



HAL
open science

Reboot the system thanks to protein post-translational modifications and proteome diversity: How quiescent seeds restart their metabolism to prepare seedling establishment

Erwann E. Arc, Marc Galland, Gwendal Cueff, Béatrice B. Godin-Pérénez, Imen Lounifi Lounifi, Dominique Job, Loïc Rajjou

► To cite this version:

Erwann E. Arc, Marc Galland, Gwendal Cueff, Béatrice B. Godin-Pérénez, Imen Lounifi Lounifi, et al.. Reboot the system thanks to protein post-translational modifications and proteome diversity: How quiescent seeds restart their metabolism to prepare seedling establishment. *Proteomics*, 2011, 11 (9), pp.1606-1618. 10.1002/pmic.201000641 . hal-01000719

HAL Id: hal-01000719

<https://hal.science/hal-01000719v1>

Submitted on 10 Jul 2024

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

REVIEW

Reboot the system thanks to protein post-translational modifications and proteome diversity: How quiescent seeds restart their metabolism to prepare seedling establishment

Erwann Arc^{1,2*}, Marc Galland^{1,2*}, Gwendal Cueff^{1,2}, Béatrice Godin^{1,2}, Imen Lounifi^{1,2}, Dominique Job² and Loïc Rajjou^{1,2}

¹ Institut Jean-Pierre Bourgin (IJPB) Unité Mixte de Recherche, Institut National de la Recherche Agronomique-AgroParisTech – Team: Physiology of Seed Germination, Versailles, France

² AgroParisTech, Paris Institute of Technology for Life, Food and Environmental Sciences – Department of Health and Life Sciences – Chair of Plant Physiology, Paris, France

Once liberated in their environment, orthodox seeds live in a quiescent dehydrated state not totally exempt of essential molecular events as, for example, the capacity of breaking dormancy during after-ripening. Upon imbibition, if internal regulatory padlocks are released and given adequate external conditions, the quiescent seed is able to “reboot” its system and, thus, germinate. Recent studies unraveled the crucial importance of protein PTMs in seed dormancy, longevity and vigor. As compared to other plant developmental stages, the seed proteome appears quite unique and diverse. Seed proteins encompass several functional classes from primary and secondary metabolism to structural and antimicrobial defense. In the dry state, oxidative damages can occur due to reactive oxygen and nitrogen species produced by non-enzymatic reactions. These reactive species can affect proteins by the oxidation of their amino acids in a post-translational manner. The hormone abscisic acid regulates major aspects of seed life including dormancy and germination. This signaling pathway has been shown to rely on several PTMs such as protein phosphorylation or ubiquitination.

Received: October 7, 2010

Revised: December 5, 2010

Accepted: January 7, 2011



Keywords:

Germination / Plant / Plant proteomics / PTMs / Seed

1 Introduction

Seeds represent the main dispersal unit of flowering plants (Angiosperms). They contain an embryo (the future plant

Correspondence: Dr. Loïc Rajjou, Institut Jean-Pierre Bourgin (IJPB) Unité Mixte de Recherche 1318 Institut National de la Recherche Agronomique-AgroParisTech – Team: Physiology of Seed Germination, Route de St-Cyr, F-78026 Versailles Cedex, France

E-mail: loic.rajjou@agroparistech.fr

Fax: +33-8-21-46-33-40

Abbreviations: ABA, abscisic acid; GAs, gibberellins; LEA, late embryogenesis abundant; PIMT, protein L-isoaspartyl methyltransferase; RNS, reactive nitrogen species; SCF, Skp1/Cullin/F-box; SnRKs, sucrose nonfermenting-1 (Snf1)-related protein kinases; SSP, seed storage protein; Trx, thioredoxin

resulting from the fusion of the gametes), storage tissues (necessary for the establishment of the seedling) and a protective outer coat. According to their desiccation-tolerance properties, three main seed groups are differentiated: (i) viviparous, (ii) recalcitrant and (iii) orthodox seeds. This last group concerns most plant species of temperate climates. Orthodox seeds acquire tolerance to desiccation during their maturation on the mother plant and are dispersed in the environment in a quiescent dehydrated state [1]. Remarkably, they may remain in this state for many years, from decades to millennia, and still retain their viability [2]. Two milestones projects, namely the Millennium Seed and the Svalbard Global Seed Banks emerged as means to preserve seeds from Earth plant diversity for

*These authors contributed equally to this work.

centuries to go [3]. If seed banks provide a valuable mean to preserve biodiversity, it becomes utterly clear that once needed, seeds stored in such facilities will have to germinate with high vigor. However, even under the best storage conditions, naturally occurring physiological features such as dormancy or aging can limit germination performance. Seed dormancy is an innate seed property ensuring that germination will only occur at the appropriate time to optimize subsequent seedling growth [4]. Dry storage (after-ripening), cold-stratification and chemical treatments (e.g. nitrate) are effective factors in breaking dormancy. Upon imbibition, non-dormant seeds reactivate their metabolism to give rise to a new plant [5]. The plant hormones abscisic acid (ABA) and gibberellins (GAs) are central regulators of various aspects of seed physiology, ABA inhibiting and GAs promoting germination [6–12]. Global gene expression profiling approaches have brought a wealth of information on seed physiology [13, 14]. These studies provided evidence that post-translational control is crucial for seed germination [15, 16]. Because of mRNA processing, protein proteolysis and PTMs, a single gene can produce many different protein variants. Indeed, proteins can be subjected to more than 300 potential PTMs in vivo (<http://www.abrf.org/index.cfm/dm.home>), which may affect their functions including localization, complex formation, stability and activity [17]. Here, we discuss recent advances in our understanding of the processes associated with germination and vigor resulting from analyses of PTMs of essential seed proteins. These studies reveal novel aspects of seed biology and provide clues that will aid future investigations into this fascinating phase of plant development.

2 Seed proteome diversity

Seed proteome investigations carried out on crops and model plant species have revealed the high complexity of seed proteomes that generates a large diversity of protein functions by means of genetic polymorphism (inter- and intra-species), post-transcriptional modifications and PTMs (Supporting Information Table S1). Seeds contain a considerable array of proteins associated with a large variety of molecular functions, a number of which being unique in the plant life cycle. Furthermore, proteins are compartmentalized within seed tissues, suggesting a cooperation of these tissues for the success of seed maturation and germination [18–20]. Precursor forms of the seed storage proteins (SSPs, e.g. prolamins, 2S albumins, 7S globulins and 11/12S globulins) are synthesized during seed maturation in the ER, and the processed forms are accumulated in protein storage vacuoles to form the protein bodies [21]. It is well established that SSPs contribute to germination vigor and support early seedling growth when mobilized upon germination [22]. In *Arabidopsis* seeds, more than 100 isoforms of 12S cruciferins (12S globulins) have been characterized by 2-D PAGE [23]. Since *Arabidopsis* 12S

cruciferins are encoded by only four genes (At4g28520, At5g44120, At1g03880 and At1g03890), this suggests that these SSPs are subjected to a wide range of PTMs [24, 25]. Proteomic analyses also disclosed the accumulation in germinating seeds of an astonishing number of proteins involved in amino acid metabolism, energy pathway, secondary and hormone metabolism, protein translation and metabolism and stress response [26–28] (Supporting Information Table S1). Proteomic data highlighted the unexpected defense response protein levels in dry and germinating seeds, which most presumably are required for protection of the embryo until the establishment of a vigorous plantlet.

3 Role of ROS in protein quality and germination control

ROS play a key role in regulating seed germination through specific oxidation phenomena [29]. Protein structure and function can be altered by ROS produced either by cell metabolism or by external oxidants. There is evidence that proteins are the most important targets for oxidants [30]. Irreversible oxidative protein carbonylation, which is the most frequent type of PTM in response to oxidative stress [31, 32], plays a major role in protein turnover by provoking the degradation of aberrant, damaged or unnecessary proteins. Correlated with an important accumulation of ROS during stress and aging, protein carbonylation can become a troublemaker by alteration of vital functions ultimately leading to cell death. The role of protein carbonylation has been investigated in the context of seed physiology (Fig. 1). In sunflower seeds, dormancy breaking is associated with an increased level in the carbonylation of specific proteins [33]. In *Arabidopsis* seeds, carbonylation also targets specific proteins including 12S cruciferins, late embryogenesis abundant (LEA) proteins and HSPs [24]. While SSPs and LEA proteins are plant specific, HSPs are ubiquitous and known to be preferential targets of oxidative modifications in various systems [34]. Their high sensitivity toward oxidation suggests that HSPs could protect nascent proteins from oxidative damage. In agreement, transgenic *Arabidopsis* seeds over-accumulating a heat stress transcription factor display enhanced accumulation of HSPs and improved germination vigor [35, 36]. During germination, the carbonylated proteome is subject to profound modifications associated with an elimination of carbonylated proteins accumulated in dry seeds and with an increase in the carbonylation level of a large number of proteins involved in energy pathway (e.g. glycolytic enzymes) [24]. These results are in harmony with previous work, suggesting that blocking glycolysis could be beneficial for germination [37]. In these conditions, the glucose flux would be reoriented to the pentose phosphate pathway (PPP), providing cells with reducing power in the form of NADPH to surmount oxidative stress.

NADPH oxidases play a crucial role in germination by modulating ABA inhibition during this process [38].

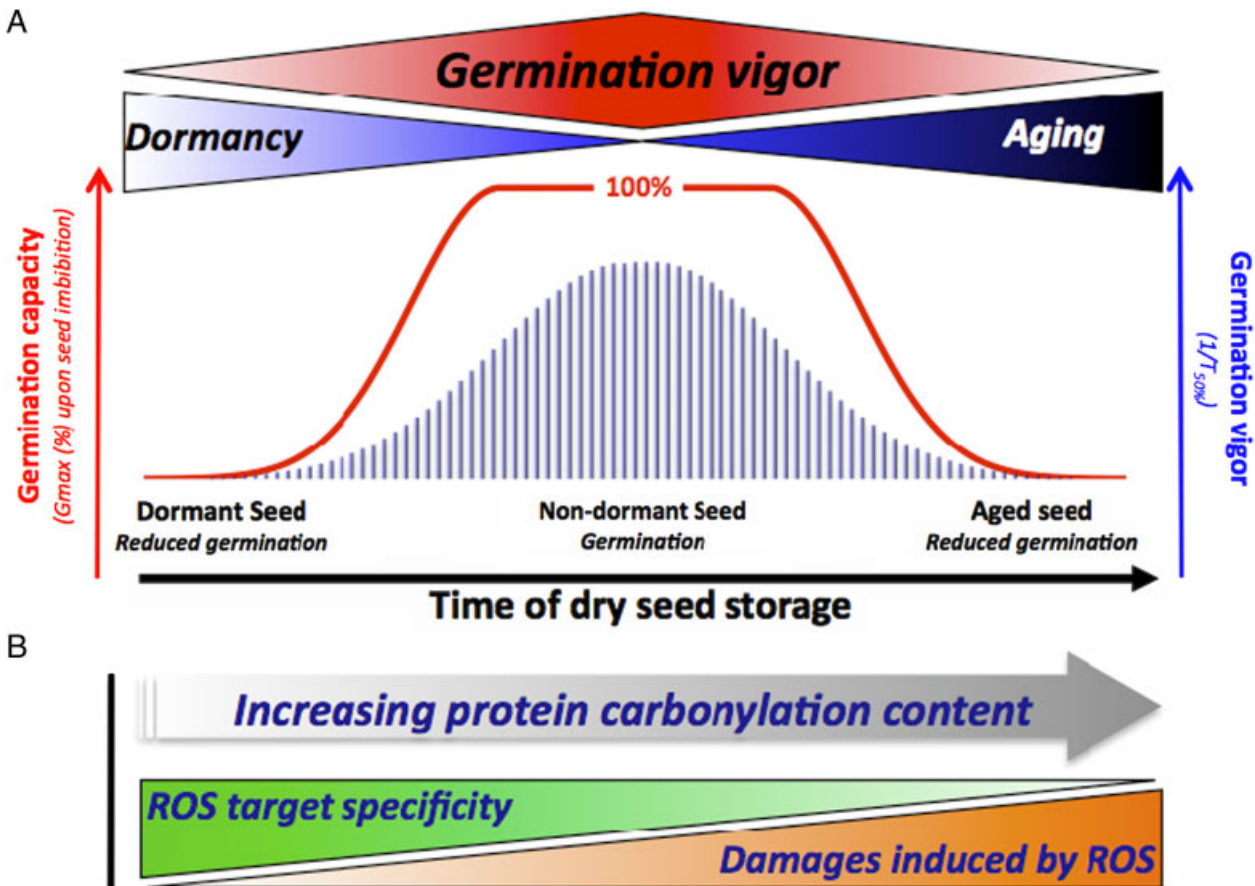


Figure 1. Model for the regulation of seed performance through protein carbonylation induced by ROS. (A) Orthodox seeds are almost metabolically inactive (quiescent) in the mature dry state. During dry storage or following seed imbibition, a wide range of PTMs can occur to modulate germination ability according to the seed environment. Red curve represents the germinative capacity (G_{\max} : maximum of germination) of a seed lot according to the time of dry storage. Blue bars correspond to 1/time to reach 50% of seed germination ($T_{50\%}$) as an indicator of seed vigor. (B) Proteins are the most important targets for ROS. The level of protein carbonylation increases during dormancy breaking by after-ripening [33] and during seed aging [40]. The specificity of the proteins targeted by ROS is reduced according with the time of storage, whereas functional damage progressively increases.

Recently, a role for the NADPH oxidase *AtrbohB* in after-ripening has been documented [39]. The *atrbohB* mutant fails to after-ripen and displays a reduced protein carbonylation pattern compared to the wild-type [39]. This suggests that ROS produced by *AtrbohB* during after-ripening could act either via ABA signaling or PTMs to control germination. Also, the progressive accumulation of oxidative damage in aged seeds was shown to contribute to the decline in germination ability [2], both during natural and artificial aging [40]. Importantly, these findings revealed that seed protein oxidation is not a random process but targets very specific proteins.

4 Nitric oxide-related protein modifications in seeds

Nitric oxide (NO^{\bullet}) is a gaseous free radical involved in numerous signalization pathways both in animals and

plants [41]. Studies on several plant species have shown that NO^{\bullet} can break seed dormancy [42] and promote germination in abiotic stress conditions [43, 44]. A transient accumulation of NO^{\bullet} has been observed during germination of soybean and sorghum seeds [45]. The same NO^{\bullet} burst was observed during the first hours of imbibition of *Arabidopsis* seeds resulting in an induction of ABA catabolism [46]. Although nitric oxide has been shown to modulate the metabolic activity in seeds, most of its direct primary targets remain unknown. NO^{\bullet} can bind to transition metals (metal nitrosylation) or cause protein PTMs such as cysteine S-nitrosylation or tyrosine nitration [41]. These modifications remain poorly characterized in plants and particularly in seeds. However, as discussed below, there is strong experimental evidence supporting the contention that nitric oxide signaling in seeds could principally rely on PTMs of specific proteins [47].

Nitration consists in the addition of a nitro group ($-\text{NO}_2$). Tyrosine nitration has been intensively studied [48]. Peroxy-

nitrite (ONOO^-) produced through the very fast reaction between NO^\bullet and O_2^- is considered as the main nitrating agent in vivo [49, 50]. Until recently, tyrosine nitration was considered as being irreversible, suggesting that the presence of nitrotyrosine in proteins represents a footprint of nitrosative stress. However, increasing evidence suggests the existence of a denitration mechanism in vivo [50]. Protein nitration can result in an alteration of diverse protein functions. For example, α -tubulin nitration was shown to affect plant cell division [51]. To our knowledge, only one study has been carried out on seeds, more precisely on sorghum embryonic axes [52]. This study revealed the appearance of several nitrated proteins after 24-h seed imbibition. Exogenous application of sodium nitroprussiate (a NO^\bullet donor) resulted in an alleviation of several oxidative damages but also in a significant increase in protein nitration [52]. Consequently, nitration may be more than a biological marker of nitrosative stress and could participate in protein turnover or signal transduction [53, 54].

S-nitrosylation consists in the covalent attachment of a NO^\bullet moiety to a reactive cysteine thiol resulting in the formation of a S-nitrosothiol group (S-NO). This PTM, which is thought to be particularly labile, is associated with a precise spatiotemporal regulation and can potentially result in the activation or inactivation of target proteins [55]. It occurs mainly through non-enzymatic reactions being dependent on the physicochemical environment of the protein cysteinyl residues and the proximity of susceptible proteins to NO^\bullet production sites in cells [56]. On the contrary, protein denitrosylation seems to be catalyzed by several enzymes, such as thioredoxins (Trxs) or Cu/Zn superoxide dismutases, as well as by reducing metals and intracellular reducing agents [56].

Due to the limited permeability of most of their outer layers, seeds can experience hypoxia [57]. Consequently, a fine regulation of oxygen consumption is necessary. This seems to be achieved through NO-mediated inhibition of seed mitochondrial activity [58]. Thus, NO^\bullet can bind to the heme a3/copper B binuclear center of cytochrome c oxidase (COX), thereby inhibiting its activity [59]. Moreover, under very low oxygen concentrations, hemeproteins, like cytochrome c and cytochrome c oxidase, could reduce nitrite to NO^\bullet [60]. Consequently, NO^\bullet -related protein modifications are likely to be increased in seed mitochondria and therefore play an important role in regulating the activity of these organelles. Many S-nitrosylated proteins identified in plants are implicated in metabolic processes [61–65], suggesting that NO^\bullet could participate in the regulation of the energy status of the seeds. In agreement, a beta subunit of the mitochondrial ATP synthase complex was found to be S-nitrosylated in dry *Arabidopsis* seeds (Supporting Information Fig. S1). Since a homologous protein was shown to be inactivated by S-nitrosylation in alcoholic fatty liver of rats [66], the seed mitochondrial ATP synthase activity might be inhibited by this PTM. Further experiments are required to assess this hypothesis.

Hypoxia also induces plant non-symbiotic class 1 hemoglobins (Hb1) expression. These hemeproteins display a very high affinity for O_2 and play a central role in the regulation of NO^\bullet internal content in plants [67, 68]. This is achieved either through an NADPH-dependent reduction of NO^\bullet to NO_3^- or through NO^\bullet scavenging potentially involving S-nitrosylation of the Hb1 protein [67, 68]. These hemeproteins are therefore important in protection against nitrosative stress and modulation of NO^\bullet signaling [69]. In cereals and *Arabidopsis*, the *Hb1* gene is induced upon seed imbibition [69, 70], concomitant with NO^\bullet release and the resumption of metabolism notably ATP synthesis [69, 71]. Consequently, Hb1 may play an important role in the germination process by regulating NO^\bullet homeostasis, thereby preventing nitrosative stress and maintaining energy and redox status [69].

5 Redox regulation of thiol groups by Trxs

In seeds, both ROS, such as hydrogen peroxide or hydroxyl and superoxide radicals, and reactive nitrogen species (RNS), such as NO^\bullet and peroxynitrite, play important roles. For example, upon imbibition, exogenous H_2O_2 can stimulate NO^\bullet production leading to an increased ABA catabolism and in turn to dormancy release [46]. On the other hand, a NO application can alleviate ROS-induced oxidative damages [43]. All these reactive species can induce protein oxidation, resulting either in signaling or protein alterations depending on their cellular concentrations. To get a clear picture of the redox mechanisms occurring in seeds, it is necessary to take ROS and RNS into account and to precisely characterize the redox status of the seeds at each developmental state. Gradual change upon time in seed ROS and RNS concentrations could have a large impact on seed developmental processes as it will regulate the redox environment of crucial proteins. Several amino acids can be affected by oxidative modifications, leading to a precise modulation of protein physicochemical properties and functions constituting the so-called “redox proteomics” [72, 73]. The redox state can influence protein levels and induce either specific reversible covalent PTMs, participating in the redox-based signaling, or oxidative irreversible damages such as protein carbonylation (see above). Therefore, focusing on a single modification does not render the complexity of the cellular response to oxidative stress or redox protein regulation [72]. Among amino acids susceptible to redox modifications, cysteinyl residues represent a major site of action due to their easily oxidized thiol groups. Consequently, “cysteine-based redox switches are now increasingly being found to underpin many different signaling systems and regulate physiological output across kingdoms” [73]. ROS- and RNS-promoted cysteine oxidation can result in S-nitrosylation (P-SNO), S-glutathionylation (P-S-S-G), intra or intermolecular disulfide bond (P-S-S-P)

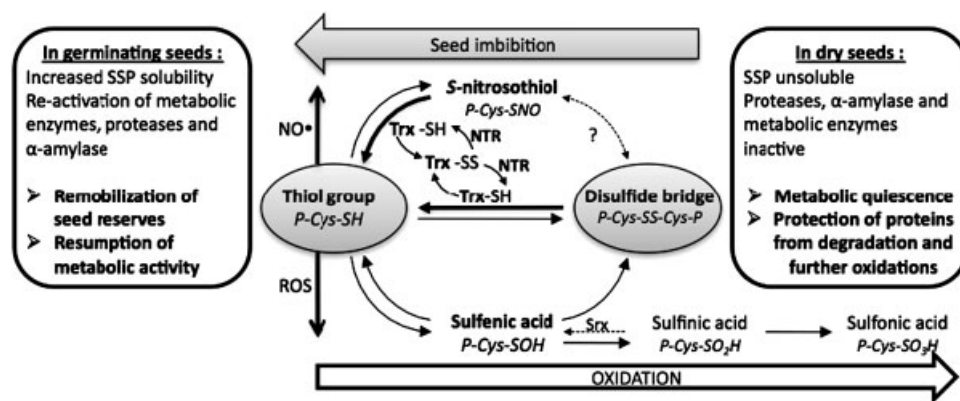


Figure 2. Current model for redox cysteine regulation during seed life. Upon seed imbibition, thioredoxins target several proteins reducing their disulfide bridges, which results in a modulation of their function. Trx: Thioredoxin; Srx: Sulfiredoxin; NTR: NADPH-dependent Trx reductase; P: Protein.

and sulfenic (P-SOH), sulfinic (P-SO₂H) or sulfonic acid (P-SO₃H) formation [72, 73]. To our knowledge, sulfinic and sulfonic acid formation have not yet been studied in seeds. On the other hand, reversible disulfide bond formation, which can be mediated via sulfenic acid formation, and reduction have been well characterized [74]. This PTM strongly impacts protein folding, stability and potential multimerization of target proteins. The resulting disulfide bridges, being relatively stable, can also constitute a protection against more damaging oxidation [73].

Protein disulfide bonds can be selectively reduced by Trxs and glutaredoxins, two protein families containing a conserved Trx motif composed of two cysteinyl residues separated by two amino acids (CxxC). This PTM is known to alter both protein target conformation and function [74, 75]. Hence, the cellular redox status is linked to the activity and regulations mediated by these enzymes. Trx activity relies on NADPH and NADPH-dependent Trx reductase catalyzing its reduction, hence the recycling of an active Trx. Many Trx targets have been identified in plants [76], especially in seeds [77, 78]. During seed development, proteins would be accumulated in their reduced state being subsequently oxidized during maturation and drying. Accordingly, many metabolic enzymes would be inactivated in dry mature quiescent seeds. In contrast, upon seed imbibition, Trx will reduce crucial oxidized proteins, thereby initiating the molecular switch leading to productive germination [77, 79]. The redox state of such important metabolic proteins could contribute to ensure maintenance of metabolic quiescence in the dry state and full resumption of metabolic activity during germination (Fig. 2). Owing to disulfide bond reduction during germination, Trx would increase the solubility of SSPs and in turn their susceptibility toward further proteolytic attack. Trxs would also inactivate several proteins such as enzyme inhibitors, notably protease or α -amylase inhibitors. In this way, Trxs can promote the remobilization of the seed storage reserves during germination [74, 76]. Studies conducted in animal systems suggested that Trxs can also reduce S-nitrosothiols [56]. Several other enzymes implicated in redox regulation and detoxification such as catalases, superoxide dismutases,

2-cys peroxiredoxins or glutaredoxins have been identified as putative S-nitrosylated proteins [61–65].

In conclusion, the accumulated evidence supports the central implication of redox-related protein modifications in seed physiology although the precise underlying mechanisms have not been entirely discovered yet. Importantly, seeds appear not only to be able to cope with reactive species but to use them as essential signaling elements.

6 Involvement of protein phosphorylation in seed physiology

Protein phosphorylation/dephosphorylation is the most widely known and well-studied form of reversible PTM. In plants, tyrosine, serine and threonine residues in polypeptide chains can be phosphorylated by protein kinases by the addition of a covalently bound phosphate group [80, 81]. Protein phosphorylation/dephosphorylation is involved in regulation of a large range of cellular functions through activation/inhibition of enzymes and transporters, protein degradation by the ATP-dependent ubiquitin/proteasome pathway and in the regulation of protein–protein interactions [80, 81]. The main studied role of protein phosphorylation is cell signaling through MAP kinase cascades for gene network regulation [82, 83]. However, the phosphoproteome of dry and germinating seeds is poorly documented. Yet, as shown in Fig. 3, de novo protein phosphorylation occurs specifically during *Arabidopsis* seed germination. A previous work showed that 12S cruciferins are the predominant phosphorylated proteins in dry mature *Arabidopsis* seeds [25]. A comparison of these previous results [25] with those shown in Fig. 3 indicates that phosphorylation of 12S cruciferins mainly occurs during seed maturation and not during germination. Considering the abundance of SSPs in mature seeds, the phosphorylation of these proteins could constitute a way to store phosphorus until germination. Thirty-three phosphorylated proteins have been revealed from a shotgun proteomic investigation of germinating maize embryos [84], including the mismatch binding protein Mus3 and the poly(ADP)-ribose

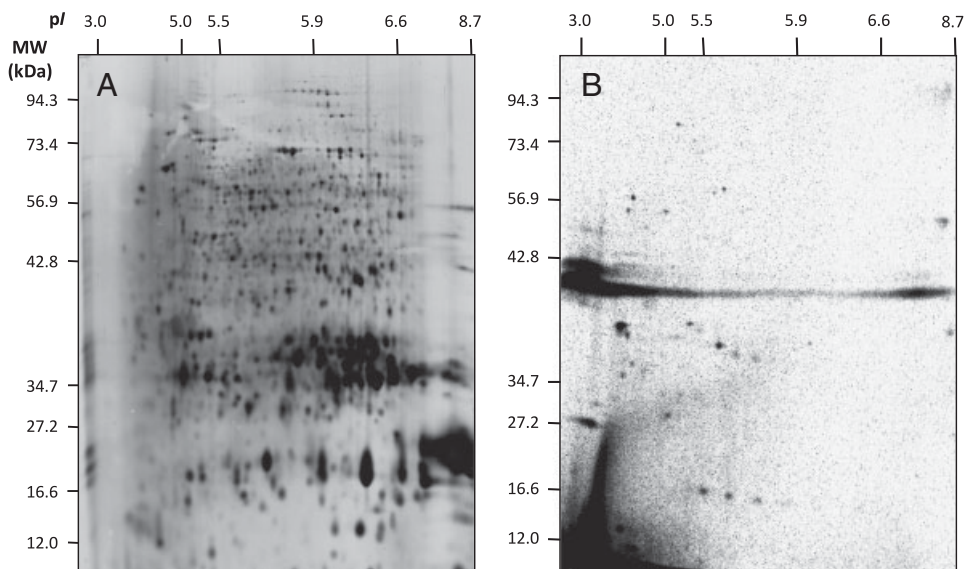


Figure 3. De novo protein phosphorylation during germination of *Arabidopsis* seeds. Seeds were incubated for 48 h in water in the presence of [^{33}P] phosphoric acid (Supporting Information text S1). Following 2-D PAGE, the radiolabeled proteins were revealed by phosphorimager analysis. (A) Silver-stained 2-D gel of total soluble proteins from *Arabidopsis* seeds incubated for 48 h in water. (B) Radiolabeled phosphoproteins from same gel as in (A).

polymerase 1 (PARP1). This suggests that Mus3 phosphorylation might be involved in mismatched DNA molecular recognition in the embryo during germination. Moreover, poly(ADP)-ribose polymerase 1 was shown to be essential to initiate early germination in *Arabidopsis* [85], supporting the finding that the maintenance of a functional DNA repair system is essential to ensure high germination vigor [2]. Thus, phosphorylation of enzymes involved in DNA repair might be crucial to allow a proper molecular control of seed germination. Protein translation plays a major role in germination vigor [14–16]. Indeed, *Arabidopsis* seed germination is strongly inhibited in the presence of cycloheximide (an inhibitor of translation) and protein translation ability is markedly reduced during seed aging [15, 40]. Initiation factors eIF4B, eIF2 α and eIF2 β are known to be differentially phosphorylated during wheat seed development and germination [86]. Phosphorylated isoforms of eIF4B strongly accumulate in the embryo at 10-h post-imbibition, a time point that correlates with the resumption of protein synthesis during germination. In contrast, eIF2 phosphorylation level in the embryo remains unchanged between 10 and 20 h of germination. Moreover, two 60S ribosomal subunits were shown to become phosphorylated upon formation of the cytosolic 60S ribosomal complex [87]. Altogether, these data suggest that during germination protein translation is under regulation of protein phosphorylation. Genetic and biochemical studies have identified a diversity of kinases, kinase inhibitors and phosphatases correlated with ABA response in seed biology [88]. For example, protein phosphorylation via mitogen-activated protein kinases (MAPKs) has been suggested to be important in ABA signaling and therefore in the control of seed maturation, dormancy and germination [88]. Furthermore, members of the sucrose nonfermenting-1 (Snf1)-related protein kinases (SnRKs) behave as key regulators of ABA signaling in seeds [89]. These kinases can be grouped

into three subfamilies: SnRK1, SnRK2 and SnRK3, the two last being unique to plants. During rice seed germination, α -amylases help to remobilize sugars by degrading the starch stored in amyloplasts [90]. In this process, SnRK1 plays a signaling role by activating the expression of the α -amylase3 gene [91]. The *Arabidopsis* genome contains 38 SnRK coding genes, 10 of which correspond to SnRK2 genes [92]. The phosphorylation of a family of basic leucine zipper transcription factors (ABFs/AREBs) by SnRK2 is involved in positive regulation of ABA-responsive gene expression [93]. Three members of the SnRK2 subclass III family (SRK2.2, SRK2.3 and SRK2.6) are mainly expressed in the nucleus during seed development and germination [94]. Although seed dormancy and germination are not affected in the *srk2.6* single mutant [95, 96], a viviparous phenotype is observed with the triple SnRK2 mutant plants when grown at high humidity [97], thus revealing a defect in the onset of dormancy [94]. Strikingly, the seed ABA content of this triple mutant is higher than that of wild type seeds. Among plant protein phosphatases 2C (PP2Cs), a group A subfamily (e.g. ABI1 and ABI2) behaves as negative regulators of SnRK2-mediated phosphorylation [98]. Indeed, *abi1* and *abi2* mutants display reduced dormancy [8]. Recently, Regulatory Component of ABA Receptor1/Pyrabactin Resistance1 (RCAR1/PYR1) and RCAR3 were highlighted as physical partners of ABI1 and ABI2 [99–101]. RCAR1/PYR1 directly binds to ABA and mediates ABA-dependent inactivation of ABI1 or ABI2 inducing a positive regulation of SnRK2-mediated phosphorylation involved in seed dormancy sustaining. Interestingly, several ethylene response mutants exhibit contrasted germination ability in close relation with ABA sensitivity [102]. Thus, *CTR1* gene, a negative regulator of the ethylene response pathway in *Arabidopsis*, encodes a Raf-like protein kinase [103]. The *ctr1* mutant seeds were less sensitive to ABA than the wild type. In opposite, the ethylene-insensitive mutants *ein2* and *etr1*

display increased seed dormancy due to an enhanced sensitivity to endogenous ABA [104]. Altogether, these data suggest that ABA and ethylene cross signaling might be involved in the control of seed germination through MAP kinase and phosphatase pathways. However, further research is needed to get a better understanding of these signaling pathways. It is also worth noting that phosphorylation and removal of phosphate groups have been demonstrated as effectors of the ABA transcription factor ABI5 that affects dormancy through the regulation of dormancy-related genes (see below) [94]. The first quantitative measurement of *in vivo* changes in the phosphoproteome following ABA treatment in planta has only been recently highlighted [105]. Several proteins have been characterized as potential early targets of the phosphorylation/dephosphorylation machinery involved in the ABA response *in vivo*. These results clearly document the major role of protein phosphorylation in the control of seed physiology through ABA signaling [94, 105].

7 Proteasome-related protein modifications

During germination, protein degradation progressively allows to switch from a quiescent developmental program to a highly active metabolic state typical of seedling establishment. The addition of at least four ubiquitin monomers to the lysine residues of a target protein triggers its degradation by the 26S proteasome [106, 107]. In plants, ubiquitin linkage to target proteins is mediated by E3 ligase enzymes in which the Skp1/Cullin/F-box (SCF) ubiquitin E3 ligase subfamily is particularly well described [106, 107]. The F-box protein belonging to the SCF complex provides protein substrate specificity. Given that as many as 700 F-box proteins are putatively present in *Arabidopsis*, it is expected that at least some of them play a role in seed physiology as demonstrated by studies on the control of germination by light or ABA [106]. First, red light is known to be a positive regulator of *Arabidopsis* seed germination through the conversion of phytochromes A and B (PHYA and PHYB) to their active Pfr isoform [108, 109]. The phytochrome interacting factor-3 (PIF3) was the first transcription factor identified among a 15-member family that proved able to interact with phytochromes and to regulate the expression of light-regulated genes [110]. Moreover, the signaling pathway encompassing light perception by PHYA and PHYB to the production of bioactive GAs needed for seed germination, through the action of PIL5, has been deciphered [89]. The PIL5 protein level is negatively regulated by the two phytochromes [89], most presumably through the action of the 26S proteasome as demonstrated for other PIL family members [111]. Second, an increasing number of studies pinpointed the ABI5 transcription factor, which is a component of the ABA signaling pathway, as a potential integrative node of PTMs [112, 113]. ABI5 is a bZIP tran-

scription factor identified as a key regulator of late seed maturation program as well as of a post-germinative control checkpoint [114]. In response to ABA, the stability of ABI5 is negatively regulated by ubiquitination through the ABI5 binding protein 1 (AFP1) [112]. Thus, this latter mechanism could represent a feedback regulatory process of the ABA signaling pathway to fine-tune the level of active ABI5. Last but not least, the ABI5 protein level was shown to be negatively regulated by the RING-type E3 ligase KEEP ON GOING (KEG), an essential regulator of ABA sensitivity. In the presence of ABA, this ligase is first phosphorylated and then subject to 26S proteasome degradation as it becomes ubiquitinated [115]. Finally, ABI5 can also be sumoylated by the E3 SUMO ligase SIZ1, which renders ABI5 inactive but also probably less sensitive to ubiquitination [113].

As stated above, GAs are major regulators of seed physiology. Central to the GA signaling pathway, the DELLA proteins, which belong to the GRAS family of transcription factors, act as repressors of GA-mediated responses [97, 116]. Among the five DELLA proteins identified in *Arabidopsis*, RGL2 (RGA-like 2) is considered as being the key player regulating germination in the light [117]. Upon imbibition, the level of bioactive GAs rises substantially while that of DELLA regulators drops [97]. GAs can bind to the GIBERELLIN-INSENSITIVE DWARF1 (AtGID1) cytosolic GA receptor thereby allowing recognition between GID1 and DELLAs [97, 118]. Then, the *Arabidopsis* F-box SLEEPY1 or its rice orthologue GIBERELLIN-INSENSITIVE DWARF1 (OsGID2) specifically interacts with DELLAs and mediate their ubiquitination via the SCF^{S_{LY}1/GID2} E3 ligase complex [97]. Upon linkage of several ubiquitin monomers, the DELLA repressors are addressed to the 26S proteasome thus releasing “the brakes” of germination [97].

When a protein exhibits destabilizing amino acids residues (N-degrons), a specialized and hierarchized protein degradation pathway named the N-end rule pathway can intervene [119–121]. Aspartate and glutamate residues located at the N-end terminal of destabilized proteins can be arginylated by the two *Arabidopsis* arginyl-tRNA:protein arginyltransferases (ATE) giving rise to secondary N-degrons [121]. Then, two UBR-box containing E3 ligases, namely PROTEOLYSIS1 (PRT1) and PRT6, recognize respectively aromatic and basic residues and address the destabilized proteins with a N-degron to the 26S proteasome [119–121]. Alternatively, PRT1 and PRT6 can function independently of ATEs. Concerning seed physiology, the *prt6* mutant displays an ABA-hypersensitive germination phenotype together with pronounced dormancy [121].

8 Other PTMs occurring in seeds

8.1 Protein L-isoaspartyl formation

Among the reversible PTMs described in seeds, it is worth mentioning the spontaneous and deleterious conversion of

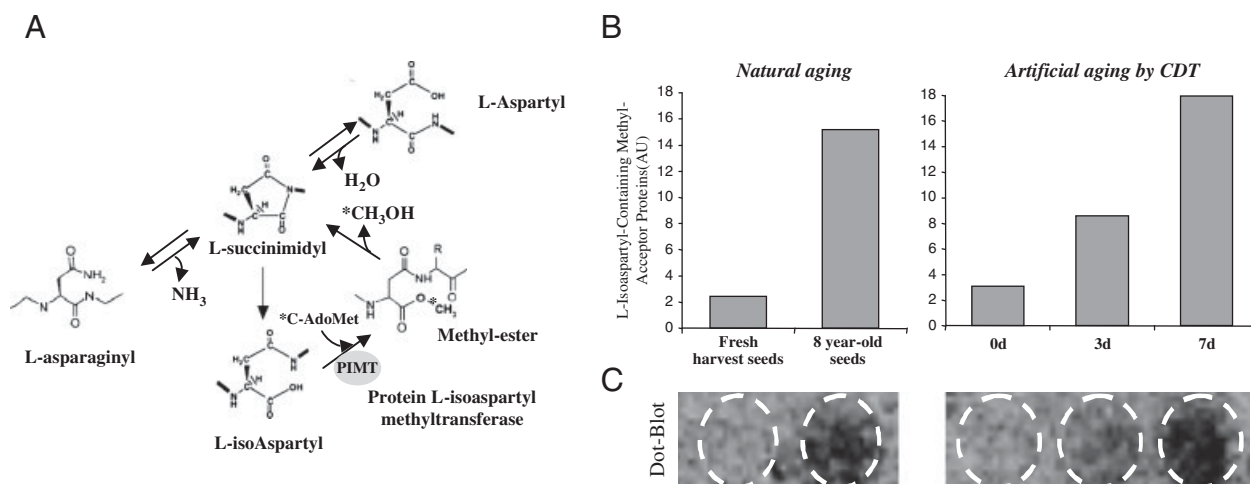


Figure 4. Formation of L-isoaspartyl residues during seed aging. (A) Mechanism of spontaneous L-isoaspartyl formation and enzymatic repair by PIMT. (B) Quantitation of L-isoaspartyl – containing methyl-accepting substrates. (C) The level of isoAsp residues was quantitated both in natural and artificial aged seed samples loaded onto a PVDF membrane. Protein extracts were methylated with recombinant PIMT and [methyl-¹⁴C]AdoMet (Supporting Information text S1). Radiolabeled proteins were revealed by phosphorimager analysis. The signal intensity of radiolabeled proteins was quantified with ImageQuant 5.2 software.

L-asparaginyl and L-aspartyl protein residues into L-iso-Asp, yielding altered proteins that could be reversibly repaired by protein L-isoaspartyl methyltransferase (PIMT) [122]. This enzyme, which catalyzes the transfer of a methyl group from S-adenosylmethionine to L-isoaspartyl residues in proteins, is widely distributed in nature. Previous work documented an increase in the extent of L-isoaspartyl residues during seed aging [123, 124]. Figure 4 shows that accumulation of L-isoaspartyl residues displays common features in artificially and naturally aged *Arabidopsis* seeds, testifying the usefulness of controlled deterioration treatment (CDT) for quickly assessing seed viability. Consistent with this, genetic modulation of *PIMT* expression in *Arabidopsis* showed that repair of age-related protein damage contributes significantly to seed longevity [125].

8.2 Gene expression regulation through histone modifications

Eukaryotic genomic DNA is wrapped around a tetramer of histone proteins that can be subject to PTMs on their N-terminal tails [126], associated with activation or repression of gene expression [126, 127]. During germination, the PICKLE SWI/SNF chromatin-remodeling factor is necessary for the repression of *ABI3* and *ABI5* expression during germination [128, 129]. Chromatin immunoprecipitation experiments showed that H3 histones bound to the *ABI3* and *ABI5* promoters are methylated on their lysine residues 9 and 27 [129]. Similarly, several genes encoding LEA proteins were shown to be transcriptionally repressed by histone deacetylases [130]. Moreover, the *reduced dormancy4* (*rdo4*) mutation proved to be affected in the HISTONE

MONOUBIQUITINATION1 (HUB1) RING finger protein that is required to covalently attach an ubiquitin monomer on histone H2B [127]. Thus, the single attachment of an ubiquitin monomer could serve as an inactivating regulation that does not necessarily drive to the target protein degradation. Thus, epigenetic regulation might help to switch from the mature seed quiescent state to seedling establishment.

8.3 Farnesylation

ENHANCED RESPONSE TO ABA 1 (*ERA1*) encodes a farnesyl transferase that transfers a 15-carbon farnesyl group together with a pyrophosphate to proteins with a specific COOH-terminal motif [131]. It is noteworthy that the first identification of *ERA1* function came from a seed-based screening assay. The *era1* mutant, which is hypersensitive to ABA, is both more dormant and more sensitive to very small ABA concentrations during germination [131]. Thus, the addition of a farnesyl group to an as yet unknown ABA-related signaling component serves as a powerful ABA-sensitivity regulation mechanism.

8.4 Glycosylation

Protein glycosylation is an enzyme-driven PTM that adds a sugar chain to a protein either on asparagine (N-glycosylation) or on serine/threonine residues (O-glycosylation) [132, 133]. In common bean seeds, SSPs have been shown to be glycosylated in the ER [134]. A substantial number of SSPs can be glycosylated (N- or O-) in the ER and the Golgi apparatus before

being incorporated in the protein storage vacuoles [21]. While the biological significance of glycosylation in seed germination remains to be determined, de-N-glycosylation, i.e. the removal of N-glycans either at the extremities or in the middle of protein sequences by specific enzymes called respectively ENGases and PNGases, was shown to occur in germinating radish and barley seeds [135, 136]. These two enzymatic activities were found to occur in dry seeds with the ENGase activity being predominant in the embryo while the PNGase activity was similar in both seed compartments. Upon imbibition, ENGase and PNGase activities increase rapidly in the embryo and endosperm, respectively [135, 136].

9 Concluding remarks

The present review underpinned several specific features on the role of PTMs in the control of seed germination. Seed proteins undergo a wide array of non-enzymatic and enzymatic PTMs during dry storage and upon imbibition. These PTMs play a central role in dormancy release, metabolism resumption during germination and aging processes. Proteome diversity and plasticity resulting from PTMs give to seeds an important adaptive capacity to their environment. However important progress remains to be realized in two directions. First, technological advancements in high throughput quantitative assays for PTMs are needed. Conception of “tool sets” for diverse classes of PTMs would help researchers in seed science research. Second, more intensive basic research is needed to gain a better understanding of the roles PTMs play in dormancy, germination vigor and aging processes. The discovery phase of these modifications should be broadly inclusive and involve a wide array of physiological conditions, mutant collections and natural genetic variations to study seed physiology.

The authors have declared no conflict of interest.

10 References

- [1] Baskin, C. C., Baskin, J. M., Germination ecophysiology of herbaceous plant species in a temperate region. *Am. J. Bot.* 1988, **75**, 286–305.
- [2] Rajjou, L., Debeaujon, I., Seed longevity: survival and maintenance of high germination ability of dry seeds. *C. R. Biol.* 2008, **331**, 796–805.
- [3] Charles, D., A “Forever” seed bank takes root in the Arctic. *Science* 2006, **312**, 1730–1731.
- [4] Finch-Savage, W. E., Leubner-Metzger, G., Seed dormancy and the control of germination. *New Phytol.* 2006, **171**, 501–523.
- [5] Bewley, J. D., Black, M., *Seeds: Physiology of Development and Germination*, Plenum Press, New York 1994.
- [6] Finkelstein, R., Reeves, W., Ariizumi, T., Steber, C., Molecular aspects of seed dormancy. *Annu. Rev. Plant Biol.* 2008, **59**, 387–415.
- [7] Nambara, E., Marion-Poll, A., Abscisic acid biosynthesis and catabolism. *Annu. Rev. Plant Biol.* 2005, **56**, 165–185.
- [8] Koornneef, M., Léon-Kloosterziel, K. M., Schwartz, S. H., Zeevaart, J. A., The genetic and molecular dissection of abscisic acid biosynthesis and signal transduction in *Arabidopsis*. *Plant Physiol.* 1998, **36**, 83–89.
- [9] Ali-Rachedi, S., Bouinot, D., Wagner, M., Bonnet, M. B. et al., Changes in endogenous abscisic acid levels during dormancy release and maintenance of mature seeds: studies with the Cape Verde Islands ecotype, the dormant. *Planta* 2004, **219**, 479–488.
- [10] Kushiro, T., Okamoto, M., Nakabayashi, K., Yamagishi, K. et al., The *Arabidopsis* cytochrome P450 CYP707A encodes ABA 8'-hydroxylases: key enzymes in ABA catabolism. *EMBO J.* 2004, **23**, 1647–1656.
- [11] Gallardo, K., Job, C., Groot, S., Puype, M. et al., Proteomics of *Arabidopsis* seed germination. A comparative study of wild-type and gibberellin-deficient seeds. *Plant Physiol.* 2002, **129**, 823–837.
- [12] Richards, D. E., King, K. E., Ait-Ali, T., Harberd, N. P., How gibberellin regulates plant growth and development: A molecular genetic analysis of gibberellin signaling. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 2001, **52**, 67–88.
- [13] North, H., Baud, S., Debeaujon, I., Dubos, C. et al., *Arabidopsis* seed secrets unravelled after a decade of genetic and omics-driven research. *Plant J.* 2010, **61**, 971–981.
- [14] Holdsworth, M. J., Bentsink, L., Soppe, W. J., Molecular networks regulating *Arabidopsis* seed maturation, after-ripening, dormancy and germination. *New Phytol.* 2008, **179**, 33–54.
- [15] Rajjou, L., Gallardo, K., Debeaujon, I., Vandekerckhove, J. et al., The effect of alpha-amanitin on the *Arabidopsis* seed proteome highlights the distinct roles of stored and neosynthesized mRNAs during germination. *Plant Physiol.* 2004, **134**, 1598–1613.
- [16] Kimura, M., Nambara, E., Stored and neosynthesized mRNA in *Arabidopsis* seeds: effects of cycloheximide and controlled deterioration treatment on the resumption of transcription during imbibition. *Plant Mol. Biol.* 2010, **73**, 119–129.
- [17] Ytterberg, A. J., Jensen, O. N., Modification-specific proteomics in plant biology. *J. Proteomics* 2010, **73**, 2249–2266.
- [18] Catusse, J., Strub, J. M., Job, C., Van Dorselaer, A., Job, D., Proteome-wide characterization of sugarbeet seed vigor and its tissue specific expression. *Proc. Natl. Acad. Sci. USA* 2008, **105**, 10262–10267.
- [19] Gallardo, K., Firnhaber, C., Zuber, H., Hélicher, D. et al., A combined proteome and transcriptome analysis of developing *Medicago truncatula* seeds: evidence for metabolic specialization of maternal and filial tissues. *Mol. Cell. Proteomics* 2007, **6**, 2165–2179.
- [20] Müller, K., Job, C., Belghazi, M., Job, D., Leubner-Metzger, G., Proteomics reveal tissue-specific features of the cress (*Lepidium sativum* L.) endosperm cap proteome and its hormone-induced changes during seed germination. *Proteomics* 2010, **10**, 406–416.

- [21] Herman, E., Larkins, B., Protein storage bodies and vacuoles. *Plant Cell* 1999, *11*, 601–614.
- [22] Müntz, K., Belozersky, M. A., Dunaevsky, Y. E., Schlereth, A., Tiedemann, J., Stored proteinases and the initiation of storage protein mobilization in seeds during germination and seedling growth. *J. Exp. Bot.* 2001, *52*, 1741–1752.
- [23] Gallardo, K., Job, C., Groot, S. P., Puype, M. et al., Proteomic analysis of *Arabidopsis* seed germination and priming. *Plant Physiol.* 2001, *126*, 835–848.
- [24] Job, C., Rajjou, L., Lovigny, Y., Belghazi, M., Job, D., Patterns of protein oxidation in *Arabidopsis* seeds and during germination. *Plant Physiol.* 2005, *138*, 790–802.
- [25] Wan, L., Ross, A. R., Yang, J., Hegedus, D. D., Kermode, A. R., Phosphorylation of the 12S globulin cruciferin in wild-type and *abi1-1* mutant *Arabidopsis thaliana* (thale cress) seeds. *Biochem. J.* 2007, *404*, 247–256.
- [26] Jerkovic, A., Kriegel, A. M., Bradner, J. R., Atwell, B. J. et al., Strategic distribution of protective proteins within bran layers of wheat protects the nutrient-rich endosperm. *Plant Physiol.* 2010, *152*, 1459–1470.
- [27] Rajjou, L., Miché, L., Huguet, R., Job, C., Job, D., in Adkins, S. W., Ahmore, S., Navie, S. (Eds.), *Seeds: Biology, Development and Ecology*, CAB International publishing, Cambridge 2006, pp. 149–158.
- [28] Dunwell, J. M., Khuri, S., Gane, P. J., Microbial relatives of the seed storage proteins of higher plants: conservation of structure and diversification of function during evolution of the cupin superfamily. *Microbiol. Mol. Biol. R.* 2000, *64*, 153–179.
- [29] Bailly, C., Active oxygen species and antioxidants in seed biology. *Seed Sci. Res.* 2004, *14*, 93–107.
- [30] Davies, M. J., The oxidative environment and protein damage. *Biochim. Biophys. Acta* 2005, *1703*, 93–109.
- [31] Møller, I. M., Jensen, P. E., Hansson, A., Oxidative modifications to cellular components in plants. *Annu. Rev. Plant Biol.* 2007, *58*, 459–481.
- [32] Nyström, T., Role of oxidative carbonylation in protein quality control and senescence. *EMBO J.* 2005, *24*, 1311–1317.
- [33] Oracz, K., El-Maarouf Bouteau, H., Farrant, J. M., Cooper, K. et al., ROS production and protein oxidation as a novel mechanism for seed dormancy alleviation. *Plant J.* 2007, *50*, 452–465.
- [34] Fredriksson, Å., Ballesteros, M., Dukan, S., Nyström, T., Defense against protein carbonylation by DnaK/DnaJ and proteases of the heat shock regulon. *J. Bacteriol.* 2005, *187*, 4207–4213.
- [35] Prieto-Dapena, P., Castaño, R., Almoguera, C., Jordano, J., Improved resistance to controlled deterioration in transgenic seeds. *Plant Physiol.* 2006, *142*, 1102–1112.
- [36] Almoguera, C., Prieto-Dapena, P., Díaz-Martín, J., Espinosa, J. M. et al., The HaDREB2 transcription factor enhances basal thermotolerance and longevity of seeds through functional interaction with HaHSFA9. *BMC Plant Biol.* 2009, *9*, 75.
- [37] Côme, D., Corbineau, F., in: Taylorson, R. B. (Ed.), *Recent Advances in the Development and Germination of Seeds*, NATO ASI series. Series A, Life sciences, New York 1989, pp. 156–179.
- [38] Kwak, J. M., Mori, I. C., Pei, Z., Leonhardt, N. et al., NADPH oxidase *AtrbohD* and *AtrbohF* genes function in ROS-dependent ABA signaling in *Arabidopsis*. *EMBO J.* 2003, *22*, 2623–2633.
- [39] Müller, K., Carstens, A. C., Linkies, A., Torres, M. A., Leubner-Metzger, G., The NADPH-oxidase *AtrbohB* plays a role in *Arabidopsis* seed after-ripening. *New Phytol.* 2009, *184*, 885–897.
- [40] Rajjou, L., Lovigny, Y., Groot, S. P., Belghazi, M. et al., Proteome-wide characterization of seed aging in *Arabidopsis*: a comparison between artificial and natural aging protocols. *Plant Physiol.* 2008, *148*, 620–641.
- [41] Moreau, M., Lindermayr, C., Durner, J., Klessig, D. F., NO synthesis and signaling in plants - where do we stand? *Physiol. Plant.* 2010, *138*, 372–383.
- [42] Bethke, P. C., Libourel, I. G., Reinöhl, V., Jones, R. L., Sodium nitroprusside, cyanide, nitrite, and nitrate break *Arabidopsis* seed dormancy in a nitric oxide-dependent manner. *Planta* 2006, *223*, 805–812.
- [43] Zhang, H., Shen, W., Xu, L., Effect of nitric oxide on the germination of wheat seeds and its reactive oxygen species metabolisms under osmotic stress. *Acta Bot. Sin.* 2003, *45*, 901–905.
- [44] Zhao, M., Tian, Q., Zhang, W., Nitric oxide synthase-dependent nitric oxide production is associated with salt tolerance in *Arabidopsis*. *Plant Physiol.* 2007, *144*, 206–217.
- [45] Simontacchi, M., Jasid, S., Puntarulo, S., in: Lamattina, L., Polacco, J. C. (Eds.), *Nitric Oxide in Plant Growth, Development and Stress Physiology*, Plant Cell Monographs, Springer, Heidelberg 2007, pp. 73–90.
- [46] Liu, Y., Shi, L., Ye, N., Liu, R. et al., Nitric oxide-induced rapid decrease of abscisic acid concentration is required in breaking seed dormancy in *Arabidopsis*. *New Phytol.* 2009, *183*, 1030–1042.
- [47] Delledonne, M., NO news is good news for plants. *Curr. Opin. Plant Biol.* 2005, *8*, 390–396.
- [48] Corpas, F. J., Chaki, M., Leterrier, M., Barroso, J. B., Protein tyrosine nitration: a new challenge in plants. *Plant Signal. Behav.* 2009, *4*, 920–923.
- [49] Ducrocq, C., Blanchard, B., Pignatelli, B., Ohshima, H., Peroxynitrite: an endogenous oxidizing and nitrating agent. *Cell. Mol. Life Sci.* 1999, *55*, 1068–1077.
- [50] Abello, N., Kerstjens, H. A., Postma, D. S., Bischoff, R., Protein tyrosine nitration: selectivity, physicochemical and biological consequences, denitration, and proteomics methods for the identification of tyrosine-nitrated proteins. *J. Proteome Res.* 2009, *8*, 3222–3238.
- [51] Jovanović, A. M., Durst, S., Nick, P., Plant cell division is specifically affected by nitrotyrosine. *J. Exp. Bot.* 2010, *61*, 901–909.
- [52] Jasid, S., Simontacchi, M., Puntarulo, S., Exposure to nitric oxide protects against oxidative damage but increases the

- labile iron pool in sorghum embryonic axes. *J. Exp. Bot.* 2008, *59*, 3953–3962.
- [53] Ischiropoulos, H., Protein tyrosine nitration-An update. *Arch. Biochem. Biophys.* 2009, *484*, 117–121.
- [54] Corpas, F., Barroso, J., Carreras, A., Valderrama, R., in: Lamattina, L., Polacco, J. C. (Eds.), *Nitric Oxide in Plant Growth, Development and Stress Physiology*, Plant Cell Monographs, Springer, Heidelberg 2007, pp. 187–205.
- [55] Hess, D., Matsumoto, A., Kim, S., Marshall, H., Stamler, J., Protein S-nitrosylation: purview and parameters. *Nat. Rev. Mol. Cell Bio.* 2005, *6*, 150–166.
- [56] Lindermayr, C., Durner, J., S-Nitrosylation in plants: Pattern and function. *J. Proteomics* 2009, *73*, 1–9.
- [57] Borisjuk, L., Rolletschek, H., The oxygen status of the developing seed. *New Phytol.* 2009, *182*, 17–30.
- [58] Borisjuk, L., Macherel, D., Benamar, A., Wobus, U., Rolletschek, H., Low oxygen sensing and balancing in plant seeds: a role for nitric oxide. *New Phytol.* 2007, *176*, 813–823.
- [59] Benamar, A., Rolletschek, H., Borisjuk, L., Avelange-Macherel, M. et al., Nitrite-nitric oxide control of mitochondrial respiration at the frontier of anoxia. *Biochim. Biophys. Acta* 2008, *1777*, 1268–1275.
- [60] Igamberdiev, A. U., Bykova, N. V., Shah, J. K., Hill, R. D., Anoxic nitric oxide cycling in plants: participating reactions and possible mechanisms. *Physiol. Plant.* 2010, *138*, 393–404.
- [61] Abat, J. K., Mattoo, A. K., Deswal, R., S-nitrosylated proteins of a medicinal CAM plant *Kalanchoe pinnatifida* 1,5-bisphosphate carboxylase/oxygenase activity targeted for inhibition. *FEBS J.* 2008, *275*, 2862–2872.
- [62] Lindermayr, C., Saalbach, G., Durner, J., Proteomic identification of S-nitrosylated proteins in *Arabidopsis*. *Plant Physiol.* 2005, *137*, 921–930.
- [63] Palmieri, M. C., Lindermayr, C., Bauwe, H., Steinhauser, C., Durner, J., Regulation of plant glycine decarboxylase by S-nitrosylation and glutathionylation. *Plant Physiol.* 2010, *152*, 1514–1528.
- [64] Romero-Puertas, M. C., Campostrini, N., Matte, A., Righetti, P. G., Proteomic analysis of S-nitrosylated proteins in *Arabidopsis thaliana* undergoing hypersensitive response. *Proteomics* 2008, *8*, 1459–1469.
- [65] Tanou, G., Job, C., Rajjou, L., Arc, E. et al., Proteomics reveals the overlapping roles of hydrogen peroxide and nitric oxide in the acclimation of citrus plants to salinity. *Plant J.* 2009, *60*, 795–804.
- [66] Moon, K., Hood, B. L., Kim, B., Hardwick, J. P. et al., Inactivation of oxidized and S-nitrosylated mitochondrial proteins in alcoholic fatty liver of rats. *Hepatology* 2006, *44*, 1218–1230.
- [67] Dordas, C., Hasinoff, B. B., Rivoal, J., Hill, R. D., Class-1 hemoglobins, nitrate and NO levels in anoxic maize cell-suspension cultures. *Planta* 2004, *219*, 66–72.
- [68] Perazzolli, M., Romero-Puertas, M. C., Delledonne, M., Modulation of nitric oxide bioactivity by plant haemoglobins. *J. Exp. Bot.* 2006, *57*, 479–488.
- [69] Hebelstrup, K. H., Igamberdiev, A. U., Hill, R. D., Metabolic effects of hemoglobin gene expression in plants. *Gene* 2007, *398*, 86–93.
- [70] Winter, D., Vinegar, B., Nahal, H., Ammar, R. et al., An “Electronic Fluorescent Pictograph” browser for exploring and analyzing large-scale biological data sets. *PLoS ONE* 2007, *2*, e718.
- [71] Borisjuk, L., Rolletschek, H., Nitric oxide is a versatile sensor of low oxygen stress in plants. *Plant Signal. Behav.* 2008, *3*, 391–393.
- [72] Rinalducci, S., Murgiano, L., Zolla, L., Redox proteomics: basic principles and future perspectives for the detection of protein oxidation in plants. *J. Exp. Bot.* 2008, *59*, 3781–3801.
- [73] Spadaro, D., Yun, B., Spoel, S. H., Chu, C. et al., The redox switch: dynamic regulation of protein function by cysteine modifications. *Physiol. Plant.* 2010, *138*, 360–371.
- [74] Buchanan, B. B., Balmer, Y., Redox regulation: a broadening horizon. *Annu. Rev. Plant Biol.* 2005, *56*, 187–220.
- [75] Meyer, Y., Buchanan, B. B., Vignols, F., Reichheld, J., Thioredoxins and glutaredoxins: unifying elements in redox biology. *Annu. Rev. Genet.* 2009, *43*, 335–367.
- [76] Montrichard, F., Alkhalifioui, F., Yano, H., Vensel, W. H. et al., Thioredoxin targets in plants: the first 30 years. *J. Proteomics* 2009, *72*, 452–474.
- [77] Alkhalifioui, F., Renard, M., Vensel, W. H., Wong, J. et al., Thioredoxin-linked proteins are reduced during germination of *Medicago truncatula* seeds. *Plant Physiol.* 2007, *144*, 1559–1579.
- [78] Wong, J., Cai, N., Balmer, Y., Tanaka, C. et al., Thioredoxin targets of developing wheat seeds identified by complementary proteomic approaches. *Phytochemistry* 2004, *65*, 1629–1640.
- [79] Yano, H., Wong, J., Lee, Y., Cho, M., A strategy for the identification of proteins targeted by thioredoxin. *Proc. Natl. Acad. Sci. USA* 2001, *98*, 4794–4799.
- [80] Kersten, B., Agrawal, G. K., Durek, P., Neigenfind, J. et al., Plant phosphoproteomics: an update. *Proteomics* 2009, *9*, 964–988.
- [81] Kersten, B., Agrawal, G. K., Iwahashi, H., Rakwal, R., Plant phosphoproteomics: a long road ahead. *Proteomics* 2006, *6*, 5517–5528.
- [82] Mishra, N. S., Tuteja, R., Tuteja, N., Signaling through MAP kinase networks in plants. *Arch. Biochem. Biophys.* 2006, *452*, 55–68.
- [83] Colcombet, J., Hirt, H., *Arabidopsis* MAPKs: a complex signalling network involved in multiple biological processes. *Biochem. J.* 2008, *413*, 217–226.
- [84] Lu, T., Meng, L., Yang, C., Liu, G. et al., A shotgun phosphoproteomics analysis of embryos in germinated maize seeds. *Planta* 2008, *228*, 1029–1041.
- [85] Hunt, L., Holdsworth, M. J., Gray, J. E., Nicotinamidase activity is important for germination. *Plant J.* 2007, *51*, 341–351.
- [86] Le, H., Browning, K. S., Gallie, D. R., The phosphorylation state of the wheat translation initiation factors eIF4B,

- elF4A, and eIF2 is differentially regulated during seed development and germination. *J. Biol. Chem.* 1998, *273*, 20084–20089.
- [87] Montoya-García, L., Muñoz-Ocoteo, V., Aguilar, R., Sánchez de Jiménez, E., Regulation of acidic ribosomal protein expression and phosphorylation in maize. *Biochemistry* 2002, *41*, 10166–10172.
- [88] Hubbard, K. E., Nishimura, N., Hitomi, K., Getzoff, E. D., Schroeder, J. I., Early abscisic acid signal transduction mechanisms: newly discovered components and newly emerging questions. *Genes Dev.* 2010, *24*, 1695–1708.
- [89] Oh, E., Yamaguchi, S., Kamiya, Y., Bae, G. et al., Light activates the degradation of PIL5 protein to promote seed germination through gibberellin in *Arabidopsis*. *Plant J.* 2006, *47*, 124–139.
- [90] Fincher, G. B., Molecular and cellular biology associated with endosperm mobilization in germinating cereal grains. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 1989, *40*, 305–346.
- [91] Lu, C., Lin, C., Lee, K., Chen, J. et al., The SnRK1A protein kinase plays a key role in sugar signaling during germination and seedling growth of rice. *Plant Cell* 2007, *19*, 2484–2499.
- [92] Hrabak, E., Chan, C., Gribskov, M., Harper, J. et al., The *Arabidopsis* CDPK-SnRK superfamily of protein kinases. *Plant Physiol.* 2003, *132*, 666–680.
- [93] Fujii, H., Verslues, P. E., Zhu, J., Identification of two protein kinases required for abscisic acid regulation of seed germination, root growth, and gene expression in *Arabidopsis*. *Plant Cell* 2007, *19*, 485–494.
- [94] Nakashima, K., Fujita, Y., Kanamori, N., Katagiri, T. et al., Three *Arabidopsis* SnRK2 protein kinases, SRK2D/SnRK2.2, SRK2E/SnRK2.6/OST1 and SRK2I/SnRK2.3, involved in ABA signaling are essential for the control of seed development and dormancy. *Plant Cell Physiol.* 2009, *50*, 1345–1363.
- [95] Mustilli, A., Merlot, S., Vavasseur, A., Fenzi, F., *Arabidopsis* OST1 protein kinase mediates the regulation of stomatal aperture by abscisic acid and acts upstream of reactive oxygen species production. *Plant Cell* 2002, *14*, 3089–3099.
- [96] Yoshida, R., Hobo, T., Ichimura, K., Mizoguchi, T. et al., ABA-activated SnRK2 protein kinase is required for dehydration stress signaling in *Arabidopsis*. *Plant Cell Physiol.* 2002, *43*, 1473–1483.
- [97] Achard, P., Genschik, P., Releasing the brakes of plant growth: how GAs shutdown DELLA proteins. *J. Exp. Bot.* 2009, *60*, 1085–1092.
- [98] Brock, A.K., Willmann, R., Kolb, D., Grefen, L. et al., The *Arabidopsis thaliana* mitogen-activated protein kinase (MAPK) phosphatase PP2C5 affects seed germination, stomatal aperture and abscisic acid-inducible gene expression. *Plant Physiol.* 2010, *153*, 1098–1111.
- [99] Ma, Y., Szostkiewicz, I., Korte, A., Moes, D. et al., Regulators of PP2C phosphatase activity function as abscisic acid sensors. *Science* 2009, *324*, 1064–1068.
- [100] Park, S., Fung, P., Nishimura, N., Jensen, D. R. et al., Abscisic acid inhibits type 2C protein phosphatases via the PYR/PYL family of START proteins. *Science* 2009, *324*, 1068–1071.
- [101] Szostkiewicz, I., Richter, K., Kepka, M., Demmel, S. et al., Closely related receptor complexes differ in their ABA selectivity and sensitivity. *Plant J.* 2010, *61*, 25–35.
- [102] Beaudoin, N., Serizet, C., Gosti, F., Giraudat, J., Interactions between abscisic acid and ethylene signaling cascades. *Plant Cell* 2000, *12*, 1103–1115.
- [103] Ouaked, F., Rozhon, W., Lecourieux, D., Hirt, H., A MAPK pathway mediates ethylene signaling in plants. *EMBO J.* 2003, *22*, 1282–1288.
- [104] Cheng, W. H., Chiang, M. H., Hwang, S. G., Lin, P. C., Antagonism between abscisic acid and ethylene in *Arabidopsis* acts in parallel with the reciprocal regulation of their metabolism and signaling pathways. *Plant Mol. Biol.* 2009, *71*, 61–80.
- [105] Kline, K. G., Barrett-Wilt, G. A., Sussman, M. R., *In planta* changes in protein phosphorylation induced by the plant hormone abscisic acid. *Proc. Natl. Acad. Sci. USA* 2010, *107*, 15986–15991.
- [106] Lechner, E., Achard, P., Vansiri, A., Potuschak, T., Genschik, P., F-box proteins everywhere. *Curr. Opin. Plant Biol.* 2006, *9*, 631–638.
- [107] Miura, K., Hasegawa, P., Sumoylation and other ubiquitin-like post-translational modifications in plants. *Trends Cell Biol.* 2010, *20*, 223–232.
- [108] Hennig, L., Stoddart, W., Dieterle, M., Whitlam, G., Schafer, E., Phytochrome E controls light-induced germination of *Arabidopsis*. *Plant Physiol.* 2002, *128*, 194–200.
- [109] Shinomura, T., Nagatani, A., Chory, J., Furuya, M., The induction of seed germination in *Arabidopsis thaliana* is regulated principally by phytochrome B and secondarily by phytochrome A. *Plant Physiol.* 1994, *104*, 363–371.
- [110] Martínez-García, J. F., Direct targeting of light signals to a promoter element-bound transcription factor. *Science* 2000, *288*, 859–863.
- [111] Shen, H., Moon, J., Huq, E., PIF1 is regulated by light-mediated degradation through the ubiquitin-26S proteasome pathway to optimize photomorphogenesis of seedlings in *Arabidopsis*. *Plant J.* 2005, *44*, 1023–1035.
- [112] Lopez-Molina, L., Mongrand, S., Kinoshita, N., Chua, N., AFP is a novel negative regulator of ABA signaling that promotes ABI5 protein degradation. *Genes Dev.* 2003, *17*, 410–418.
- [113] Miura, K., Lee, J., Jin, J. B., Yoo, C. Y. et al., Sumoylation of ABI5 by the *Arabidopsis* SUMO E3 ligase SIZ1 negatively regulates abscisic acid signaling. *Proc. Natl. Acad. Sci. USA* 2009, *106*, 5418–5423.
- [114] Lopez-Molina, L., Mongrand, S., Chua, N. H., A post-germination developmental arrest checkpoint is mediated by abscisic acid and requires the ABI5 transcription factor in *Arabidopsis*. *Proc. Natl. Acad. Sci. USA* 2001, *98*, 4782–4787.

- [115] Liu, H., Stone, S. L., Abscisic acid increases *Arabidopsis* ABI5 transcription factor levels by promoting KEG E3 ligase self-ubiquitination and proteasomal degradation. *Plant Cell* 2010, 22, 2630–2641.
- [116] Dill, A., Jung, H., Sun, T., The DELLA motif is essential for gibberellin-induced degradation of RGA. *Proc. Natl. Acad. Sci. USA* 2001, 98, 14162–14167.
- [117] Lee, S., Cheng, H., King, K. E., Wang, W. et al., Gibberellin regulates *Arabidopsis* seed germination via *RGL2*, a GA/RGA-like gene whose expression is up-regulated following imbibition. *Genes Dev.* 2002, 16, 646–658.
- [118] Schwegheimer, C., Willige, B. C., Shedding light on gibberellic acid signalling. *Curr. Opin. Plant Biol.* 2009, 12, 57–62.
- [119] Stry, S., Yin, X., Potuschak, T., Schlogelhofer, P. et al., PRT1 of *Arabidopsis* is a ubiquitin protein ligase of the plant N-end rule pathway with specificity for aromatic amino-terminal residues. *Plant Physiol.* 2003, 133, 1360–1366.
- [120] Garzón, M., Eifler, K., Faust, A., Scheel, H. et al., *PRT6/At5g02310* encodes an *Arabidopsis* ubiquitin ligase of the N-end rule pathway with arginine specificity and is not the *CER3* locus. *FEBS Lett.* 2007, 581, 3189–3196.
- [121] Holman, T. J., Jones, P. D., Russell, L., Medhurst, A. et al., The N-end rule pathway promotes seed germination and establishment through removal of ABA sensitivity in *Arabidopsis*. *Proc. Natl. Acad. Sci. USA* 2009, 106, 4549–4554.
- [122] Reissner, K. J., Aswad, D. W., Deamidation and isoaspartate formation in proteins: unwanted alterations or surreptitious signals? *Cell. Mol. Life Sci.* 2003, 60, 1281–1295.
- [123] Mudgett, M. B., Clarke, S., Hormonal and environmental responsiveness of a developmentally regulated protein repair L-isoaspartyl methyltransferase in wheat. *J. Biol. Chem.* 1994, 269, 25605–25612.
- [124] Mudgett, M. B., Clarke, S., A distinctly regulated protein repair L-isoaspartylmethyltransferase from *Arabidopsis thaliana*. *Plant Mol. Biol.* 1996, 30, 723–737.
- [125] Ogé, L., Bourdais, G., Bove, J., Collet, B. et al., Protein repair L-isoaspartyl methyltransferase 1 is involved in both seed longevity and germination vigor in *Arabidopsis*. *Plant Cell* 2008, 20, 3022–3037.
- [126] Chinnusamy, V., Gong, Z., Zhu, J., Abscisic acid-mediated epigenetic processes in plant development and stress responses. *J. Integr. Plant Biol.* 2008, 50, 1187–1195.
- [127] Liu, Y., Koornneef, M., Soppe, W. J., The absence of histone H2B monoubiquitination in the *Arabidopsis hub1 (rdo4)* mutant reveals a role for chromatin remodeling in seed dormancy. *Plant Cell* 2007, 19, 433–444.
- [128] Ogas, J., Kaufmann, S., Henderson, J., Somerville, C., PICKLE is a CHD3 chromatin-remodeling factor that regulates the transition from embryonic to vegetative development in *Arabidopsis*. *Proc. Natl. Acad. Sci. USA* 1999, 96, 13839–13844.
- [129] Perruc, E., Kinoshita, N., Lopez-Molina, L., The role of chromatin-remodeling factor PKL in balancing osmotic stress responses during *Arabidopsis* seed germination. *Plant J.* 2007, 52, 927–936.
- [130] Tai, H. H., Tai, G. C., Beardmore, T., Dynamic histone acetylation of late embryonic genes during seed germination. *Plant Mol. Biol.* 2005, 59, 909–925.
- [131] Cutler, S., Ghassemian, M., Bonetta, D., Cooney, S., McCourt, P., A protein farnesyl transferase involved in abscisic acid signal transduction in *Arabidopsis*. *Science* 1996, 273, 1239–1241.
- [132] Yan, A., Lennarz, W. J., Unraveling the mechanism of protein N-glycosylation. *J. Biol. Chem.* 2005, 280, 3121–3124.
- [133] Helenius, A., Aebi, M., Intracellular functions of N-linked glycans. *Science* 2001, 291, 2364–2369.
- [134] Bollini, R., Vitale, A., Chrispeels, M., *In vivo* and *in vitro* processing of seed reserve protein in the endoplasmic reticulum: evidence for two glycosylation steps. *J. Cell Biol.* 1983, 96, 999–1007.
- [135] Vuylsteker, C., Cuvellier, G., Berger, S., Faugeron, C., Karamanos, Y., Evidence of two enzymes performing the de-N-glycosylation of proteins in barley: expression during germination, localization within the grain and set-up during grain formation. *J. Exp. Bot.* 2000, 51, 839–845.
- [136] Berger, S., Menudier, A., Julien, R., Karamanos, Y., Regulation of de-N-glycosylation enzymes in germinating radish seeds. *Plant Physiol.* 1996, 112, 259–264.