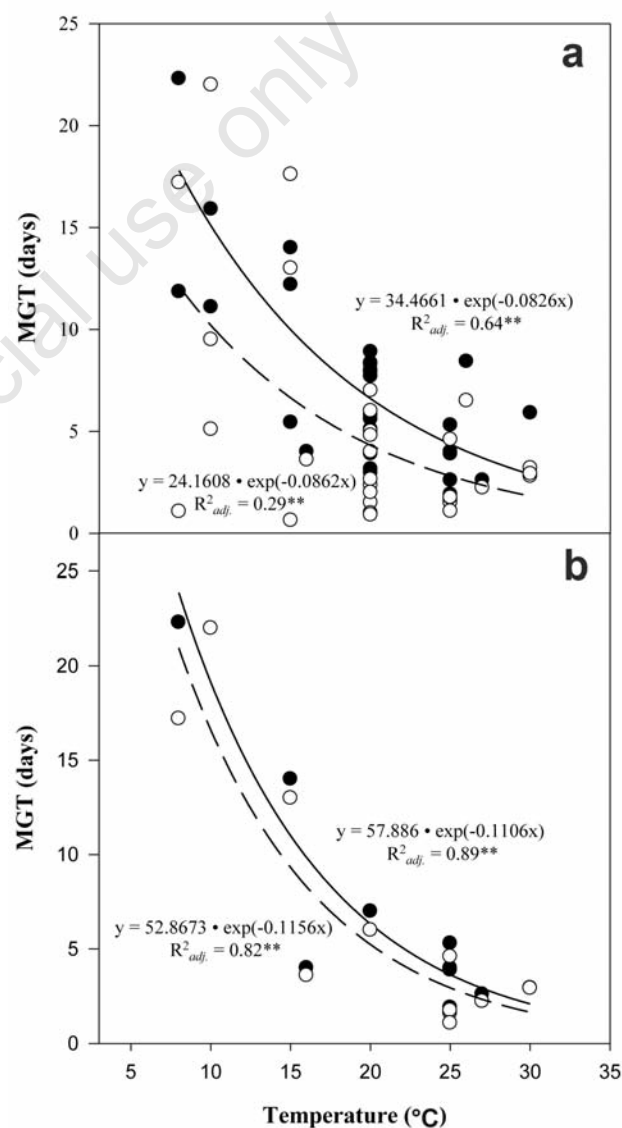
A direct effect of temperature was not observed on percent germination of both unprimed and primed seed, which is consistent with the high inter-specific variation in thermal requirements among tested plants in the complete data set (35 cases). Conversely, a trend may be observed in the gramineous species (17 cases): in this group, the linear relation between temperature and percent germination exhibits a steeper slope in unprimed than in primed seed ( $b=2.1$  and  $1.1$  in the two respective cases;  $R^2=0.51^{**}$  in both). This indicates a lower dependence of primed seed on temperature to achieve a good germination, which is a proof of treatment effectiveness despite the heterogeneity among the surveyed species and priming conditions.

In the same survey (Table 1), the effect of priming on MGT appears stronger than on percent germination. MGT tended to decrease under priming, pointing to a faster germination. The high variation observed in the complete data set (average MGT= $7.6 \pm 5.0$  days in unprimed seed *vs*  $4.9 \pm 5.2$  days in primed seed) is consistent with the high inter-specific variation among cases. The same MGT pattern repeats when monitoring only the gramineous species (*data not shown*).

MGT was significantly influenced by germination temperature: exponential decay functions describing MGT response to increasing temperature fit both unprimed and primed seed behaviour (Figure 1a), although in the latter case the regression was quite weaker ( $R^2_{adj.}=0.29$  *vs*  $0.64$ ). This indicates a lower dependence of MGT of primed seed on temperature, which is consistent with the effects expected from the treatment. Likewise, the two curves tend to converge at high temperatures, indicating a lower effectiveness of priming when germination conditions improve. If only the gramineous species are monitored (12 cases out of 34), the two curves get closer and their regressions get tighter ( $R^2_{adj.}=0.89$  and  $0.81$  in unprimed and primed seed, respectively) (Figure 1b). It is perceived, therefore, that MGT is the most relevant trait in seed germination to be influenced by priming.

## Triphasic model of seed imbibition

In orthodox seeds the dry seed, ready for germination, exhibits a triphasic pattern of water uptake (Figure 2) (Bewley and Black, 1978). Phase I is the rapid water uptake that is largely a consequence of the matric forces exerted by the seed. During this phase, DNA and mitochondria are repaired and proteins are synthesized using existing messenger ribonucleic acid (mRNA) (McDonald, 2000). Phase II is a lag phase, in which seed water potential is in balance with that of the environment. In this phase the major metabolic changes preparing the embryo for germination occur, including the synthesis of mitochondria and proteins by new mRNA. Thus, phase II is also called activation phase. In phase II the germination process is completed *stricto sensu*; however, only in phase III the radicle emerges and the so called *visible*



**Figure 1.** Relationships between temperature and mean germination time (MGT) in all the experiments reported in Table 1 (34 cases) (a), or only in the gramineous species (12 cases) (b), in unprimed (closed symbols) and primed (open symbols) seed. Exponential decay curve, unprimed (solid line) and primed (dashed line) seeds.

**Table 1. Experiments on seed priming carried out in a series of crop species, reporting data of percent germination and mean germination time.**

Crop	Priming conditions	Traits		Notes	References
		Germination	MGT		
Sweet corn ( <i>Z. mays</i> L.)	PEG 6000 -1.02 MPa 25°C x 24 h	x	x	4 germination temperatures (10, 15, 20 and 25°C)	Sung and Chang, 1993
	KH <sub>2</sub> PO <sub>4</sub> -0.5 MPa 25°C x 24 h	x	x		Ghiyasi <i>et al.</i> , 2008
	KNO <sub>3</sub> -0.5 MPa 25 °C x 24 h	x	x		Ghiyasi <i>et al.</i> , 2008
	PEG 8000 -0.5 MPa 25 °C x 24 h	x	x		Ghiyasi <i>et al.</i> , 2008
Sorghum [ <i>S. bicolor</i> (L.) Moench]	PEG 6000- 0.86 MPa 15°C x 48 h	x	x	3 germination temperatures (8, 16 and 25°C)	Foti <i>et al.</i> , 2002
Tomato ( <i>S. lycopersicum</i> L.)	PEG 6000 -0.7 MPa 20°C x 6 d	x	x		Mauromicale and Cavallaro, 1995
	KNO <sub>3</sub> +K <sub>2</sub> PO <sub>4</sub> -1.1 MPa 20°C x 6 d	x	x		Mauromicale and Cavallaro, 1995
Wheat ( <i>T. aestivum</i> L.)	PEG 8000 -0.9 MPa 25°C x 12 h	x	x		Salehzade, 2009
Rice ( <i>O. sativa</i> L.)	Hydropriming 30°C x 24 h	x	x		Basra <i>et al.</i> , 2005
	Sand priming 3.8% (v/w)	x	x		Hu <i>et al.</i> , 2005
Leek ( <i>A. porrum</i> L.)	PEG 6000 -1.0 MPa		x	2 germination temperatures (8 and 15°C)	Bray <i>et al.</i> , 1989
	PEG 6000 -1.5 MPa at 15°C		x		means of 3 priming durations (7, 10 and 14 days)
Celery ( <i>A. graveolens</i> L.)	PEG 6000 -1.5 MPa at 15°C		x	means of 3 priming durations (7, 10 and 14 days)	Gray <i>et al.</i> , 1991
Onion ( <i>A. cepa</i> L.)	PEG 6000 -1.5 MPa at 15°C		x	means of 3 priming durations (7, 10 and 14 days)	Gray <i>et al.</i> , 1991
Carrot ( <i>D. carota</i> L.)	PEG 6000 -1.5 MPa at 15°C		x	means of 3 priming durations (7, 10 and 14 days)	Gray <i>et al.</i> , 1991
	PEG 6000 -0.59 MPa at 20°C x 7 d		x		2 germination temperatures (10 and 20°C)
Sunflower ( <i>H. annuus</i> L.)	PEG 8000 -2.0 MPa 15°C x 7 d		x		Bailly <i>et al.</i> , 2000
	NaCl (1000 mg l <sup>-1</sup> ) for 8 h	x	x		Wahid <i>et al.</i> , 2008
Parsley ( <i>P. crispum</i> Mill.)	PEG 6000 -0.59 MPa for 7 d		x	2 germination temperatures (10 and 20°C)	Biniek, 1993
Surgabeet ( <i>B. vulgaris</i> L.)	PEG 8000 -2.0 MPa 25°C x 14 d	x			Capron <i>et al.</i> , 2000
Perennial regrass ( <i>L. perenne</i> L.)	PEG 8000 -1.1 MPa for 48 h	x		3 germination temperatures (5, 10 and 15°C); data after 7 days of germination	Danneberger <i>et al.</i> , 1992
Kentucky bluegrass ( <i>P. pratensis</i> L.)	PEG 8000 -1.5 MPa 20°C x 4 d	x			Pill and Korengel, 1997
	KNO <sub>3</sub> -1.5 MPa 20°C x 4 d	x			Pill and Korengel, 1997
Tickseed ( <i>C. lanceolata</i> L.)	K <sub>3</sub> PO <sub>4</sub> [50 mM] 16°C x 3 or 6 d	x		3 germination temperatures (15, 20 and 25°C); data after 12 from sowing	Samfield <i>et al.</i> , 1991
<i>Primula acaulis</i> (L.)	PEG 6000 -1.5 MPa 15°C x 10 d	x	x		Finch-Savage <i>et al.</i> , 1991
<i>Impatiens wallerana</i> Hook.	PEG 6000 -0.75 MPa 15°C x 14 d	x	x		Finch-Savage <i>et al.</i> , 1991
<i>Verbena x hybrida</i>	PEG 6000 -1.5 MPa 15°C x 14 d	x	x		Finch-Savage <i>et al.</i> , 1991
<i>Salvia splendens</i>	PEG 6000 -1.5 MPa 15°C x 14 d	x	x		Finch-Savage <i>et al.</i> , 1991
<i>Petunia x hybrida</i>	PEG 6000 -1.0 MPa 15 °C x 14 d	x	x		Finch-Savage <i>et al.</i> , 1991
<i>Viola x wittrockiana</i>	PEG 8000 -1.0 MPa 15°C x 7 d	x		3 germination temperatures (10, 15 and 25°C)	Carpenter and Boucher, 1991
<i>Rudbeckia fulgida</i> Ait.	PEG 8000 -0.5 MPa 30°C x 7 d	x	x		Fay <i>et al.</i> , 1994
	KNO <sub>3</sub> -1.0 MPA 30°C x 7 d	x	x		Fay <i>et al.</i> , 1994

MGT, mean germination time.

germination can be assessed (Bewley and Black, 1978; Bradford, 1995). In phase III, a second rapid uptake of water occurs. Phase I and II represent the most delicate phases for the process of germination and are crucial for a successful seed priming (Bewley, 1997).

The triphasic model has deep implications for seed viability. The seed tolerates a return to the initial moisture necessary for storage, a process known as drying-back or re-drying, when it is in phase I or II, whereas phase III is too advanced to allow a drying-back without seed damage (Taylor *et al.*, 1998). According to the triphasic model, the start of germination is associated with a rapid synthesis of RNA and proteins, to carry out the repairing processes before the beginning of DNA replication (Osborne, 1983). Seed priming typically involves an extension of phase II, which in turn permits the completion of more repair processes (Bray, 1995), and allows the drying-back, which is necessary when the final sowing is postponed (industrial seed production). Differences in the imbibition phases of a normal germination process compared to priming followed by drying-back and subsequent germination are shown in Figure 3 (Bradford and Bewley, 2002). Under favourable conditions, the postponement of phase III involved by priming plus re-drying results in a better seed performance.

## Priming methods

### Hydropriming

In this method, seeds are submerged in water with or without aeration. Therefore, water is freely available to seeds, its uptake only being governed by the affinity of the seed tissue for water (Taylor *et al.*, 1998). As a main drawback, seed germination can proceed until radicle protrusion. Thus, the process needs to be stopped at a precise moment, before phase III begins. Another disadvantage is that seeds are not equally hydrated, which results in a non-uniform activation of the physiological processes necessary to synchronize and improve germination (McDonald, 2000). A variant of hydropriming is drum priming, where seed water uptake can be regulated, overcoming the limits of hydropriming. In drum priming the seed is slowly spun in a rotating drum in which nebulized water is injected. The drum is linked to an electronic scale continuously monitoring the weight, thus the level of hydration. The process is stopped when the level of hydration set for a specific seed lot is reached (Rowse, 1996; Warren and Bennett, 1997). Drum priming consists of four stages: i) calibration necessary to determine the desired level of hydration of the seed; ii) hydration, consisting in the addition of water at different times; iii) incubation, where the seed keeps the level of hydration acquired during the priming process; iv) drying-back, where the hydrated seed is brought back to the pre-treatment moisture (Rowse, 1996). This method allows the treatment of large quantities of seed, but the most difficult step is the calibration, which is necessary to determine the right amount of water required to hydrate the seed.

### Solid matrix priming

Solid matrix priming (SMP) involves the use of a wet organic or inorganic material (Parera and Cantliffe 1994), which simulates the natural imbibition processes taking place in the soil (McDonald, 2000). The substrate must possess given characteristics: low matric potential; high seed safety; high specific surface (*i.e.*, high surface to volume ratio); negligible water solubility; high adhesiveness to seed surface; high capacity to retain water (Khan, 1991). The materials used include peat or vermiculite, or some commercial substrates such as Celite® or Micro-cel®. The seed is placed on or mixed with the hydrating substrate which gradually moisturizes the seed (McDonald, 2000). In order to improve the control of imbibition, pure water may be replaced by an osmotic solution, as in osmotic priming (Khan, 1991).

## Osmotic priming

Osmotic priming (osmopriming) is the process that involves the use of osmotic solutions with a low water potential to control seed water uptake. The most common substances used for osmopriming are inorganic salts, polyethylene glycol (PEG), mannitol and glycerol. The following salts are commonly used: NaCl, NaNO<sub>3</sub>, MnSO<sub>4</sub>, MgCl<sub>2</sub>, K<sub>3</sub>PO<sub>4</sub> and KNO<sub>3</sub> (Alvarado *et al.*, 1987; Haigh and Barlow, 1987; Tiryaki and Buyukcingil, 2009). Some salt solution may also exert direct/indirect

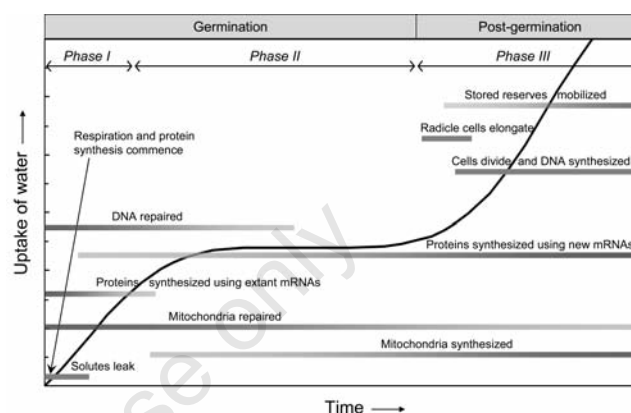


Figure 2. Events associated with germination and post-germination phases. The time for events to be completed varies from several hours to many weeks, depending on plant species, germinating conditions and seed lot quality. From: Bewley (1997), *Seed germination and dormancy*. Plant Cell. 9:1055-1066; www.plant-cell.org. Copyright American Society of Plant Biologists.

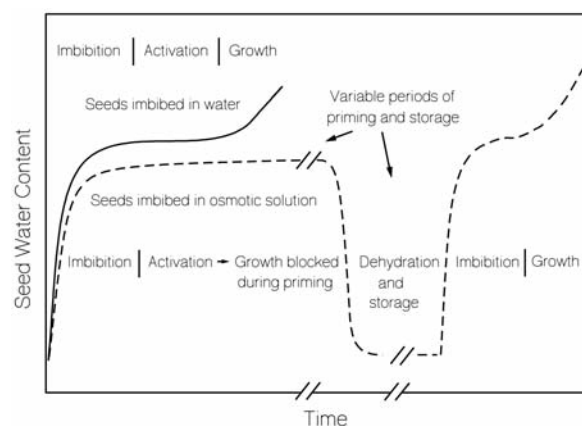


Figure 3. Time course of a standard germination process compared to a germination process including seed priming, dehydration and storage. Seed soaking in water determines a normal course of imbibition, activation and radicle protrusion (phase I, II and III of the germination process, respectively). Priming with an osmotic solution extends the activation phase (phase II) for a certain time, without leading to radicle protrusion (phase III). Seed dehydration after priming (drying-back) allows to store the seed for a variable time without losing the advantages achieved with priming. At sowing, the seed must be able to soak and develop the radicle. From: Bradford and Bewley (2002), *Seeds: Biology, Technology and Role in Agriculture*. In: M.J. Chrispeels and D.E. Sadava (Eds.) *Plants, Genes and Crop Biotechnology*, second edition. Copyright 2003, Jones & Barlett Learning, Sudbury, MA, USA (www.jblearning.com). Reprinted with permission.

nutritional effects, described a long time ago: Dastur and Mone (1958) observed that nitrogen, potassium and phosphate concentration in embryos of cotton seed did not change after osmopriming with such salts, whereas the concentration of some micronutrients (manganese and copper) increased. The accumulation of salts in the seed could determine toxicity (Bradford, 1995), reduce the osmotic potential and induce a high water absorption during treatment (Parera and Cantliffe, 1994), resulting in a more likely radicle protrusion.

PEG as an inert material can prevent embryo toxicity problems during priming (Cantliffe, 1983). The large size of PEG molecule (6000 to 8000 mw) also prevents its penetration into seed tissues, avoiding to lower the osmotic potential (Michel and Kaufman 1973; Brocklehurst and Dearman, 1984). The major disadvantage resulting from the use of PEG is the reduction of oxygen in the solution, because of its viscosity (Mexal *et al.*, 1975); aerating the solution during PEG osmopriming can overcome this problem (Akers, 1990; Bujalski and Nienow, 1991).

Inorganic salts determine effects on germination which are different from those carried out by PEG, depending on seed species. For instance, osmopriming with inorganic salts resulted toxic to sorghum seeds (Haigh and Barlow, 1987), whereas it was as effective as PEG in asparagus (Pill, 1995) and performed better than PEG in tomato (Alvarado and Bradford, 1988; Mauromicale and Cavallaro, 1997; Ellis, 1963). The difference in the response of different species to salts or PEG may be due to a selective semi-permeable layer that surrounds the embryo: when this layer is present, it allows the absorption of water, but prevents salt diffusion; when it is absent, ions can be absorbed and cause embryo damages (Welbaum *et al.*, 1998). For example, tomato (*Solanum lycopersicum* L.), melon (*Cucumis melo* L.), lettuce (*Lactuca sativa* L.) and *Capsicum annuum* seeds possess this layer and may be safely subjected to osmopriming with inorganic salts (Welbaum and Bradford, 1990; Taylor *et al.*, 1997). Conversely, these treatments are harmful to broccoli and cabbage seeds (*Brassica oleracea* L.), which lack this layer (Taylor *et al.*, 1997).

Priming with osmotic solution involves a control of water uptake, through the adjustment of the osmotic potential ( $\psi$ ) (Taylor *et al.*, 1998). The  $\psi$  of an osmotic solution with inorganic salts can be calculated according to the van't Hoff equation (Salisbury and Ross, 1985):

$$\psi = -imRT \quad \text{Eq. 1}$$

where  $i$  is the van't Hoff coefficient (adimensional), taking into account the number of molecules or ions in the solution;  $m$  is the molality;  $im$  is the osmolality, *i.e.* the number of osmoles of solute per kg of solvent;  $R$  is gas constant and  $T$  is absolute temperature ( $^{\circ}\text{K}$ ).

In a PEG 6000 solution,  $\psi$  (expressed in MPa) can be calculated through the Michel and Kaufmann equation (1973):

$$\psi = -(1.18 \times 10^{-2})C - (1.18 \times 10^{-4})C^2 + (2.67 \times 10^{-4})CT + (8.39 \times 10^{-7})C^2T * 10^{-1} \quad \text{(Eq. 2)}$$

where  $C$  is the PEG concentration in  $\text{g kg}^{-1}$  of  $\text{H}_2\text{O}$  and  $T$  is the temperature in  $^{\circ}\text{C}$ . Temperature is a important factor affecting  $\psi$ . For example, a PEG 6000 solution in a  $250 \text{ g L}^{-1}$  concentration, at  $15^{\circ}\text{C}$  has an osmotic potential of  $-0.85 \text{ MPa}$ ; at  $5^{\circ}\text{C}$ ,  $-0.97 \text{ MPa}$ ; at  $25^{\circ}\text{C}$ ,  $-0.73 \text{ MPa}$ . Therefore, at low temperature an osmotic solution becomes stronger than at high temperature, as  $\psi$  exerts a progressively higher retention (*i.e.*, a more negative potential).

The  $\psi$  of dry orthodox seeds is very low (between  $-350$  and  $-50 \text{ MPa}$ ) (Roberts and Ellis, 1989) and water uptake gradient is high when the environmental  $\psi$  is between and  $0$  and  $-2 \text{ MPa}$  (Bradford, 1995). Reducing the environmental  $\psi$  slows water uptake and extends the duration of phase II, which is the aim of seed priming (Bray, 1995) (Figure 4). Tarquis and Bradford (1992) showed that the duration of phase I was similar in lettuce seed imbibed in water or in a PEG 8000

( $-1.5 \text{ MPa}$ ) solution. Conversely, the duration of the lag phase was longer in the PEG solution and seed moisture grew more slowly in time. Finally, PEG-imbibed seeds did not reach phase III, in contrast to water-imbibed seeds.

Seed priming is influenced by many factors (aeration, light, temperature, time and seed quality), which are often tested in specific experiments. Aeration, especially in a PEG solution, is considered important to assist seed respiration (Bujalski *et al.*, 1989; Bujalski and Nienow, 1991), which is essential for seed viability and contributes to synchronize the emergence (Heydecker *et al.*, 1975). The effect of aeration, however, varies according to species: in onion, aeration of the PEG solution increased the germination percentage, compared to non-aerated treatment (Heydecker and Coolbear, 1977; Bujalski *et al.*, 1989). By contrast, no difference was observed in the germination of lettuce between aerated and non-aerated  $\text{K}_3\text{PO}_4$  priming (Cantliffe, 1981). In most of the reviewed cases, aerated priming is preferred, as it ensures a safer seed habitat. In small-scale experiments, the aeration is often provided by an aquarium pump (Akers and Holley, 1986).

Concerning light, the best results with lettuce were obtained with priming in the dark (Cantliffe *et al.*, 1981), but photoblastic seeds such as lettuce and celery (*Apium graveolens* L.), needing light to germinate, may be illuminated during priming to reduce dormancy (Khan *et al.*, 1978).

Temperature is another important variable, as it affects the speed of chemical reactions and  $\psi$  value, according to Eq. 1 and Eq. 2. Temperatures of about  $15^{\circ}\text{C}$  during priming were shown to improve the overall seed performance in most species (Bradford, 1986), whereas lower temperatures slowed the germination processes, requiring longer times to achieve the same results (McDonald, 2000). The range of temperatures normally used in priming varies between  $15$  and  $20^{\circ}\text{C}$ . Treatment duration in priming mainly depends on the type of osmotic solution, osmotic potential, temperature and crop species. It also depends on the specific time and likelihood of radicle protrusion: a long priming can more easily lead to this occurrence, creating irreversible damage during drying-back (Parera and Cantliffe, 1994).

Seed quality is a key aspect influencing the effects of priming. A vigorous seed, free from pathogens is an essential requisite for a good priming result (Cantliffe *et al.*, 1987), in contrast to the belief that this technique may improve the performance of seeds of intrinsic modest quality. Other seed characteristics may influence priming effects. For

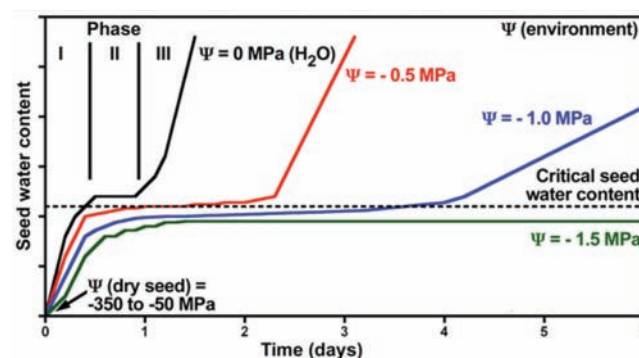


Figure 4. Influence of varying osmotic potential ( $\psi$ ) of a solution, on the duration of phase II (activation phase) in seed germination. By reducing  $\psi$ , phase II is extended and the occurrence of phase III is delayed. Copyright 2006, G. Leubner - The Seed Biology Place ([www.seedbiology.de](http://www.seedbiology.de)).

instance, osmopriming with PEG solution is not suitable for seed treatment of sorghum with high content of tannin, because tannins can be removed with the solution treatment and determine a reduction of germination (Patanè *et al.*, 2008). In fact, tannins reduce seed susceptibility to insects, birds and mould diseases and protect from weathering (McMillan *et al.*, 1972; Harris and Burns 1973; Beta *et al.*, 1999). In this specific case, it is advisable to adopt treatment solutions different from PEG or others technique, such as bio-priming (Patanè *et al.*, 2008).

Another key aspect is seed maturity. Melon seeds harvested 40 days after anthesis (daa) were more responsive to osmopriming (KNO<sub>3</sub>) than seeds harvested 60 daa (Welbaum and Bradford, 1991a). Since seeds were primed just after being harvested, it may be speculated that the physiological processes activated by the treatment contributed to complete the maturation in those seeds that had been harvested still unripe (at 40 daa). These results suggest that priming could be beneficial to standardize lots of seeds harvested at a non-uniform level of maturity. In fact, priming also enhances the vigour of immature broccoli seeds (Jett and Welbaum, 1996). Moreover, since priming melon seeds harvested at 40 daa had the same effect as after-ripening in dry storage, the treatment appeared to reduce the post-ripening dormancy (Welbaum and Bradford, 1991b).

### Biochemical changes induced by priming

The positive effects of priming on the germination performance of many species are attributed to the induction of biochemical mechanisms of cell repair: the resumption of metabolic activity can restore cellular integrity, through the synthesis of nucleic acids (DNA and RNA), proteins (Bewley and Black, 1994) and the improvement of the antioxidant defence system.

### Effects on DNA

Assuring DNA integrity is a factor of key importance to avoid errors of replication and synthesis of DNA. A strong increase in DNA synthesis only occurs at the end of germination (phase III) in both primed and unprimed seed, as shown in wheat seed (Dell'Aquila and Taranto, 1986). However, subsequent studies showed some effects of priming on DNA synthesis also in phase II. Leek (*Allium porrum* (L.) J. Gay) seed exhibited a small increase of DNA content in the embryo during this phase, because of plastid- and mitochondrial-DNA replication for the repairing processes (Osborne, 1983). Thereafter, an increase in DNA was observed 14 days after the treatment in both primed and unprimed treatment, when the seed had entered the irreversible germination phase (Bray, 1995).

It also appears that the pre-replication repair of damaged DNA favours DNA synthesis (Varier *et al.*, 2010). A further proof of the positive effects of priming on DNA is offered by a study on *Brassica oleracea* L., where an aerated hydration determined an advance in DNA synthesis (Thornton *et al.*, 1993).

In spite of its crucial role, the amount of DNA which is needed in the repair processes is only 20-30% of the total DNA synthesized during priming. The rest is mainly represented by mitochondrial DNA; in fact, the number of mitochondria was shown to rapidly increase during priming in leek seeds (Ashraf and Bray, 1993).

In addition to DNA synthesis for the repair processes and new plastids and mitochondria, in several species such as tomato (Lantieri *et al.*, 1994; Ozbingol *et al.*, 1999), pepper (*Capsicum annum* L.) (Lantieri *et al.*, 1993, 1994) and sugar beet (*Beta vulgaris* L.) (Redfean and Osborne, 1997), a DNA synthesis for cell division was also

observed. This synthesis involves the DNA fraction (4C DNA) active in the phases of synthesis (S) and preparation for cell division (G<sub>2</sub>) within the cell cycle.

The enhancement of DNA replication during priming depends on species, cultivar, seed lot quality (Lantieri *et al.*, 1994) and treatment conditions (Ozbingol *et al.*, 1999). Priming *per se* has no direct effect on cell division, but advances its beginning (G<sub>1</sub> and G<sub>2</sub> phase of mitosis) from phase III to phase II of seed imbibition (Ozbingol *et al.*, 1999). This advance is enabled by an accumulation of  $\beta$ -tubulins in primed seed, which are proteins involved in maintaining the cell cytoskeleton and forming the microtubules necessary to cell division (De Castro *et al.*, 1995, 2000). The accumulation of tubulins is associated with the synchronization of cells on the G<sub>2</sub> phase; in the subsequent phase III, cell division takes simultaneously place in all cells.

On the other hand, tubulins are sensitive to re-hydration, so they are not so tolerant to drying-back (Bartolo and Carter, 1991). Such circumstance may explain the drawbacks found in this procedure. This assumption is corroborated by the findings of De Castro *et al.* (2000), who showed that drying-back in primed tomato seed was responsible for the depolarization of the  $\beta$ -tubulins formed during the treatment, disabling their functions.

### Effects on RNA

Osmopriming increases the RNA content in the embryo and reserve tissues of leek (Bray *et al.*, 1989), tomato (Coolbear and Grierson, 1979) and lettuce (Khan *et al.*, 1980). Bray *et al.* (1989) proved that this accumulation involved rRNA (ribosomal RNA; 85% of total RNA), in a turnover between degradation of damaged rRNA and synthesis of new rRNA, while the level of mRNA (messenger RNA; 0.5% of total RNA) remained constant. rRNA is as much necessary to repair cell damages as DNA. Priming allows the recovery of rRNA integrity (Coolbear *et al.*, 1990), in turn ensuring a correct coding of amino acids for the synthesis of proteins during seed germination.

### Effects on protein synthesis

In protein synthesis, a compensation mechanism has been elucidated. Protein synthesis, an essential requisite for germination, starts several minutes after hydration (Cheung *et al.*, 1979). At this stage, osmopriming depresses protein synthesis in the embryo and reserve tissues, compared to seed soaked in water for the same time. Then, priming induces a higher level in the subsequent germination. In fact, Bray (1995) showed that the amount of synthesized protein observed 2 d after germination in primed leek seed was the same as that observed 4 d after germination in unprimed seed. Chen *et al.* (2011) observed an increase of dehydrin in spinach (*Spinacia oleracea* L.) during osmopriming. This increase was also observed during germination of primed seeds in chilling stress and desiccation stress, suggesting that osmopriming may play a positive role in the tolerance to these stresses.

On the other hand, priming does not appear to induce the synthesis of specific proteins, as it has been demonstrated by the qualitative analysis of protein pattern in pea seeds (Dell'Aquila and Bewley, 1989).

### Effects on enzymes

Osmopriming induces the synthesis and activation of enzymes catalyzing the breakdown and mobilization of reserve substances (Varier

*et al.*, 2010). This occurrence has been observed in several species. In sugar beet, for instance, the degradation product of the reserve globulin 11-S was shown to cumulate after osmopriming (Job *et al.*, 1999). Other enzymes are activated for the mobilization of reserve carbohydrates ( $\alpha$  and  $\beta$  amylases) and lipids (isocitrate liase) (Sung and Chang, 1993). This effect is associated with the water deficit induced by osmopriming, which is supposed to determine a mobilization of reserve proteins (Varier *et al.*, 2010).

Seed deterioration during storage is another domain of enzyme activity related to priming. This deterioration is associated with the accumulation of active oxygen species (AOS), such as oxygen peroxide ( $H_2O_2$ ), superoxide anion ( $O_2^{\bullet-}$ ) and hydroxyl radical ( $OH^{\bullet}$ ). AOS react with most organic molecules, causing oxidation and carbonylation of amino acid residues and DNA mutation (Bailly, 2004); they also react with polyunsaturated fatty acids found in cell membranes, leading to lipid peroxidation and consequent disruption of membrane integrity (Bailly *et al.*, 1998). In plants, the defence system includes antioxidant enzymes, *i.e.* scavengers of AOS such as superoxide dismutase (SOD), catalase (CAT) and glutathione reductase (GR) (Bailly *et al.*, 1998). Priming appears to strengthen this defence system: in fact, the treatment was associated with an increase in CAT expression in *Arabidopsis* (Gallardo *et al.*, 2001) and sunflower (*Helianthus annuus* L.) (Kibinza *et al.*, 2011), and CAT activity in soybean (Posmyk *et al.*, 2001) and maize seeds (Chiu *et al.*, 2002). In sunflower seeds, Bailly *et al.* (1998; 2000) showed that osmopriming with PEG led to an increase of SOD and CAT, in response to the rise of metabolic activity during priming, which is responsible for a secondary production of AOS from mitochondrial respiration and/or lipid peroxidation. It appears, therefore, that the defence system of the antioxidant enzymes is enhanced in response to a higher amount of potential threats.

Examples are offered in literature (Table 2), showing a relevant decrease of malondialdehyde (MDA), a product of lipid peroxidation: -20% in echinacea (*Echinacea purpurea* L.) (Chiu *et al.*, 2006), and -40% in spinach (Chen and Arora, 2011). In addition to reduced MDA, also SOD, CAT and GR concentrations in echinacea were increased by priming (Table 2), in agreement with Bailly *et al.* (1998 and 2000). In contrast to this, spinach SOD and CAT declined (Table 2), although this species possesses alternative antioxidant mechanisms, such as the Asa-GSH cycle, an important AOS-scavenging pathway (Bailly *et al.*, 2001; De Tullio and Arrigoni, 2003; Garnczarska and Wojtyła, 2008).

It may be concluded from all these clues that the physiological processes activated by priming, involving nucleic acids, proteins and enzymes, are devised to withstand multiple stresses and still result in a vigorous germination, thus reducing the time to emergence and promoting early plant development.

## Drying-back and seed longevity

Priming is connected with practical interest in subsequent seed handling and storage. Direct seeding of primed seed is often unfeasible (*e.g.*, wet seeds bridging within seeders), risky (*e.g.*, rains delaying the seeding date), or unaffordable at a small farm scale. Therefore, drying-back is necessary to allow seed storage after priming and represents a crucial phase, as the benefits achieved with priming may be lost (Parera and Cantliffe, 1992). The effects of priming on seed longevity appear somewhat contradictory: the treatment was shown to enhance seed longevity in pepper (Georghiou *et al.*, 1987), onion (*Allium cepa* L.) (Dearman *et al.*, 1986) and Brussels sprouts (Burgass and Powell, 1984), whereas it was responsible for a depression of longevity in seeds of leek (*A. porrum* L.) (Bray, 1995), carrot (*Daucus carota* L.) (Dearman *et al.*, 1987), wheat (*Triticum aestivum* L.) (Nath *et al.*, 1991), lettuce (Kraak and Weges, 1989; Tarquis and Bradford, 1992) and tomato (Alvarado and Bradford, 1988; Argerich and Bradford, 1989; Owen and Pill, 1994).

However, these contrasting effects appear to depend on the conditions of drying-back and storage. A rapid drying-back may alter the content of soluble carbohydrates, in turn reducing the tolerance to desiccation and seed longevity (Gurusinghe and Bradford, 2001). Conversely, a slow drying-back may improve seed longevity after priming (Bruggink *et al.*, 1999). Therefore, the two aspects of tolerance to desiccation and seed longevity are clearly related (Ellis and Hong, 1994; Hay and Probert, 1995).

Sugars and their derivatives play an important role in desiccation tolerance and seed longevity (Horbowicz and Obendorf, 1994; Brenac *et al.*, 1997; Obendorf, 1997; Obendorf *et al.*, 1998), since they are, supposedly, involved in maintaining membrane integrity (Crowe *et al.*, 1988; Hoekstra *et al.*, 1992; Oliver *et al.*, 1998) and three-dimensional structure of proteins (Crowe *et al.*, 1992; Wolkers *et al.*, 1998) during drying-back. The sugars playing this role are sucrose and some raffinose family oligosaccharides (RFOs), namely raffinose, stachyose and verbascose (Obendorf, 1997). These sugars interact with the lipids and proteins of cell membrane forming glycolipids and glycoproteins, respectively. Furthermore, they form a glassy layer at the membrane level (Leprince *et al.*, 1993), countering degradation during desiccation and storage (Gurusinghe and Bradford, 2001).

During hydration, RFOs are the first sugars to be metabolized. Their consumption during priming and a lack of accumulation during drying-back, are responsible for a reduced formation of the glassy layer, resulting in accelerated deterioration (Gurusinghe and Bradford, 2001). In support to this hypothesis, Gurusinghe and Bradford (2001) found a decreasing sucrose and raffinose concentration with increasing hydropriming duration in lettuce seed. More to this, in the same work

**Table 2. Effects of priming on cell antioxidant activity in terms of malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT) and glutathione reductase (GR) in two experiments reported in literature.**

Species	Treatment	MDA nmol g <sup>-1</sup>	SOD Unit <sup>o</sup>	CAT Unit <sup>#</sup>	GR Unit <sup>#</sup>	References
<i>Echinacea purpurea</i> L.	Control	17.0*	13.3*	11.8*	5.1*	Chiu et al., 2006
	Primed <sup>§</sup>	13.4*	27.8*	15.6*	9.1*	
<i>Spinacia oleracea</i> L.	Control	4.1	0.9	0.1	-	Chen and Arora, 2011
	Primed <sup>^</sup>	1.7	0.5	0.04	-	

<sup>o</sup>The SOD activity was expressed as: i) the amount of enzyme needed to inhibit the reaction by half per seed per minute, in *E. purpurea*; ii) the amount of enzyme needed to reach 50% inhibition of the reaction in the "minus enzyme extract" control, in *S. oleracea*. <sup>#</sup>One unit represents: i) 1 mol of substrate undergoing reaction per mg protein per minute, in *E. purpurea*; ii) the degradation of 1  $\mu$ M  $H_2O_2$  in 1 minute, in *S. oleracea*. <sup>§</sup>Primed in non-aerated solution of PEG 6000 (-0.5 MPa) at 25°C x 6 days. <sup>^</sup>Primed in solution of PEG 8000 (-0.6 MPa) at 15°C x 4 days. \*Average values of large- and small-size seeds (thousand-seed weight, 5.31 $\pm$ 0.24 g and 4.42 $\pm$ 0.21 g, respectively).

the p50 value, a longevity index representing the number of days of ageing needed to reduce seed viability by 50%, was directly related to the concentration of sucrose and raffinose, meaning a better longevity at a higher content of such sugars.

In addition to sugars, proteins may act to increase the desiccation tolerance during drying-back. In seeds of *Brassica oleracea*, a slow drying-back was associated with an increased expression of two genes of stress tolerance (Soeda *et al.*, 2005). These genes, *Em6* and *RAB 18*, encode proteins belonging to the LEA (Late Embryogenesis Abundant) group, induced by various stresses and conferring desiccation tolerance during seed maturation. *Em6* and *RAB 18* were expressed to a lesser extent in a rapid drying-back (Soeda *et al.*, 2005). In tomato, a post-priming treatment allowing a gradual reduction of seed moisture (incubation at 30 or 40°C for 2-4 h) proved a sound way to restore longevity (Gurusinghe and Bradford, 2001; Gurusinghe *et al.*, 2002). This treatment was accompanied by an increase in BiP proteins (Immunoglobulin Binding Protein), another group contributing to restore the functions of proteins damaged by the processes of seed wetting and drying (Gurusinghe *et al.*, 2002).

## Conclusions

Priming allows the synchronization of the metabolic events in a seed lot, improving the speed and uniformity of field emergence. During the treatment, seed damage due to various metabolic and genetic events may be repaired through the completion of phase I and II of seed imbibition.

Optimal priming ensures a flawless replication and transcription of nucleic acids, promotes the activation of enzymes mobilizing reserve proteins, and prepares the cells for division. A major drawback is that seed longevity is endangered by priming, although this depends on the conditions during drying-back; a slow drying-back counters the deteriorating processes, enabling a recovery of seed longevity after the treatment.

The adoption of priming fosters an overall increase in percent germination (+11%) associated with a shorter mean germination time (-36%). These simple figures demonstrate priming value in the perspective of practical utilization for commercial purposes (seed firms), but also in programmes of agricultural development where yields are constrained by poor seedling establishment.

Despite such advantages, priming has not achieved a widespread diffusion. The time course of scientific literature suggests some trends in the research on the subject, hinting at the problems that remain to be solved: of the 133 references cited in this review, only 13 date back to the 1970s or earlier, 27 to the 1980's, 60 to the 1990's, 33 from the year 2000 to present. This pattern outlines an increasing, then decreasing interest in the topic, which is counterbalanced by the fact that recent research has more closely addressed the subject of biochemical changes, than the issues of priming methods, effects and seed longevity after the treatment: in fact, sub-dividing the cited references by subject, biochemical changes result the main focus in 30% of the references published in the 1980s, 50% in the 1990s, 55% from the year 2000 to present. Therefore, a decreasing effort in quantitative terms has been compensated by an increasing commitment to unravel the complex metabolic response to the treatment.

In conclusion, the basic mechanisms of priming are generally acknowledged, whereas the underpinning physiological processes are not always elucidated and described in a sufficient number of species. This reflects in the contrasting effects sometimes observed after priming, which are responsible for the uncertainties still surrounding this technique. It appears, therefore, that providing further clues on the subject is the only way to achieve meaningful and reliable benefits

from priming. The association with other techniques of seed vigour enhancement such as seed sorting and physical treatments (polishing, coating, *etc.*) stands out as the ultimate approach in the quest for high and consistent seed performance.

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