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Seed Priming: New Comprehensive Approaches for an Old Empirical Technique

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Additional information is available at the end of the chapter

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Abstract

Seed priming is a pre-sowing treatment which leads to a physiological state that enables seed to germinate more efficiently. The majority of seed treatments are based on seed imbibition allowing the seeds to go through the first reversible stage of germination but do not allow radical protrusion through the seed coat. Seeds keeping their desiccation tolerance are then dehydrated and can be stored until final sowing. During subsequent germination, primed seeds exhibit a faster and more synchronized germination and young seedlings are often more vigorous and resistant to abiotic stresses than seedlings obtained from unprimed seeds. Priming often involves soaking seed in predetermined amounts of water or limitation of the imbibition time. The imbibition rate could be somehow controlled by osmotic agents such as PEG and referred as osmopriming. Halopriming implies the use of specific salts while "hormopriming" relies on the use of plant growth regulators. Some physical treatments (UV, cold or heat...) also provide germination improvement thus suggesting that priming effects are not necessarily related to seed imbibition. A better understanding of the metabolic events taking place during the priming treatment and the subsequent germination should help to use this simple and cheap technology in a more efficient way.

Keywords: germination, omics approaches, priming, seedling growth, stress resistance

1. Introduction

Efficient seed germination is important for agriculture. Successful establishment of early seedling indeed requires a rapid and uniform emergence and root growth. Germination of orthodox seeds commonly implies three distinct phases (**Figure 1**) consisting in (1) Phase I: seed hydration process related to passive imbibition of dry tissues associated with water movement first occurring in the apoplastic spaces; (2) Phase II: activation phase associated with the re-establishment of metabolic activities and repairing processes at the cell level; and (3) Phase III: initiation of growing processes associated to cell elongation and leading to radicle protrusion. Phases I and III both involve an increase in the water content while hydration remains stable during Phase II. It is commonly considered that before the end of Phase II, germination remains a reversible process: the seeds may be dried again and remain alive during storage and able to subsequently re-initiate germination under favorable conditions.

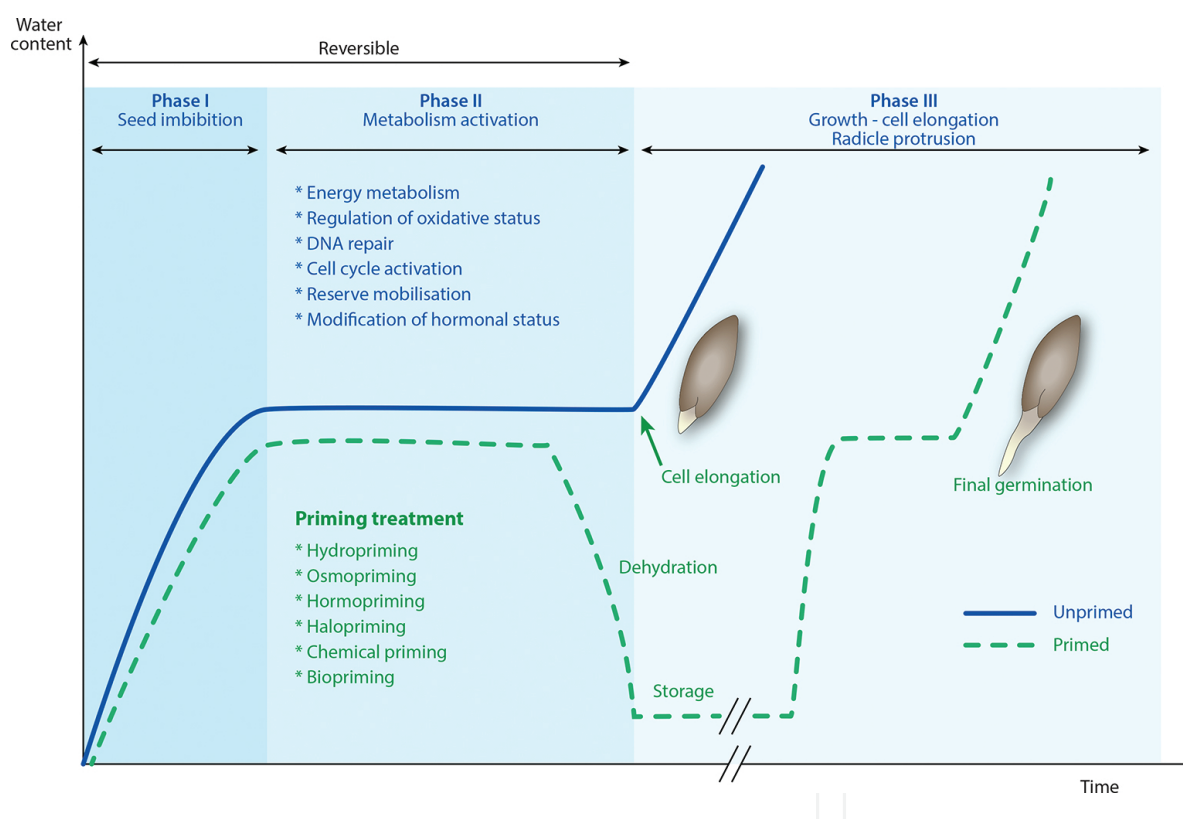


Figure 1. Seed hydration curves and germinating phases in unprimed and primed seeds.

Water-based seed priming is defined as a pre-sowing treatment that partially hydrates seeds without allowing emergence [1]. Various treatments may indeed be applied during the reversible phase of germination (point 3). They widely differ according to the osmotic potential of the priming solution, the duration, the external temperature, and the presence of specific chemical compounds. The efficient treatments trigger metabolic processes activated during the phase II of germination, which are then temporally stopped before a loss of desiccation occurs (**Figure 1**) [2].

The overall consequence of seed priming consists in an increased seed vigor defined as the whole set of properties conditioning seed lots performance in a wide range of environment [3]. Priming strategies may afford several economic and agronomic advantages to cultivated plants (point 4). Numerous data published in the literature indeed reported an improvement in the rate and uniformity of germination but also an obvious improvement in the behavior of the obtained seedlings in terms of plant growth and stress resistance.

Although priming is used since decades by farmers and seed companies to improve germination, it can also occur under natural plant conditions. This is more especially the case in serotinous plants growing in deserts and able to retain their seeds for a long time. These seeds indeed undergo several hydration-dehydration cycles enhancing subsequent germination after final seed dispersion caused by heavy rain [4]. From a general point of view, the priming process not only concerns seeds but also the whole plant system itself and may be defined as an induced state whereby a plant reacts more rapidly and more efficiently to a stress [5]. In this acception, plants exposed to a primary constraint are triggering a set of temporary metabolic adaptation leading to a stress memory and allowing them to adapt more efficiently to subsequent episodes of stress [6, 7].

Although the interest of seed priming has been demonstrated since a long time, the underlying physiological and biochemical basis of this fascinating process remain poorly understood. Holistic approaches related to omics tools now provide new opportunities to elucidate the molecular components of the priming phenomena. Similarly, nondestructive and noninvasive methods such as digital image technology may be used in a more precise way to study the kinetics of imbibition in relation to the modification of the seed ultrastructure. This chapter reviews the most recent progresses accomplished in the understanding of the seed priming-induced modifications.

2. A brief history of seed priming

Man established contact with seed physiology since the beginning of agriculture and quickly realized that many seeds do not germinate easily and uniformly. Ancient civilization was fascinated by the capacity of an apparently « dead seed » to resurrect and to produce a viable young and healthy seedling after germination. The Greek Theophrastus (ca. 372–287 BC) already focused on seed physiology and suggested that germination process may be temporarily interrupted [8]. Pre-hydration of legume seeds before sowing was performed by Roman farmers in order to increase the germination rate and synchronize germination as reported by the Roman naturalist Gaius Plinius Secundus. Several centuries later, these techniques were still used for a wide range of species according to the French agronomist Olivier de Serres (1539–1619) [8]. In 1664, Evelyn [9] mentioned that temperature prior sowing may have an impact on further germination while one century later, Ingenhousz [10] analyzed the impact of light on seedling emergence.

During the nineteenth century, numerous botanists started to describe morphological processes associated with seed germination [11, 12]. Sachs [13] experimented the impact of various

compounds (including tyrosine and asparagine) before and during germination. The discovery of plant hormones in the 1920s underlined the crucial role of these compounds in seed desiccation tolerance, reserve mobilization, as well as cell division and cell elongation occurring during germination. The possibility to influence final germination as a consequence of pre-sowing treatment has led to a wide range of empirical methods for numerous cultivated plant species during the year 1970s [14].

3. Priming methods and priming agents

Several methods of seed priming have been developed in order to invigorate seeds and alleviate the environmental stresses. A common feature of water-based priming techniques, which distinguishes them from other pre-sowing treatments, is partial seed pre-hydration and the activation of early germination events in seed. Priming efficiency is affected by many factors and strongly depends on treated plant species and chosen priming technique. Physical and chemical factors such as osmotica and water potential, priming agent, duration, temperature, presence or absence of light, aeration, and seed condition also influence priming success and determine germination rate and time, seedling vigor, and further plant development [15, 16].

3.1. Hydropriming

Hydropriming is the simplest method of seed priming, which relies on seed soaking in pure water and re-drying to original moisture content prior to sowing. No use of additional chemical substances as a priming agent makes this method a low-cost and environmentally friendly. The main disadvantage of hydropriming is uncontrolled water uptake by seeds. This is a consequence of free water availability to seeds during hydropriming, so that the rate of water uptake depends only on seed tissue affinity to water [17]. Moreover, this technique may result in unequal degree of seeds hydration thus leading to lack of simultaneous metabolic activation within seeds followed by unsynchronized emergence [18]. Considering these limiting factors, it is highly important to define accurate treatment duration, temperature, and water volume used in hydropriming to ensure desired level of seed hydration and to prevent radicle protrusion. Despite the aforementioned limitations, many reports indicated beneficial effect of hydropriming on seed germination and seedling growth under both optimal and stress conditions, in various crop plants such as chickpea, maize [19], wheat [20], Indian mustard [21], canola [22], sunflower [23], rice [24], mung bean [25], capsicum [26], and durum wheat [27].

One of the commercially used types of hydropriming is the system named “drum priming”, patented in the early 1990s [28, 29]. In this technique, seeds are gently rotating in drum and gradually hydrated by addition of water in vapor form. Drum priming allows seed imbibition in a controlled manner and could be an attractive alternative to conventional hydropriming. Specially designed apparatus enables monitoring of the seed weight, precise regulation of time, and water amount during hydration process, what ultimately results in an appropriate and

uniform moisture level of the seeds [30]. Drum priming with 24-epibrassinolide shows positive effect on germination time and seedling growth of bell pepper concomitant with improved superoxide dismutase (SOD), catalase (CAT), and peroxidase (POX) activities [31]. Another variant of hydropriming, so-called “on-farm priming”, consist of seed soaking in water followed by surface drying and subsequent sowing. The duration of treatment obligatorily cannot be longer than “safe limit” (maximum time of priming without risk of seed or seedling damage by premature germination) [32]. The positive impact of this method on crop emergence and yield was confirmed by Harris et al. [33]. On-farm priming is especially useful for resource-poor farmers in marginal tropical environment [34].

3.2. Osmopriming

Osmopriming involves soaking seeds in osmotic solution with low water potential instead of pure water. Due to low water potential of osmotic solutions, water enters seed slowly which allows gradual seed imbibition and activation of early phases of germination but prevents radicle protrusion [35]. Usually water potential of priming agent varies from -1.0 down to -2.0 MPa [36]. However, values of water potential together with duration of the priming treatment should be always adjusted to species, cultivar, and sometimes seed lot. Different compounds are used in osmopriming procedure including polyethylene glycol (PEG), mannitol, sorbitol, glycerol, and inorganic salts such as NaCl, KCl, KNO_3 , K_3PO_4 , KH_2PO_4 , MgSO_4 , and CaCl_2 [37]. Priming with salt solutions is often referred as “halopriming”. Most common chemical employed in osmopriming treatment is PEG, mainly owing to its specific characteristic. Large molecular size of PEG prevents its penetration into the seed thus avoiding induction of potential cytotoxic effect and reduction of osmotic potential within seed [35]. Nevertheless, PEG exhibits some undesirable features including high viscosity, which restrict diffusion of oxygen in the solution so in PEG priming aeration system is preferred [2]. Seed priming with PEG has been shown as an effective method to improve seed germination, seedling emergence, and stress tolerance of several crop plants under unfavorable conditions such as salt, water, chilling, and nano-ZnO stresses [1, 3, 36–38].

3.3. Solid matrix priming

Solid matrix priming (SMP, matricconditioning), in which water uptake by seeds is controlled, has been developed as an alternative method to osmopriming because of high cost of osmotic agents and technical problems with aeration [2]. During solid matrix priming, seeds are mixed and incubated with wet solid water carrier for a certain period. Afterward, seeds are separated from matrix, rinsed, and back-dried. The use of solid medium allows seeds to hydrate slowly and simulates natural imbibition process occurring in the soil [18]. To successfully accomplish SMP, materials utilized as matrices should possess specific physical and chemical features such as low matrix potential, minimal water solubility, high water holding capacity and surface area, no toxicity to seeds, and ability to adhere to seed surface. In fact, vermiculite, peat moss, charcoal, sand, clay, and some commercially offered substrate such as Celie or Micro Cell are exemplary solid carries applied in solid matrix priming [2, 35]. In order to obtain the best

priming performance, time of treatment and optimal water content must be determined separately for each matrix [39].

Positive effects of SMP on crop seeds have been noted in many reports. Solid matrix priming enhanced field performance of carrot [40] as well as improved germination and seed vigor of soybean [41]. Study on onion showed that matricconditioning improved seed germination rate, seedling emergence, and growth under optimal and low temperature conditions [42]. Sand priming increased the activities of antioxidant enzymes such as catalase (CAT), peroxidase (POX), and soluble sugar content in waxy maize concomitant with improved rate of germination and seedling growth under high-salt stress conditions [43].

It is well established that integration of SMP with biological and chemical factors may greatly enhance seed performance [18]. Adoreoli and de Adnrade [44] indicated that inclusion of gibberellins/fungicide/*Bacillus subtilis* to matricconditioning leads to improved stand establishment and productivity of some vegetable crops under tropical conditions. Similarly, matricconditioning with GA₃ enhanced the quality of hot pepper seeds [45]. More recently published data demonstrated that solid matrix priming with *Trichoderma viride* improved seedling emergence and yield of okra under low temperatures [46].

3.4. Hormopriming

During hormopriming, seeds imbibition occurs in the presence of plant growth regulators, which can have direct impact on seed metabolism. The following regulators are commonly used for hormopriming: abscisic acid, auxins, gibberellins, kinetin, ethylene, polyamines, and salicylic acid (SA). Gibberellic acid (GA₃) and PEG priming improved photosynthetic properties, antioxidant system, seedling emergence, and growth of white clover on heavy metal polluted soil [47]. Priming spring wheat seeds with GA₃ increased grain yield and salt tolerance by modulating hormone homeostasis together with alterations of ion uptake and accumulation between shoots and roots [48]. Enhanced salt tolerance, growth, and grain yield of wheat were also observed after kinetin-priming [49]. Among the different techniques of seed priming (hydro-, osmo-, and halopriming), spermidine pretreatment appeared to be the most effective method for induction drought tolerance in rice [50]. High efficiency of polyamines-priming on the improvement of rice tolerance to drought has been demonstrated also by Farooq et al. [51]. Critical role of phytohormones exogenously supplied into seeds for plant response to salinity stress was stated in wheat seeds primed with ascorbic acid and salicylic acid, as this pretreatment method increases the ability of wheat to grow successfully under salt stress, whereas hormonal priming with ABA was not effective in this case [52].

3.5. Biopriming

Biopriming involves seed imbibition together with bacterial inoculation of seed [53]. As other priming method, this treatment increases rate and uniformity of germination, but additionally protects seeds against the soil and seed-borne pathogens. Hydration of seeds infected with pathogens during priming can result in a stronger microbial growth and consequently impairment of plant health. However, applying antagonistic microorganisms during priming

is an ecological approach to overcome this problem [54]. Moreover, some bacteria used as biocontrol agents are able to colonize rhizosphere and support plant in both direct and indirect way after germination stage [55]. It was found that biopriming is a much more effective approach to disease management than other techniques such as pelleting and film coating [56]. Nowadays, the use of biopriming with plant growth-promoting bacteria (PGPB) as an integral component of agricultural practice shows great promise [57, 58]. In pearl millet, biopriming with *Pseudomonas fluorescens* isolates enhanced plant growth and resistance against downy mildew disease [59]. Biopriming with rhizobacteria improved germination parameters of radish seeds under saline conditions [60].

3.6. Others

Chemical priming refers to seed treatment with different chemical solutions used as priming agents. This approach includes priming with wide range of both natural and synthetic compounds such as antioxidants (ascorbic acid, glutathione, tocopherol, melatonin, and proline), hydrogen peroxide, sodium nitroprusside, urea, thiourea, mannose, selenium, chitosan, fungicide etc. Positive impact of chemical priming with various priming agents in a wide range of environmental conditions was indicated by numerous studies [26, 61–64]. Seed priming with β -amino butyric acid increased drought and salt tolerance of green gram [65]. Application of ascorbic acid as a seed priming agent induced drought and salt resistance of wheat [66, 67]. Analysis conducted by Fercha et al. [67] revealed that priming with ascorbate counteracts the negative effects of salinity stress by changes in abundance of proteins involved in metabolism, protein destination, and storage.

Nutripriming is a technique in which seeds are soaked with solutions containing the limiting nutrient instead of pure water. The idea of this method is to obtain nutritional effect together with biochemical advantages of priming in order to improve seed quality, germination parameters, and seedling establishment [68]. Seed priming with Zn improved productivity of chickpea and wheat [69], germination and early seedling growth of rice [70], development and root growth of maize seedling exposed to low root zone temperatures [71], while K-priming brought favorable effect on growth and nutrient status of cotton seedling under saline conditions [72]. Some nutripriming techniques are commonly used by seed companies in the process of seed production and preparation for growers. One of this methods, broad spectrum nutrient seed priming (BSN), is based on imbibing seeds in mixture of minerals, such as zinc, copper, manganese, molybdenum, and phosphorus, which has been proved to fertilize the seed and provides the nutrients for early growth, which positively affects germination, seedling vigor, and root system development (<http://seedprimer.com/>).

4. Seed priming and agriculture

Pre-sowing priming induces a particular physiological status in seeds and has emerged as a promising strategy to improve plant behavior in the field. There is a strong interest for farmers

and seed companies to find suitable cheap priming treatments but also to precisely identify the agronomical properties improved as a result of priming in cultivated species.

4.1. Hastening and synchronization of germination

Primed seeds often exhibit an increased germination rate and greater germination uniformity. An enhanced and uniform seedling emergence may contribute to regular crop establishment. Priming may enhance events taking place at the beginning of the germination, but the whole process is interrupted at a given state, which is the same for all concerned seeds. Priming may also induce structural and ultrastructural modifications that could facilitate subsequent water uptake and attenuate initial differences between the seeds in terms of imbibition, thus resulting in a more uniform germination [47].

A faster emergence may help to improve competitiveness of cultivated plants against weed species as recently demonstrated by Jalali and Salehi [73] for sugar beets. In mung bean plants, a faster seedling establishment resulting from priming may contribute to a total increase in yield up to 45% [74].

Priming-induced increase in germination may be associated to a change in plant hormone biosynthesis and signaling. Priming has been reported to increase gibberellins (GA)/abscisic acid (ABA) ratio [75], and this may be a direct consequence of a priming impact in gene expression pattern [76]. A more uniform GA endogenous concentration in primed seeds may help to synchronize endosperm weakening, embryo cell elongation, and reserve mobilization [77]. Ethylene also directly influences germination speed and percentage. Increase in ethylene production during priming may promote endo- β -mannase activity facilitating endosperm weakening and post-priming germination [78]. Priming has been reported to initiate repair and reactivation of pre-existing mitochondria and to initiate the biogenesis of new ones [79]. It may thus afford a higher level of energy over a short time to sustain final germination [80].

4.2. Plant growth

Plants issued from primed seeds often exhibit a faster growth than those issued from unprimed ones. Determine whether such growth stimulation is the consequence of a more rapid seedling establishment or result from a long-term specific physiological status induced by priming still remains an unresolved question. In numerous cases, the beneficial impact of priming on plant growth is more obvious under nonoptimal than under optimal conditions, leading to the global concept that a major advantage of priming consists in an increase in stress resistance (point 4.10). Thus, in direct relation to memory events, the main question is related to the remanence of priming-induced modifications. Imram et al. [71] showed that such modifications remain intact several weeks after germination in maize.

In rice, priming with 5-aminolevulinic acid improved shoot elongation [81] while priming with picomolar rutin augmented both root and shoot length in relation to an increase in photosynthetic pigments, phenolic and flavonoid contents [82]. In wheat, priming with sodium prusside stimulated plant growth as a consequence of improved capacity to scavenge free radicals by

antioxidants [83], and a similar observation was reported for rice as a result of an increase in glutathione peroxidase (GPX) activity [24] and other antioxidant enzyme activities [84].

The beneficial impact of priming on plant growth may be due to an improved nutrient use efficiency allowing a higher relative growth rate [85] and to an improved regulation of the plant water status [86]. Jisha and Puthur [65] confirmed that the priming effect of β -aminobutyric acid on seeds of *Vigna radiata* further get carried over the seedlings. A higher growth of seedlings issued from primed seeds may also be analyzed in relation to a direct impact of pretreatment on cell cycle regulation and cell elongation processes (point 7) [77, 78].

4.3. Mineral nutrition

Modification of nutrient uses efficiency by young seedlings may be a consequence of priming-induced overexpression of genes encoding for specific transporters, although only few transporters appear specifically induced by priming itself [36]. An efficient strategy to improve mineral nutrition of young seedling is to use nutrient-based seed priming strategy. Phosphorous seed priming supported crop development at early stages and may compensate for P deficiency in the soil [87, 88]. Jamil et al. [89] demonstrated that improvement of mineral status of P-primed cereals reduced strigolactone exudation and thus sensitivity to the parasite weed *Striga hermonthica*. Muhammad et al. [85] recently performed experiments using Zn, Mn, B, and P priming. These authors demonstrated that nutrient seed priming allowed maize plants to maintain Zn and Mn supply for at least 3 weeks in highly calcareous soils characterized by a low nutrient availability. Similarly, Pame et al. [90] showed that P accumulation in rice may be increased by using P-primed seeds, which is of special interest in Asia where about one-third of the area of rainfed rice is situated on P-deficient soils. Such a higher absorption could not be explained only by nutrient accumulation in the seeds during the primed phase since it is still observed in plants several weeks after sowing. It may therefore be hypothesized that priming interferes with regulation of acquisition mechanisms and further research is crucially needed to identify the molecular mechanisms involved in these processes. Priming with boron improves seedling emergence in rice and, on a long-term basis, increases panicle fertility in relation to an improvement in stigma receptivity [91]. Seed priming may also contribute to improve N nutrition, mainly through an enhanced nitrate reductase activity in plants [40]. Priming with nonessential beneficial elements, such as Si, leads to an increase in Si content of cultivated plants and has a protective impact on plant development [86].

Beside the improvement of essential elements uptake, priming also helps to reduce accumulation of putatively toxic elements. Chromium (VI) accumulation is reduced in maize seedlings issued from salicylic acid primed seeds and cultivated in the presence of this toxic element [82]. Osmopriming with PEG and hormopriming with GA improved germination and early seedling growth of white clover maintained on a heavy metal-contaminated soil, but the impact on Cd accumulation by plants may differ according to the considered treatment since GA₃ increased Cd accumulation while PEG reduced it [47]. Liu et al. [92] demonstrated that PEG increases Ca²⁺ cytosolic concentration through hyperpolarization-activated calcium permeable channels, which could explain a lower Cd accumulation as a consequence of an improved selectivity toward calcium.

Numerous data are also available concerning the priming effect on the plant behavior exposed to salinity. It is frequently reported that priming-induced stress resistance may be a consequence of an improved discrimination for K^+ over Na^+ nutrition. Both osmo- and hydropriming were efficiently used to influence K^+ selectivity of seedlings, but the underlying molecular basis of this improvement still needs to be identified, especially in terms of regulation of monovalent cation transporters.

4.4. Yield-related parameters

A huge amount of studies is devoted to the impact of seed priming on the seed germination phase and early seedling growth. Most of those studies are conducted under controlled environmental conditions in plant growth chambers or greenhouses. Data reporting a real improvement under field conditions remain rare. Yield effect may be linked to a faster plant establishment allowing a longer growth period. Khan et al. [93] reported that plant issued from primed seed benefits from a longer period of assimilates accumulation in sugar beet. Conversely, in some cases, phenological evolution of cultivated plants may be modified by priming: in chickpea, plants issued from priming encountered an earlier seed maturity allowing them to escape disease or heat terminal stress in the season [94]. Yield increase may also result from a higher plant density observed as a consequence of priming-induced increase in germination percentage [95].

Since less than a decade, several data started to be available for priming-induced yield improvement in rice. Shah et al. [96] demonstrated that priming had a positive effect on the weight of 1000 grains in this species. Boron priming induced an obvious decrease in panicle sterility and consequently improved the number of grains per inflorescence [91]. Binang et al. [97] also demonstrated that priming had a significant effect on the number of tillers, number of fertile panicles, and consequently grain yield of new NERICA rice varieties. Promising yield improvement has also been reported for maize [85, 98], onion [99], okra [100], and sugar beet [73]. Beside its impact on quantitative parameters, priming may also improve the quality of harvested plants, as recently reported by Janecho et al. [101] for the vitamin content and nutritional value of legumes.

4.5. Stress resistance

Most of the studies performed on the seedlings issued from primed seeds demonstrated a clear improvement of resistance to environmental constraints. **Table 1** is providing a nonexhaustive list of recent publications dealing with stress resistance improvement on cultivated plant species. Frequently, such improvement is obvious just after emergence at the seedling level, but progressively disappears at the adult stage. For example, some young plants issued from priming treatments displayed improvement of resistance to chilling [84], low temperature [75], salinity [43, 102], high temperature [80], drought [24, 65, 103], and UV exposure [82]. Some interesting studies also demonstrated that priming may afford resistance to biotic stresses such as *Fusarium oxysporum* in tomato [104], viral disease in *Brassica rapa* [105], and downy mildew in pearl millet [106]. Such a large set of data suggests that seed priming may elicit numerous pathways contributing to stress resistance. The molecular basis involved in this stress resist-

ance remains intact during the dehydration phase following priming and may contribute to stress resistance during the final germination step. Moreover, some data suggest that a single priming treatment may induce resistance to various stresses.

Environmental constraint	Plant species	Priming treatment	Reference
Salinity	<i>Brassica juncea</i>	hydro/osmopriming	[21]
	<i>Brassica napus</i>	PEG	[120]
		Halopriming	[22]
	<i>Helianthus annuus</i>	KNO ₃	[23]
	<i>Triticum durum</i>	ascorbic acid	[27]
	<i>Medicago sativa</i>	PEG	[37]
	<i>Zea mays</i>	Sand priming	[43]
	<i>Triticum aestivum</i>	ABA + SA	[52]
		NO (nitroprusside)	[63]
		Ascorbic acid	[67]
		Biopriming	[102]
		KCl + CaCl ₂	[167]
	<i>Raphanus sativus</i>	Biopriming	[60]
	<i>Gossypium arvense</i>	Potassium	[72]
	<i>Citrus sinensis</i>	NO (nitroprusside)	[185]
Drought/water stress	<i>Oryza sativa</i>	KH ₂ PO ₄	[24]
		Dry priming	[50]
		Polyamines	[51]
		Salicylic acid	[134]
	<i>Zea mays</i>	Urea; KNO ₃	[64]
	<i>Vigna radiata</i>	BABA	[65]
	<i>Triticum aestivum</i>	Ascorbic acid	[66]
		Silicon	[86]
		PEG	[178]
	<i>Brassica napus</i>	Hydropriming	[117, 118]
		Osmoconditioning	[187]

Environmental constraint	Plant species	Priming treatment	Reference
Chilling and low temperatures	<i>Cicer arietinum</i>	Osmo/hydropriming	[170]
	<i>Vigna radiata</i>	Hydropriming/proline	[25]
	<i>Zea mays</i>	chitosan	[62]
		Nutrient priming	[71]
	<i>Glycine max</i>	Osmopriming	[79]
	<i>Oryza sativa</i>	Salicylic acid	[84]
	<i>Beta vulgaris</i>	Osmopriming	[93]
	<i>Spinacea oleracea</i>	Osmopriming	[107]
High temperatures	<i>Nicotiana tabacum</i>	Putrescine	[166]
	<i>Lactuca sativa</i>	Hydropriming	[76]
	<i>Daucus carota</i>	PEG	[80]
Heavy metals	<i>Trifolium repens</i>	PEG	[47, 179]
	<i>Poa pratensis</i>	PEG, Gibberellins	[179]
Biotic stresses			
<i>Phythium ultimum</i>	<i>Zea mays</i>	Biopriming	[53]
<i>Verticillium</i>	<i>Brassica napus</i>	Biopriming	[56]
Downy mildew	<i>Pennisetum glaucum</i>	Biopriming	[59]
		BABA	[106]
Yellow mosaic virus	<i>Vigna radiata</i>	On farm priming	[75]
<i>Fusarium oxysporum</i>	<i>Solanum lycopersicum</i>	Methyl jasmonate	[104]
Double-stranded DNA virus	<i>Brassica rapa</i>	Biopriming	[105]

Table 1. Nonexhaustive list of recent studies devoted to priming-induced increase in the stress resistance of cultivated plant species.

The priming procedure itself implies frequently the use of stressing agent, as it is the case for PEG and salt. In some cases, priming may be performed at low temperature to reduce the kinetics of seed hydration. A slow hydration may be considered as a stressing process since the water content is too low to allow radicle elongation (see point 5). It may thus induce defense responses within embryos. This is especially the case for biochemical processes involved in protection against reactive oxygen species (point 8). Several components of the ROS-mediated signaling pathways are activated during the first hydration phase of the priming process. The ultimate stress resistance in the seedlings may then be linked to the persistence of the antiox-

idative defenses after final germination. Since management of oxidative stress is an important component of resistance to a wide range of stress, this observation may, at least partly, explain the cross-resistance phenomena.

The dehydration step that follows the partial hydration phase is also a major stressing phase. Numerous studies focus on late embryogenesis abundant proteins (LEA) normally involved in the acquisition of desiccation tolerance. Chen et al. [107] showed that the major dehydrins disappear during osmopriming while Maia et al. [108] conversely suggested that PEG may induce LEA synthesis. Water deprivation associated with the dehydration phase may also trigger accumulation of transcription factors, some of them being specifically involved in stress resistance [109, 110]. Molecular chaperones such as heat shock proteins (HSP) also explain a priming-induced improvement of stress resistance [78]. It may also be hypothesized that priming-induced modification of the seed hormonal status, mainly an increase in ABA, may somewhat influence the seed and young seedling response to environmental constraints in relation to a faster activation of ABA-responsive genes involved in stress acclimation [108, 109].

The beneficial impact of priming treatments relies on numerous properties as indicated in **Figure 2**.

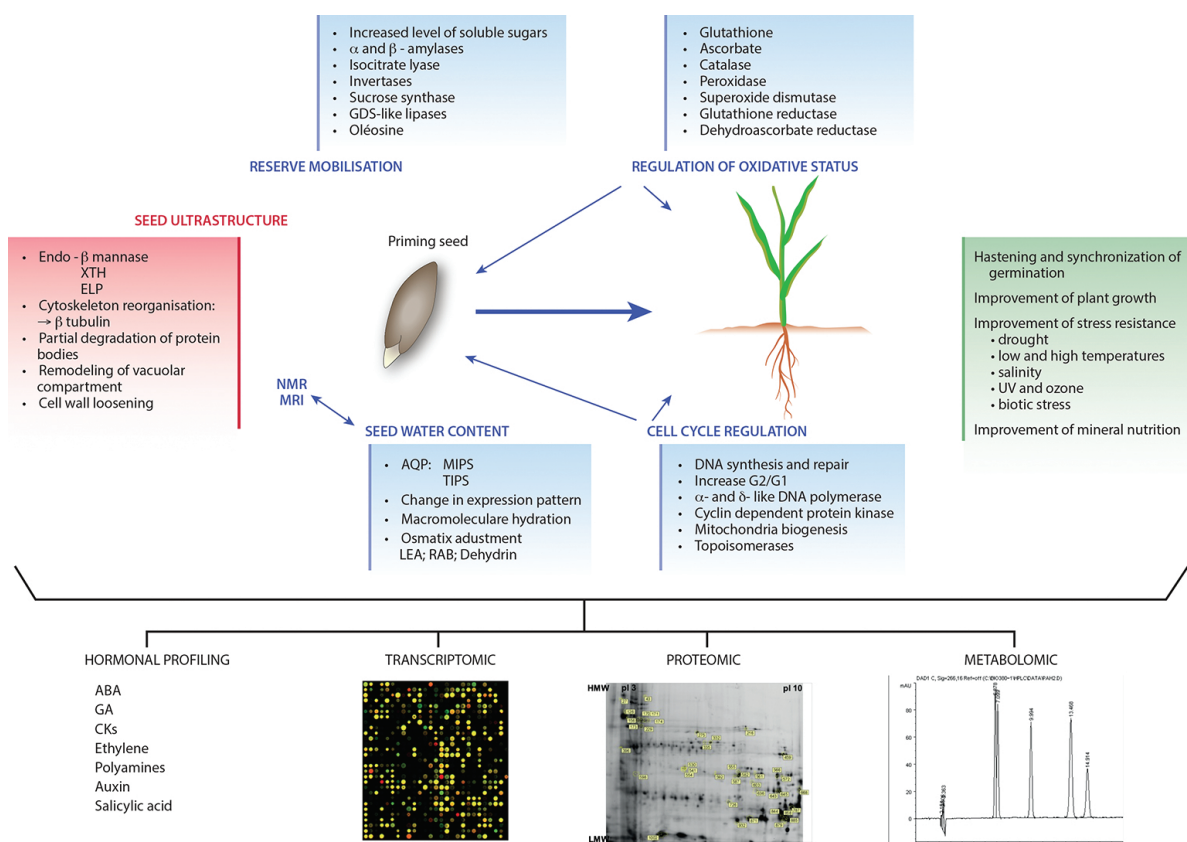


Figure 2. General overview of biochemical and physiological basis of priming effects. Priming modifies seed ultra-structure, reserve mobilization, regulation of oxidative status and cell cycle, and seed water content. The obtained seedlings may be improved for growth, mineral nutrition, and stress resistance. The components of priming effect may be revealed through an integrated convergent proteomic, transcriptomic, and metabolomics holistic approach.

5. Priming and seed water content

Seed germination is characterized by triphasic kinetic of water uptake with a rapid initial uptake (imbibition), followed by an apparent lag phase. A further increase in water uptake occurs only after germination is completed, as the embryonic axis elongates [111] (**Figures 1 and 2**). Early imbibition of seeds involves the fastest and most drastic changes in tissue hydration observed during germination. The water content of seeds and tissues within seeds depends of the composition of stored reserves. Seed imbibition and subsequent embryo growth depend on water exchanges and water potential gradients represent the motive force for water flow and finally tissue expansion. Water transport across cell membranes is essential for the initiation of metabolism. This intracellular water transport is mediated by aquaporins.

Aquaporins (AQPs) are transmembrane proteins, members of the major intrinsic protein (MIP) family that facilitate rapid and passive water transport across cell membranes and play a crucial role in plant water relations [112–114]. Plant aquaporins are remarkably diverse with several subfamilies of MIPs identified in dicots and monocots. Among them, the plasma membrane intrinsic proteins (PIPs) and the tonoplast intrinsic proteins (TIPs) subfamilies constitute the largest number of AQPs and correspond to AQPs that are abundantly expressed in the plasma and vacuolar membranes, respectively. Both PIP and TIP subfamilies are believed to play a key role in transcellular and intracellular plant water transport.

To gain insight into the role of water channel in germination, the expression profiles of *AQP* genes were studied in *Arabidopsis* [115], *Oryza sativa* [116], and *Brassica napus* [117] during seed imbibition and early embryo growth. These results have demonstrated the possible role of several AQPs in seed germination also in response to abiotic stresses. Moreover, Liu et al. [116] have shown the reduced seed germination rate via *OsPIP1;3* silencing and the promotion of seed germination via *OsPIP1;3* over-expression under drought conditions demonstrating that *OsPIP1;3* is required for normal seed germination.

Seed priming involves imbibing seeds with restricted amounts of water to allow hydration sufficient to permit pre-germinative metabolic events to proceed while preventing radicle protrusion. This treatment can extend phase II of water uptake while preventing seeds from entering into phase III. The completion of radicle emergence is prevented by restricted amount of water provided to the seed (hydropriming, solid matrix priming) or decreased water potential (Ψ_w) of the imbibition medium by the use of osmotic solutes such as PEG or salts (osmopriming) [111]. In a study on *Brassica napus* osmopriming, Kubala et al. [36] revealed that at the beginning of the soaking period and at the end of drying phase, the seed water content was as low as 5%. The soaking treatment allowed seed imbibitions up to 50%, which should be enough to re-initiate metabolism.

As AQPs regulate water movement, it can be supposed that these proteins play an important role both in priming treatment (seed soaking) and post-priming germination under both favorable and unfavorable conditions. A role for aquaporin-controlled water transport across cell membranes in primed seeds of *Brassica napus* during germination was demonstrated by Gao et al. [118]. Seed priming with PEG or ABA resulted in an enhanced germination,

particularly under salt and osmotic stresses at low temperature. Priming treatment induced expression of *BnPIP1* but had no effect on transcript level of *Bnγ-TIP2*. However, transcripts of both *BnPIP1* and *Bnγ-TIP2* genes during germination were present earlier in primed seeds than nonprimed ones. Gao et al. [118] speculated that *BnPIP1* was involved in water transport required for the activation of enzymatic metabolism of storage nutrients in the early stages of rapeseed germination, while *Bnγ-TIP2* expression was correlated with cell growth during radicle emergence. Changes in the expression pattern of *SoPIP1;1*, *SoPIP1;2*, *SoPIP2;1*, and *SoδTIP* during *Spinacia oleracea* seeds osmopriming and post-priming germination under optimal conditions, chilling and drought have been reported by Chen et al. [119]. The authors have stated that all these genes were up-regulated within 2–4 d of priming (phase II-imbibition). Therefore, the high expression of those AQPs might contribute to water transport across plasma and vacuolar membranes to facilitate water supply to expanding tissues and to increase germination potential of primed seeds. The down-regulation of all AQPs genes expression was observed under chilling and drought. However, the expression of some AQPs genes was elevated in primed seeds that also exhibited greater chilling and drought tolerance [119]. Kubala et al. [36] revealed up-regulation of two genes encoding tonoplast AQPs (*TIP4.1* and *TIP1.2*) in *Brassica napus* seeds in relation to osmopriming. In this study, expression of *TIP1.2* increased approximately 20 fold during post-priming germination as compared to unprimed seeds. In addition, the same authors have also stated facilitated water uptake and higher stress tolerance of germinating primed *Brassica napus* seeds [26, 120]. The above-mentioned results have demonstrated that water transport and sufficient water supply for embryo during post-priming germination regulated by AQPs may be one of the crucial components modulated by pre-sowing seed priming that influences germination rate and stress resistance.

As PIPs, but not TIP, are generally found at the plasma membrane, PIPs are thought to play a key role in seed water uptake. Nevertheless, both microarray [121] and macroarray experiments [115] with the complete set of genes encoding major intrinsic proteins revealed that out of 13 PIPs encoded by the *Arabidopsis* genome, transcripts for only three isoforms (PIP1;2, PIP1;4, and PIP1;5) were detectable in seeds. Mapping of TIPs in germinating *Arabidopsis* seeds has revealed that isoforms TIP3;1 and TIP3;2 detected in embryos, appear to localize to both the plasma membrane and tonoplast [122]. Vander Willigen et al. [115] have observed that during germination, very high level of TIP3 protein was coincident with decreased level of PIP1;2 and PIP2;1 polypeptides until phase III of water uptake. As stated by Vander Willigen et al. [115], it is intriguing how such low concentrations of PIP protein during the early phases of germination can achieve basic transcellular water transport in the seed. Gattolin et al. [122] have speculated that TIP3 may be the only AQP involved in seed water intake, and that the presence of TIP3 at the plasma membrane may compensate for the absence (or low concentration) of PIPs. In the light of these results, the enhanced germination potential of primed *Brassica napus* seeds could be partially explained by the up-regulation of TIPs during priming and post-priming germination [36]. However, the involvement of apoplastic water movement and simple diffusion of water across membranes during seed imbibition cannot be ruled out. The treatment of *Arabidopsis* seeds with mercury, a general blocker of aquaporins, reduced the speed of seed germination but did not affect its developmental sequence or basic aspects of seed water relations. Vander Willigen et al. [115] suggested that aquaporins functions are not

involved in early seed imbibition but would rather be associated with water uptake accompanying expansion and growth of the embryo.

The transmembrane water transport via the regulation of AQP quantity and activity endows seeds with a remarkable capacity to modulate water absorption, transport, and compartmentation within tissues. Nuclear magnetic resonance (NMR) spectroscopy has provided insights into changes in the physical states of seed water during germination [123–126]. In particular, magnetic resonance imaging (MRI) has revealed a precise spatial distribution of water within tissues of germinating seeds and different patterns between species [125, 127, 128] highlighting the tight control of water transport. Water status of primed seeds was characterized by Nagarajan et al. [129] in study on tomato halo- and osmopriming. Nagarajan et al. [129] pointed out that better performance of primed seeds may be attributed to the modifications of seed water-binding properties and reorganization of seed water during imbibition, so as to increase the macromolecular hydration water required for various metabolic activities related to the germination process. In the future, it will be crucial to see how the spatial pattern of aquaporin expression can fit the hydration pattern revealed by MRI during both priming and post-priming germination, therefore enabling a comprehensive understanding of water transport in seeds.

Several studies have reported that water uptake is improved by priming treatment as primed seeds exhibited a faster imbibition in comparison with nonprimed ones, although pre-treated seeds were dried after priming to reach the same water content as nonprimed ones [36, 47, 107]. Although MRI studies revealed that water penetrates seeds through the hilum and micropyle [125, 130], Galhaut et al. [47] did not observe any particular modification of these structures after *Trifolium repens* priming, despite a faster seed hydration. However, scanning electron microscopy analysis showed that primed seeds of *Trifolium repens* exhibited seed coat tears and circular depressions that can favor seed imbibition. Moreover, X-ray photographs revealed tissue detachment in dry primed seeds that formed free space between the cotyledons and radicle, making water flow easier, thus contributing to tissue hydration [47]. Similarly, formation of free space surrounding the embryo in dry primed seeds of tomato was noticed by Liu et al. [131]. In brief, these observations suggest that structural modifications might contribute to rapid seed germination by improving water uptake.

The maintenance of favorable water status is critical for survival of germinating seeds under environmental stresses leading to tissue dehydration. The accumulation of nontoxic, compatible solutes within seed tissues, that is, osmotic adjustment is a major trait associated with maintenance of high cell turgor pressure potential in response to stress conditions. Priming treatment itself may generate a moderate abiotic stress during soaking (e.g. osmotic stress, salt, and drought created by the priming agents) [36, 132]. The accumulation of osmotically active solutes such as amino acids (e.g. proline) ammonium compounds (e.g. glycine betaine), sugars (e.g. glucose, fructose, sucrose) during priming was noticed in several species and was shown to improve seed germination under subsequent water stress [3, 21, 133, 134].

Seeds can also experience dehydration in the course of priming treatment, that is, during drying after soaking. Late embryogenesis abundant proteins (LEAs) can stabilize cell structure and macromolecules upon cell dehydration by preventing inactivation and aggregation of

proteins and the loss of membranes integrity. This could be realized due to the ability of most LEA proteins to either coat intracellular macromolecules with a coherent layer of water or to interact with the surface of proteins and thus acting as water replacement [135]. As LEA proteins accumulate at a high level in response to cell/tissue dehydration, they may contribute to acquisition of tolerance to drought and related stresses such as osmotic, salt, and cold stress. In support of this, several studies revealed changes in the pattern of expression/accumulation of LEA transcript/protein in seeds caused by priming treatment and suggested their association with improved stress tolerance of primed seeds [36, 107, 136–138]. For example, the transcripts of two genes: *Em6*, encoding LEA group 1 protein and *RAB18*, encoding responsive to ABA 18 protein, belonging to LEA group 2, declined during osmopriming (soaking in PEG solution), reaccumulated after slow drying and again degraded during *Brassica oleracea* seeds germination [138]. The up-regulation of *RAB18* and *Em6* expression during slow seed drying suggests that they play a role in drought tolerance. Chen et al. [107] reported transient accumulation of four dehydrins-like proteins (32, 30, 26, 19-kD) in seeds of *Spinacia oleracea* during early stages of osmopriming followed by progressive degradation to a lower level in primed dry seeds compared to unprimed ones. A similar trend was confirmed to cold acclimation protein CAP85. In contrast to protein concentration, relative expression of CAP85 was greater in primed dry seeds than in unprimed ones. Recently, Kubala et al. [36] revealed accumulation of LEA transcripts (*LEA4-1*, *LEA4-5*) and LEA3 proteins during soaking in PEG solution. The authors proposed that soaking in PEG with a low osmotic potential should not be considered only as a rehydration phase: water uptake may be sufficient to reinitiate a physiological activity from a previous quiescent stage but water content of 50% remained low enough to represent a water stress situation, especially when it is maintained during several days [36].

6. Priming and seed ultrastructure

In general, the ability of seeds to germinate seems to be critically determined by a change in the balance between growth potential of the embryo and the mechanical resistance of the surrounding tissues. In many species, the endosperm tissue enclosing the embryo restrains the germination process acting as a physical barrier, which restricts radicle emergence.

Weakening of tissues surrounding elongating radicle by cell separation due, for instance, to the activity of cell wall hydrolases may occur as a consequence of priming (**Figure 2**). It was determined that osmopriming induced hydrolysis of the endosperm tissue of *Cucumis melo* seeds [139] and increased the endo- β -mannanase activity in the endosperm cap and decreased its mechanical restraint on the elongating tomato embryo [140]. A strong correlation was observed between lowering of the mechanical restraint and the activity of endo- β -mannanase [141].

Penetration of the structures surrounding the embryo is a consequence of radicle cells elongation. Up-regulation of the gene encoding xyloglucan endotransglucosylase/hydrolase (XTH) in response to osmopriming and accumulation of transcript for extensin-like protein

(ELP) during post-priming germination was observed in rapeseed [36]. As XTHs have the ability to cleave xyloglucans and rejoin the cut ends with new partners, they are engaged in cell wall loosening during growth and in the restructuring of the cell walls after extension.

Cytoskeleton reorganization is also necessary to achieve large rates of cells elongation that precedes radicle protrusion. The component of microtubules (β -tubulin) accumulated in tomato seeds during germination and priming and the expression preceded visible germination [142]. Higher level of β -tubulin protein accumulation was shown in rapeseed during PEG soaking, drying, and post-priming germination. The up-regulation of genes encoding γ - and β -tubulins was also noticed during post-priming germination [36].

Ultrastructural observations performed during the 6-d period of solid matrix priming (SMP) of carrot (*Daucus carota*) seeds indicated the breakdown of storage materials, specific to the catabolic phase of germination *sensu stricto*, both in the axis and in the micropylar endosperm covering the radicle tip [143]. It was found that complete degradation of storage protein and lipid bodies and subsequent starch accumulation occurred in the radicles of carrot seeds after 8-d SMP. In the endosperm, the catabolic changes were limited to the micropylar area, where extensive breakdown of storage cell walls, partial degradation of protein bodies, and no storage lipid hydrolysis were observed [144].

During seed germination, storage proteins, which provide a source of reduced nitrogen, and inorganic minerals need to be mobilized to support seedling growth. In addition, a lytic aqueous vacuolar compartment building up the turgescence necessary for cell expansion and to promote radicle protrusion and embryo elongation has to be formed (**Figure 2**). Bolte et al. [145] investigated the features and the dynamics of the vacuoles during the early stages of *Arabidopsis* seed germination and indicated the successive occurrence of two different lytic compartments in the protein storage vacuoles (PSV). The first one corresponds to globoids specialized in mineral storage and the second one is at the origin of the central lytic vacuole in these cells [145]. Different mechanisms for the transformation of PSV into lytic vacuole in the root tip cell of germinating tobacco (*Nicotiana tabacum*) seeds were proposed by Zheng and Staehelin [146]. Ultrastructural studies demonstrated that the radicle cells of tobacco contain only one type of vacuole at particular time of development. Upon rehydration, the radicle cells only contain PSVs, but during subsequent root development, the PSVs are systematically transformed into lytic vacuoles via cell type specific pathways.

At present, we do not have a complete view of ultrastructural changes occurring during seed priming. One would expect that similar vacuole remodeling might occur during priming, especially in embryonic axis. The maintenance of metabolism over, for instance, several days of seed priming requires mobilization of embryo reserves. It is speculated that accumulation of endogenous osmotica, cell vacuolization, together with cell wall loosening initiated during priming, might determine the embryonic axis extension and radicle protrusion during post-priming germination. Deeper and more detailed studies should be continued in order to completely clarify this phenomenon.

7. Seed priming and cell cycle regulation

Some of the hypotheses proposing explanation for priming-induced improvement are based on its effect on DNA in relation to activation of DNA repair mechanisms, synchronization of the cell cycle in G_2 and preparation to cell division (**Figure 2**). During seed maturation, most of the embryo cells are stopped at G_1 or G_0 phase of cell cycle and only some species have a small proportion of cells in the G_2 phase [111]. During seed imbibition, meristematic activity is limited; however, some preparation to cell division occurs. In embryos of dry tomato seeds, most cells have 2C DNA level and are in G_1 phase of nuclear division [147]. The authors observed that DNA synthesis preceded germination as during imbibition in water, 4C signal was found mostly in the embryonic root tip, which suggests that cell enters S phase. They also primed seeds for 14 d in PEG-6000, which enhanced the rate and uniformity of germination. The 4C DNA signal of root tip cells increased during priming starting from 3 d incubation in PEG and was constant after re-drying the seeds to the initial moisture content. This observation suggests that priming increased the ratio of cells in G_2 phase to G_1 phase and indicates that the beneficial effects of priming on seedling performance are associated with the replicative DNA synthesis prior to germination [147]. This is accompanied by increase in α - and δ -like DNA polymerase activities in primed seeds and during germination.

The initiation of cell cycle and proceeding the cell to S phase may depend also on a G_1 checkpoint control. Most, if not all, cell cycle proteins responsible for cell cycle control appear to be already present in dry mature seeds, although some of them should be synthesized *de novo*. However, not only protein synthesis but also their modification may play a regulatory function for cell cycle control [148]. Cell division starts just after radicle protrusion, thus, seed priming, which prolongs Phase II of seed germination and is finished just before Phase III, does not affect cell division in itself [16]. Seed priming extends Phase II, when DNA repair mechanisms and expression of genes encoding proteins needed in cell cycle control and commencement are activated and overreach the level observed in unprimed seeds. Pre-activation of cell cycle by priming could be through regulation of the activity of cell cycle proteins such as cyclin-dependent protein kinases and proliferating-cell nuclear antigens [16]. It was found that osmopriming of *Brassica napus* seeds induced expression of cell division control protein 48 homolog C, cyclin P4;1, cyclin like protein and topoisomerase II in dry seeds, as well as proliferating-cell nuclear antigen 2 and cyclin dependent kinase 3;2 during imbibition [36]. Accumulation of proliferating-cell nuclear antigen during maize seed imbibition was associated with transition of the cells from G_1 to G_2 [149]. Moreover, microtubules, apart from cytoskeleton formation, cytoplasmic streaming, organellar movement, and cell wall formation, function in mitotic spindle formation during mitosis. Microtubules in dry seeds are depolymerized and form discrete granular bodies, which become organized into the cytoskeleton during imbibition [111, 142]. Higher expression of genes encoding microtubule-associated protein 65-1 and 70-2 as well as tubulin subunits γ -1, β -1, β -3 and microtubule motor activity proteins belonging to kinesin family was also observed during PEG soaking and in dry osmoprimed *Brassica napus* seeds [36]. Enhanced expression of tubulin genes was associated with accumulation of β -tubulin protein during osmopriming and subsequent germination [36]. Also in pre-hydrated seeds of *Arabidopsis thaliana* and *Solanum lycopersicum* accumulation of

tubulins (mainly β -tubulin) was stated during germination as compared to unprimed seeds [136, 142].

During Phase II of seed germination, when water uptake is severely limited, major metabolic processes are activated [111]. One of the most important events undergoing during Phase II is DNA repair, which precedes cell cycle activation [142, 150]. The process of DNA replication is preceded by repair of DNA damage caused mainly by reactive oxygen species, which are accumulated during seed storage and aging [151]. DNA repair covers first period of DNA synthesis, while the second period of DNA synthesis (replication) is observed before cell division. DNA synthesis in Phase II of germination and also during seed priming corresponds rather to DNA repair, mainly in organelle such as plastids and mitochondria [152]. Increased number of mitochondria in leek embryo cell of osmoprimed seeds was observed by Ashraf and Bray [153]. Mitochondria biogenesis before mitochondria division involved the transition of promitochondria to mature mitochondria. This process is accompanied by the expression of genes of nucleotide biosynthesis, transport, and organelle RNA- and DNA-related functions [154, 155]. Pre-sowing seed osmopriming induced higher expression of genes corresponding to mitochondria biogenesis such as translocases of the inner membrane (TIM) complex TIM10 and TIM23-1, mitochondrial ribosomal protein and translational elongation factor EF2, which is targeted into mitochondria [36].

There are still some gaps in comprehensive understanding of pre-sowing seed priming impact on DNA repair and cell cycle regulation. Activation of different DNA repair mechanism has been observed during seed imbibition preceding germination and they are believed to be essential for successful reactivation of cell cycle [111]. They include α and β tyrosyl-DNA phosphodiesterase 1, α and β DNA topoisomerase I [156], 8-oxoguanine DNA glycosylase/lyase and formamidopyrimidine-DNA glycosylase [157], transcription elongation factor II-S [158], DNA ligase VI and IV [159]. Varier et al. [16] have suggested that in primed seeds DNA damage is repaired before replication, primarily through DNA synthesis. However, in a study on *Cicer arietinum* primed seeds, the role of DNA repair genes in enhancing the physiological quality of seeds was postulated [160]. The authors tested the expression level of genes encoding proteins with already proved function on DNA repair mechanisms in relation to priming methods and seed size. Moreover, enhanced accumulation of transcripts was found in dry and imbibed osmoprimed *Brassica napus* seeds [36] for genes involved in DNA repair according to function description in databases, such as DUTP-pyrophosphatase-like 1, endonuclease V family protein, ribonucleoside-diphosphate reductase subunit M2 (TSO), casein kinase II, replicon protein A2, DNA glycosylase DEMETER (DME), BARD1, RECQ helicase L4B, and MUTS homolog 2. Thus, activation of DNA repair mechanisms in seeds occurs prior to their germination and contributes to enhanced germination rate and better quality of seeds undergoing pre-sowing seed priming.

8. Management of oxidative status

Management of oxidative status is also considered as an important part of primed seeds physiology [18, 36, 161, 162] (**Figure 2**). Beginning with the seed development through

maturation and germination, the seed moisture content as well as seed metabolic activity is subjected to dramatic changes. The biochemical and cellular events triggered by water uptake and subsequent loss are accompanied by a generation of reactive oxygen species (ROS) [151, 163]. During seed imbibition and early stages of germination, ROS production occurs mainly through respiratory activities of mitochondria, activities of β -oxidation pathways and enzymes such as NADPH oxidases, extracellular peroxidases, and oxalate oxidases [163]. ROS accumulation and associated oxidative damage can be regarded as a source of stress that may affect the successful completion of germination. As ROS, particularly H_2O_2 can act as signaling molecules, seeds must be endowed with a ROS removing system that tightly regulates their concentration. Scavenging of ROS is carried out by antioxidant system, a multifunctional machine, which includes enzymes (i.e. catalase (CAT), superoxide dismutase (SOD), and ascorbate peroxidase (APX), monodehydroascorbate reductase (DHAR), and glutathione reductase (GR)) as well as nonenzymatic compounds (i.e. ascorbic acid (AsA) or reduced glutathione (GSH) [1, 151]. Metabolism of ROS, mainly H_2O_2 , which is believed to play a central role in oxidative status signaling, is strictly associated to other reactive species and signaling molecules such as nitric oxide and hydrogen sulfide, which contribute and regulate the transition from dormant to germination phase [151, 163, 164].

Kubala et al. [36] have considered the priming as a process, which consists of two main phases: controlled hydration of the seeds and drying back to the initial moisture content. Seed pre-hydration followed by re-drying during priming treatment, similarly to seed maturation and germination, exerts changes in moisture content, which leads to ROS production and activation of the antioxidant system. The activation of APX and the accumulation of AsA and GSH during osmopriming of spinach seeds (*Spinacia oleracea*) were accompanied by the repression of SOD and CAT activity [1]. These results indicated that activation of AsA-GSH cycle during osmopriming of spinach seeds can decrease the level of lipid peroxidation products in primed seeds. Moreover, the same authors have suggested that the renewal of antioxidant defense system, possibly required by seedling establishment, occurred during the late stages of germination as a result of up-regulation of CAT activity after initial reduction and overall antioxidant activation [1]. Kubala et al. [36] have indicated through an integrated transcriptomic and proteomic approach that the priming-induced germination can be linked with the activation of antioxidant system. The authors showed that during osmopriming of *Brassica napus* seeds CAT2 and PER21 encoding peroxidase 21 were up-regulated and GR protein was accumulated. Moreover, the same authors have observed up-regulation of PER13 gene and accumulation of peroxidase 12, DHAR and peroxiredoxin proteins during post-priming germination. Furthermore, Kubala et al. [162] have postulated a correlation between activation of antioxidant metabolism in osmoprimed *Brassica napus* seeds and increased tolerance to salt stress during germination. The enhanced activity of APX, SOD, and CAT corresponded with increased expression rate of APX, SOD, and CAT genes. Similar result has been obtained by Nouman et al. [165], who showed that priming of *Moringa oleifera* seeds with *Moringa* leaf extract (MLE) improves growth under saline condition mainly by activation of antioxidant system (SOD, CAT, and POD). Priming of mung bean seeds (*Vigna radiata* L. Wilczek) with β -amino butyric acid (BABA) enhanced the activities of SOD and POX leading to improved tolerance to NaCl and PEG 6000 stresses [65]. Enhanced chilling stress tolerance in two tobacco

varieties (MSk326 and HHDJY) was due to increased activity of antioxidant enzymes (SOD, POD, CAT, and APX) as a result of priming seeds with putrescine [165]. Results obtained by Islam et al. [167] showed that in haloprimed wheat (*Triticum aestivum*) seeds, increased activity of CAT, POD, and APX enhanced tolerance to salinity stress. Osmopriming with PEG has improved sorghum (*Sorghum bicolor*) seed germination and seedling establishment under adverse soil moisture conditions and has been correlated with antioxidant system activation (APX, CAT, POD, and SOD) [3]. Rice (*Oryza sativa*) seeds primed with polyethylene glycol (PEG) showed increased activity of APX in parallel with decreased activity of SOD, POD, and CAT under ZnO nanoparticles stress [38]. The same authors have also observed down-regulation of genes encoding the antioxidant enzymes (*APXa*, *APXb*, *CATa*, *CATb*, *CATc*, *SOD2*, and *SOD3*) in PEG primed seeds under nano-ZnO stress. They have concluded that priming with PEG significantly alleviates the toxic effects of nano-ZnO through improved cell structures of leaf and roots.

Seed aging during storage is associated with ROS production. Appearance of oxidative stress results in a decrease of seed quality. Kibinza et al. [161] showed that priming plays an important role in seed recovery from aging through CAT activation. Their results revealed accumulation of hydrogen peroxide (H_2O_2) and reduction of CAT at the gene expression level and protein content during sunflower (*Helianthus annuus* L.) seed aging. Interestingly, the adverse results of aging were recovered by seed osmopriming, which led to induction of CAT synthesis by activating gene expression and translation of the enzyme.

Summing up, the management of oxidative status in primed seeds plays a very important role as a machinery, which leads to protection against oxidative stress, recovery from aging, and regulation of ROS production/accumulation. Alleviation in ROS level exerts a signal, which could be perceived, transduced, and crosstalk with other signaling pathways, thus executing physiological response by activation or repression of molecular processes.

9. Reserve mobilization

Recent transcriptome and proteome study of cabbage (*Brassica oleracea*) and *Arabidopsis* seeds as well as integrated transcriptomic and proteomic approach in study of rapeseeds (*Brassica napus*) osmopriming revealed that germination and priming altered similar processes [36, 136, 138]. Indeed, it is proposed that germination-related processes such as respiration, energy metabolism, and early reserve mobilization can also occur during priming [16] (**Figure 2**). Faster and uniform rice (*Oryza sativa*) seed germination due to priming was related to improved activity of α -amylase, resulting in increased level of soluble sugars in primed kernels [168]. Sung and Chang [169] have shown that priming of maize (*Zea mays*) seeds leads to increased activity of enzymes for carbohydrates (α and β amylases) and lipids (isocitrate lyase, ICL) mobilization. Priming of chickpea (*Cicer arietinum* L. Cv PBG-1) seeds with mannitol led to increased activity of amylase, invertases (acid and alkaline), sucrose synthase (SS), and sucrose phosphate synthase (SPS) in shoots of primed seedlings. The higher amylase activity in shoots suggests a rapid hydrolysis of transitory starch formed in the shoots of primed

seedlings leading to more availability of glucose for seedling growth. [170]. Higher content of soluble protein, aldolase, and ICL activity has been observed in haloprimed pepper (*Capsicum annum* L) seeds than in control seeds [171]. Moreover, the α -glucosidase accumulation and increased level of globulin degradation products were observed during the priming process of sugar beet (*Beta vulgaris* L.) seed [172]. Similar observation on primed sugar beet seed has been done by Capron et al. [173] who showed increased solubilization of 11S-globulin- β -subunit in response to hydro- and osmopriming. Gallardo et al. [136] have also observed higher polypeptides content in both hydro- and osmoprimed *Arabidopsis* seeds. They were identified as products of 12S-cruciferin- β -subunit degradation.

Kubala et al. [36] have shown that during *Brassica napus* seeds osmopriming and post-priming germination accumulation of transcript and proteins for seed storage proteins occurred. The authors have observed up-accumulation of cruciferin CRU1. The group of six genes encoding GDSL-like lipases, playing a role in triacylglycerols (major storage lipids in rape seeds) catabolism, were strongly up-regulated during post-priming germination while the other three genes for GDSL-like lipases as well as extracellular lipase 6 were up-regulated in osmoprimed seeds [36]. The activation of lipid catabolism-related genes was correlated with the activation of genes involved in lipid transport such as genes encoding bifunctional inhibitor/lipid-transfer protein/seed storage 2S albumin superfamily protein [36]. Priming can also reduce a level of oleosins-proteins, which surround oil bodies. In osmoprimed *Brassica napus* seeds as well as during post-priming germination, down accumulation of oleosin S4-3 protein and down-regulation of *OLEOSIN2* gene were observed, respectively [36].

Activation of respiration and rapid ATP production is primary metabolic events occurring during priming [18], and higher respiratory activity is required to cover energy pool for speed up germination. The increased ATP level/energy charge after priming was observed in tomato (*Solanum lycopersicum*), eggplant (*Solanum melongena*), araucaria (*Araucaria columnaris*), spinach, oat (*Avena sativa*), and cabbage (*Brassica oleracea*) [174, 175]. Primed seed needs a large amount of fuel, which supplies energy required for higher reserve mobilization rate. All together lead to improved energy turnover and increased metabolism rate of primed seed and contribute to better germination and stress tolerance.

10. Holistic “omic” approaches of seed priming

The identification of biomarkers of seed priming effect is a relevant goal for plant physiologists. The sequence of events associated with water-based priming involves a limited hydration step during the soaking period followed by a more or less rapid dehydration phase (**Figure 1**). It may be considered that each phase has its own profile of activation/deactivation. Transcription is not necessarily coordinately associated with translation, resulting in some cases in a limited correspondence between mRNA levels and protein abundance. Some proteins synthesized during incubation may also be degraded during dehydration, although the rate of degradation might somewhat be influenced by the rate of drying. Finally, protein synthesis may also occur as a result of the translation of long-lived mRNA previously synthesized during seed maturation [17, 37, 103, 110].

The advantage of these complementary holistic approaches is that they provide a large set of data allowing physiologist to obtain a global view of all parameters of the metabolism associated with priming. The major disadvantage, however, is that these techniques are rather expensive. As a consequence, samples are frequently analyzed after a given duration of treatment and the time-dependent evolution of parameters are rarely considered. It is still difficult to reconstitute a kinetic approach, but published studies rather provide a large set of information at a given moment while the priming procedure is a dynamic process.

10.1. Transcriptomics

Transcriptomics is the study of the transcriptome—the complete set of RNA transcripts that are produced by the genome under specific circumstances—using high-throughput methods. Several techniques are available for transcriptomic approaches, including microarray, c-DNA amplified fragment length polymorphism (cDNA-AFLP), expressed sequence tag sequencing, serial analysis of gene expression, RNAseq, and massive parallel signature sequencing [176]. According to Buitink et al. [109], more than 1300 genes may be differentially regulated during priming with PEG at -1.7 MPa in *Medicago truncatula*. Genes whose expression are regulated during the priming process are commonly categorized according to the function of the corresponding protein (metabolism and regulation of metabolism, cell cycle regulation, DNA processing, transcription regulation, cellular transport and communications, stress responses, etc.). However, a consistent part of the identified genes is still not annotated.

In *Brassica napus*, numerous priming-regulated genes are involved in gluconeogenesis, which is essential for triacylglycerol breakdown into small molecules. Another important category of genes involved in water-based priming of rapeseed encodes for transcription factors, and this is especially the case after hormoprimering with ABA [138]. In the same species, it was demonstrated that germination of primed seeds involves a specific set of genes comparatively to germination of unprimed ones [36]. In young seedlings obtained from primed seeds, the expression of stress-related genes is often more rapid than in plants from unprimed ones, as recently exemplified for cold tolerance [26]. Not only genes related to protein synthesis but also genes involved in protein degradation may be induced by priming. Some transcription factors may be down-regulated during soaking and up-regulated during drying [36]. Genes involved in the synthesis of osmoprotectant, such as proline, may also be regulated at various steps of priming [120].

In *Brassica oleracea*, transcripts that are abundant in dry seeds rapidly decline during osmo-priming and germination expression programs are initiated during osmo-priming [138]. For these authors, genes expressed during slow drying following the soaking period are correlated with the stress resistance properties of primed-material. This may especially concern cell-cycle-regulated genes, enzymes involved in C metabolism, and components of the translation machinery. Acquisition of the desiccation tolerance after the soaking period is a crucial trait for priming. Transcriptomic data demonstrated that the molecular determinism associated with such trait may be, at least partly, similar to those involved during a « normal » seed dehydration process during maturation. In *Medicago truncatula*, several regulatory genes expressed during drought stress are also up-regulated during seed maturation. During

priming, these genes are involved in post-soaking dehydration while genes conditioning cell cycle, cell wall biogenesis, and energy metabolism are repressed [109]. Transcripts accumulated after PEG-priming treatment comprise those encoding for LEA and seed storage proteins, or genes controlling seed dormancy while genes involved in photosynthesis and cell wall modifications are commonly repressed [108].

Beside involvement of transcription factors, it could be of primary importance in the future to analyze the priming-induced epigenetic changes. Indeed, DNA methylation may be directly involved in « stress memory » and some changes, such as cytosine methylation or overexpression of histone deacetylase was reported to occur during germination [78].

10.2. Proteomics

Proteome is the entire set of proteins present at a given moment in a given biological sample and proteomic is the large-scale study allowing identification and quantification of these proteins. Proteomics has now been practiced in different plant systems through the separation of proteins by two-dimensional gel electrophoresis followed by peptide mass fingerprinting. Tandem mass spectrometry can get sequence information from individual isolated peptides. Quantitative proteomics represent an important extension enabling the comparison of changes in protein levels across different samples [177]. Gel-free shotgun proteomic is an alternative for identification and quantification of protein in large-scale studies. This method was recently used to reveal potential biomarkers of priming-induced salt tolerance in durum wheat with a concomitant use of protein fractionation and hydrogel nanoparticles enrichment technique [27]. According to these authors, hydropriming was accompanied by a significant change in 72 proteins. Most of them are involved in proteolysis, protein synthesis, metabolism regulation, and disease or defense response. Priming with ascorbic acid changed the pattern of proteome signature and most of identified proteins are involved in metabolism regulation, antioxidant protection, repair processes, and methionine-related metabolism.

Cycloheximide, a potent inhibitor of protein synthesis, may be added to priming or to germination solution to analyze the precise requirement of protein synthesis during these steps. It appears that protein synthesis is not necessarily required for normal germination process, which could be related to the fact that in some cases, germination relies only on translation of pre-stored long-lived mRNA produced during seed maturation [14, 178]. Cycloheximide may drastically affect osmopriming-induced improvement of Cd resistance in *Poa pratensis* and *Trifolium repens* in relation to a decrease in α and β -amylase [179]. Protein concentration in primed seeds is the result of two contrasting pathways: the first implies specific protein synthesis while the second involves the breakdown of storage proteins through protease activation. It was demonstrated that priming may act on protease activities and also influence the expression of protease encoding-gene [180].

Some strategies are also used to separately identify proteins in embryos and in embryo-surrounding tissues. In monocots, a special attention is often paid to aleurone layers involved in hydrolytic enzyme synthesis [181]. Aleurone layer is easily separated in some plant material such as wheat or barley but is more difficult with other monocot such as turfgrasses with very

small seeds. In wheat, it was demonstrated that the set of proteins regulated by priming is quite different in embryos and in surrounding tissues and the number of proteins affected by priming was by far higher in the embryos than in the other parts of the seed. Proteins regulated in embryos involve those directly linked to metabolism regulation (especially methionine biosynthesis, glutamate/glutamine metabolism, amino acids synthesis, etc.), energy supply, cell growth and maintenance of cell structure while proteins up-regulated in surrounding tissues are mainly involved in reserves mobilization or in the management of the oxidative stress [67]. In barley, however, ascorbate peroxidase was observed only in the embryo while several other redox-related proteins differed in spatio-temporal patterns at the onset of radicle elongation [181].

Priming is considered as an invigoration treatment and several proteomic approaches were performed to unravel the biomarker of seed vigor. Catusse et al. [182] found that 18 proteins are accumulated during hydropriming of sugarbeet seeds and that the same proteins appear directly reduced during aging. Seed vigor appears directly related to lipid and starch mobilization, protein synthesis and methionine cycle. In poplar, more than 81 proteins showed a significant change in abundance when comparing the proteomes among seed with different vigor [183]. According to these data, the decrease in seed vigor is an energy-dependent process, which requires protein synthesis and degradation as well as cellular defense and rescue. Salicylic acid (SA) was proposed as an invigorating elicitor promoting seed germination under saline conditions: SA re-induced the late maturation program during early stages of germination, induced the synthesis of antioxidant enzymes, and improved the quality of protein translation [184]. Similarly, the “prime-ome” approach performed by Tanou et al. [185] confirmed the importance of redox proteomic and processes such as N-nitrosilation, tyrosine nitration, and mitogen-activated protein kinase MPK3 signaling in priming effects.

Osmotic priming was reported to trigger desiccation tolerance in *Medicago truncatula* [110]. Proteomic analysis demonstrated that such trait is directly linked to the synthesis of late-embryogenesis abundant proteins from different groups. Secondary structure of some proteins was compared in the hydrated and dry state after fast or slow drying using Fourier transform infrared spectroscopy, which confirms that these proteins adopted α helices and β -sheets conformation during drying process.

Some proteomic approaches are conducted on plant species whose genomes are not sequenced. A recent 2DE-MS/MS-based proteomic study was conducted on pearl millet seeds primed by β -aminobutyric acid and showed an over-representation of proteins belonging to glucose metabolism, and a majority of induced proteins are directly related to energy [106]. Seedlings issued from those seeds are more resistant to downy mildew (*Sclerospora graminicola*). It is interesting to note that several of the elicited proteins are present in the extracellular space and in organelles (mainly mitochondrion and chloroplast).

10.3. Metabolomics

Metabolome refers to the complete set of small-molecule metabolites present within a plant tissue or organ at a moment. Metabolomic should thus be considered as the quantitative

measurements of the whole set of compounds involved in the metabolism of a given biological sample. Plant metabolomics has become an essential part of functional genomics. It often appears difficult to analyze the entire range of metabolites by a single analytical method, and several tools are thus commonly combined for this purpose: high performance liquid chromatography (HPLC), gas chromatography (GC), electrospray ionization coupled with mass spectrometry, capillary electrophoresis, atmospheric pressure chemical ionization (APCI), and secondary ion mass spectrometry (SIMS). Metabolomic approach, however, still remains difficult to perform on seed material considering the high proportion of molecule issued from reserve mobilization. An excess of soluble sugar in gramineae and an excess of small lipids issued from oil digestion in oleaginous plant material may greatly hamper isolation of other compounds involved in hastening the germination of primed seeds. Similarly, some important compounds might be present only in specific tissues or cell compartments and react with sugars during extraction processes; this is especially the case of gibberellins which play a crucial role in germination but are believed to conjugate with sugar or phenol compounds, which greatly compromise isolation of and identification procedures of numerous metabolites [186].

Priming process may drastically modify the synthesis and accumulation of endogenous antioxidant such as glutathione, ascorbic acid, and even α -tocopherol. A modification in reducing sugars concentration, such as glucose resulting from partial starch digestion during priming, may influence protein glycation, a nonenzymatic reaction between reducing sugars and amino groups in protein [150]. The amount of proline was reported to be modified in osmoprimed seeds [120]. Some other data reported modifications in aspartate, leucine, threonate, glutamate, fumarate, or pinitol content in primed seeds from different species [1, 2, 35, 104, 152]. Methionine may also play a key role in priming process, mainly as a precursor of both ethylene and polyamine synthesis. Polyamines are small aliphatic molecules influencing all aspects of plant metabolism and development, including the germination processes. Ethylene is also involved in various aspects of seed germination, and priming was reported to modify the kinetics of ethylene synthesis from its precursor 1-aminocyclopropane-1-carboxylic acid (ACC) [186]. Other phytohormones are expected to assume important functions in priming effect (ABA, gibberellins, cytokinins, auxin, etc.) [50, 52], but the quantitative impact of priming procedure on these compounds is still not well established.

11. Main limitations of priming techniques

Seed priming has emerged as an effective approach for increasing seed vigor. The optimal treatment differs between species, cultivar, and seed lots. Such variability is a major limitation of the priming method since numerous trials are required to identify the most appropriate strategy for each situation. There is no “general rule” concerning modalities of seed priming and there is no clear trend of priming response according to the taxonomic position of the species [188]. This, undoubtedly, limits the commercial implementation of priming treatments.

Some priming treatments may imply a risk of medium contamination by fungi and bacteria, which may heavily impair subsequent seed germination [189]. This may especially be the case

for PEG and sometimes requires the simultaneous use of pesticide, although the impact of these compounds on the priming efficiency remains unknown. After priming treatment, seeds are dried back to their initial moisture content, but this dehydration phase is usually performed rapidly and occurs faster than classical dehydration of maturing seeds. It has been hypothesized that this brutal desiccation procedure alters the beneficial effect of priming [2].

The major drawback of priming is that it reduces the longevity of primed seeds as compared with the nonprimed seeds [190–192]. Storability of primed seed material is consequently reduced, and this results in higher costs for material maintenance for farmers and seed companies. The loss of viability, however, appears quite variable depending on the species, cultivars, and seed lots. In extreme cases, priming-induced advantages may even disappear after only 14 d of storage and the obtained seedling may then perform worse than those issued from unprimed seeds [193]. One of the limitations of these studies, however, is that experiments are commonly performed on artificially aged seeds using short-term exposure to high temperature in a moist environment, which is not necessarily fulfilling relevant from a real aging process. Some studies, using classical long-term strategies, reported that longevity is not necessarily affected or may even be increased by priming treatments [194]. Hussain et al. [195] recently demonstrated that post-priming temperature plays a key role in maintenance of seed longevity, which is indeed rapidly compromised in rice seeds maintained at room temperature while it remains intact when the material is stored at 4°C. According to these authors, the deleterious effect observed at 25°C storage was related to hampered starch metabolism in primed seeds. It was also suggested that maintenance of the seed longevity at 4°C may be due to a high viscosity that strongly reduces molecular mobility in the cytoplasm and thus limits the impact of deteriorative process even in seeds exhibiting low water content [196]. Oxygen during storage may trigger metabolic processes in primed seeds, which did not re-establish a true quiescent stage after dehydration when stored at room temperature while oxygen had no influence at low temperatures [195].

In some cases, repeat priming treatment after storage may partly remove the damaging effect on seed viability [193] while, in other cases, such a loss is permanent and not reversible [194]. Whatever, the fact that an additional treatment may be required to restore full germination potential represents an additional cost and source of variability.

12. Conclusions

Seed priming is an old empirical strategy used since centuries by farmers, and since decades by seed companies, to improve germination processes in cultivated plant species. The underlying mechanisms involved in this positive impact of pre-sowing treatments remained obscure for a long time. The present review aimed to summarize recent information provided by various tools allowing the identification of molecular cues conditioning priming efficiency.

Data obtained from molecular approaches applied to some well-known plant species (rice, rapeseed, tomato, etc.) are now available. The role of genes associated to metabolic and cell cycle events now starts to be deciphered, mainly those encoding for translational components

such as ribosomal subunits, translational initiation factor, enzyme involved in carbon metabolism, histones, and transcription factors. A putative epigenetic basis of priming effects should also be considered. Some authors identified enzyme activity changes in relation to priming while others also reported numerous changes in the seed storage protein. Priming has also been reported to increase proteins related to the cell cycle activities such as α - and δ -DNA polymerase. Protecting proteins like dehydrins or HSP is expected to assume protective functions during the dehydration step. Similarly, proteins involved in water transport, cell wall modification, cytoskeletal organization, and cell divisions, may be to some extent regulated during priming. Gene expression and enzyme activities involved in osmocompatible solute synthesis may be of primary importance to regulate tissue protection during the dehydration step and water fluxes during the final germination phase. Measurements of water uptake by primed seeds suggest a reduction in the lag time of imbibition. Water uptake and its subsequent cell-to-cell movement during germination might be controlled by aquaporins and expression of the corresponding genes constitutes a specific target of the priming treatment.

Among the different hypothesis proposed to explain the biochemical basis for germination improvement, DNA replication and cell cycle advancement during priming treatment as well as synchronization of the cell cycle at the G2 phase are supported by some experimental evidences. DNA synthesis is involved during priming treatment itself but also during post-germinative events. Changes are also observed with the modification of the membrane structure and reorganization of mitochondrial integrity. Activation of antioxidative properties by priming treatment may also explain the improved behavior of plant material, especially when final germination and/or growth occur under stress condition.

The priming-induced decrease of the storage capacity is a major limitation for the application of the priming technique by seed companies. Partial vacuum storage may be useful for extending the longevity of primed seeds. Improved longevity may be related to enhanced antioxidative activity that minimizes the accumulation of total peroxide during long-term storage. Another challenge for private seeds company is to identify appropriate treatments able to restore vigor of old dry seed lots in order to increase their mean percentage of germination to values compatible with commercial purposes.

If priming may undoubtedly be considered as a valuable strategy to improve stand establishment, its impact on final yield and crop production has not be always confirmed. Most studies devoted to “omics” approaches of seed priming are performed on young seedling cultivated under fully controlled environmental conditions. The link between seedling behavior in plant growth chambers and the adult plant performance in field conditions is far from being clear. As a consequence, there is an urgent need to focus on transcriptomic, proteomic, and metabolomics of adult plants issued from primed seeds, especially at the reproductive stage, in order to assess the long-term impact of priming on cultivated plants throughout the plant cycle.

In cultivated plant species, a given priming treatment also has contrasting effects on various cultivars. It may be hypothesized that the ability to respond to priming treatment might be genetically controlled but, to the best of our knowledge, no data are available concerning this important aspect. Thus, further progresses are needed not only to identify the set of

genes that are regulated by priming, but also the set of genes that putatively regulate priming response and efficiency themselves.

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