Review

Advances in the Biology of Seed and Vegetative Storage Proteins Based on Two-Dimensional Electrophoresis Coupled to Mass Spectrometry

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Abstract: Seed storage proteins play a fundamental role in plant reproduction and human nutrition. They accumulate during seed development as reserve material for germination and seedling growth and are a major source of dietary protein for human consumption. Storage proteins encompass multiple isoforms encoded by multi-gene families that undergo abundant glycosylations and phosphorylations. Two-dimensional electrophoresis (2-DE) is a proteomic tool especially suitable for the characterization of storage proteins because of their peculiar characteristics. In particular, storage proteins are soluble multimeric proteins highly represented in the seed proteome that contain polypeptides of molecular mass between 10 and 130 kDa. In addition, high-resolution profiles can be achieved by applying targeted 2-DE protocols. 2-DE coupled with mass spectrometry (MS) has traditionally been the methodology of choice in numerous studies on the biology of storage proteins in a wide diversity of plants. 2-DE-based reference maps have decisively contributed to the current state of our knowledge about storage proteins in multiple key aspects, including identification of isoforms and quantification of their relative abundance, identification of phosphorylated isoforms and assessment of their phosphorylation status, and dynamic changes of isoforms during seed development and germination both qualitatively and quantitatively. These advances have translated into relevant information about meaningful traits in seed breeding such as protein quality, longevity, gluten and allergen content, stress response and antifungal, antibacterial, and insect susceptibility. This review addresses progress on the biology of storage proteins and application areas in seed breeding using 2-DE-based maps.

Keywords: seed proteomics; seed phosphoproteomics; seed glycoproteomics; seed quality traits; seed molecular breeding

1. Introduction

Storage proteins accumulate during seed development within membrane-bound organelles called protein bodies and serve as a reservoir of amino acids, reduced nitrogen, carbon, and sulfur required for germinating seedlings [1–5]. Storage proteins also play a crucial role in human nutrition and livestock feed. Plants provide most (ca. 58%) of the dietary protein consumed worldwide compared to animal-based protein sources, although with marked variations depending on the region and economic status [6–9]. In particular, seeds are a major source of the dietary protein content that varies approximately from 10% (dry weight) in cereals to 40% in some legumes and oilseeds [1]. Storage
proteins determine to a great extent the seed nutritional quality because they account for a major part of the total protein content. By way of illustration, approximately 70–80% of the total amount of reduced nitrogen in cereals and legume grains can be attributed to seed storage proteins (SSPs) [10]. In addition, some SSPs and vegetative storage proteins (VSPs) can exhibit additional enzymatic activities such as lipid acyl hydrolase, acyltransferase, esterase and acid phosphatase activities capable of assuming useful supplementary biological functions, including defense and antioxidant functions [11–14].

The model species *Arabidopsis thaliana* L. has played a key role in identifying gene regulatory networks that govern seed development and germination. A wide repertoire of genetic technologies enabled the identification of essential regulatory genes during seed development and germination in Arabidopsis as well as the identification of orthologous genes in other plant species [15–20]. These technologies include forward genetic screens of lines obtained by T-DNA insertional mutagenesis for tagged mutants that produce a knockout phenotype, microarray RNA transcriptional profiling, and identification of seed-specific transcription factors (TFs). Genes involved in the regulatory networks responsible for the synthesis, accumulation and mobilization of seed storage proteins have been identified in Arabidopsis and other plants [20,21]. Dormancy induction and germination are greatly regulated by the dynamic balance between the functional antagonist abscisic acid (ABA) and gibberellic acid (GA) phytohormones [22]. Considerable progress has been achieved in unraveling the regulatory mechanisms underlying ABA response [23–26]. In particular, a number of protein-coding genes and TFs have been associated with the hormonal regulation involved in the synthesis and accumulation of storage proteins [20].

Seed proteome comprises a heterogeneous collection of functionally differentiated proteins that undergo highly dynamic qualitative and quantitative changes in order to meet seed requirements during development and germination. Storage proteins are typically multimeric proteins encoded by multi-gene families constituted by highly homologous genes clustered on one or various chromosomes [14,20,27,28]. They often undergo abundant glycosylations and phosphorylations, two types of co- and/or post-translational modifications (PTMs) that notably increase the diversity of isoforms [29,30]. Proteomics encompasses a wide range of technologies with sufficient potential for the detailed characterization of the broad set of storage protein isoforms. There have been a large number of gel-based and gel-free MS-driven proteomic studies focused on seed proteome [31–37]. The 2-DE proteomic technology initially developed by O’Farrel [38] opened the way to numerous studies addressing the characterization of storage proteins. Reference maps of many storage proteins have been constructed based on the separation of total seed proteins by 2-DE and protein identification by downstream MS analysis.

2-DE-based maps of storage proteins have been obtained using two different experimental strategies with strengths and weaknesses. Hundreds of publications have used experimental protocols for the study of global seed proteins with very different relative abundance [31–35,39–41]. This is an optimal experimental approach to assess the interplay between storage proteins and other seed proteins, but it entails the loss of definition of storage protein isoforms on 2-DE gels. Alternatively, a minority of studies used 2-DE specific protocols aimed at obtaining high-resolution profiles of storage proteins [29,30,42–44]. This approach is very useful to characterize storage protein isoforms and their response to internal and external seed stimuli at higher level of resolution, although the information it provides is decoupled from the rest of seed proteins. Overall, the application of these two strategies has provided most of the advances in the biology of storage proteins. These advances cover facets as diverse as the identification of isoforms and their relative abundance, the identification, mapping and quantitation of phosphorylated and glycosylated isoforms and the assessment of qualitative and quantitative changes of isoforms during seed development and germination. Seed breeding programs have benefited from these advances for the improvement of many seed traits of interest such as protein quality, longevity, gluten and allergen content, stress response and antifungal, antibacterial and insect susceptibility [45–51].
This review focuses on the use and importance of 2-DE-based maps to obtain insights into the biology of storage proteins and application areas in seed breeding.

2. Terminology and Classification of Storage Proteins

SSPs are currently denominated according to profoundly heterogeneous criteria: extraction/solubility in distinct solvents (e.g., albumins), sedimentation coefficients (e.g., 7S), generic names in Latin (e.g., hordeins from barley, *Hordeum vulgare* L.), trivial names (cactin from *Cereus jamacaru* DC.) and specific terminology for polypeptide subunits encoded by multigene families (e.g., phaseolin α-type polypeptide from common bean, *Phaseolus vulgaris* L.) [34]. However, most storage proteins have traditionally been classified into four main groups on the basis of their solubility in different solvents as proposed by Osborne [52]: water (albumins), dilute saline (globulins), alcohol-water mixtures (prolamines) and dilute acid or alkali (glutelins). New bioinformatics algorithms have recently been proposed for a higher classification accuracy using specific sequences available in public databases [53,54].

VSPs are a differentiated set of plant storage proteins located in vegetative tissues (tubers, stems, roots or leaves) of plants such as the sweet potato (sporamins), the potato (patatins) and *Oxalis tuberosa* Mol. (ocatins) [2,13,55,56]. For example, the patatin multigene family can be divided into class-I and class-II gene subfamilies with differential tissue expression patterns: class I transcripts are potato (*Solanum tuberosum* L.) tuber specific while class II transcripts are expressed not only in tubers but also in roots but much less abundant than class I transcripts [57,58]. VSPs are not grouped together with SSPs because they belong to a family of unrelated proteins and exhibit certain different characteristics such as a distinct form of mobilization [2,13,55,59].

A representative list of storage proteins (SSPs and VSPs) that includes important worldwide agricultural crops is shown in Table 1.

### Table 1. List of seed and vegetative storage proteins in different crop types.

| Crop                        | Storage Proteins | Percentage of Total Protein | Molecular Weight Subunits (kDa) | References |
|-----------------------------|------------------|-----------------------------|--------------------------------|------------|
| Maize (*Zea mays* L.)       | Globulins        | 12–16                       | 63, 45, 26, 23                 | [3,60–64]  |
|                             | globulin-1       |                             |                                |            |
|                             | globulin-2       |                             |                                |            |
|                             | Prolamins        | 50–70                       |                                |            |
|                             | α-zeins          | 25–49                       | 22, 19                         |            |
|                             | β-zeins          | 1–4                         | 14–16                          |            |
|                             | γ-zeins          | 6–13                        | 27, 16, 50                     |            |
|                             | δ-zeins          | 1–4                         | 10                             |            |
| Wheat (*Triticum aestivum* L.) | Prolamins        | 80                          | 30–80                          | [65–73]    |
|                             | gliadins         | 30–50                       |                                |            |
|                             | α-gliadins       | 15–30                       |                                |            |
|                             | β-gliadins       |                             |                                |            |
|                             | γ-gliadins       |                             |                                |            |
|                             | ω-gliadins       |                             |                                |            |
|                             | glutenins        |                             |                                |            |
|                             | LMW-GS           | 12                          | 42–51 (B), 30–40 (C), 58 (D)   |            |
|                             | HMW-GS           |                             | 80–130 (A)                     |            |
|                             | Globulins        | 11-12S triticins            | 5                              |            |
|                             |                 |                             | 58 (D), 22 (B), 52 (A), 23 (α) |            |
| Rice (*Oryza sativa* L.)    | Glutelins        | 60–80                       | 35–40, 20–22                   | [74–76]    |
|                             | Prolamins        | 20–30                       | 10, 13, 16                     |            |
|                             | Globulins        | α-globulins                 | 2–8                            |            |
|                             |                 |                             | 26                             |            |
| Crop | Storage Proteins | Percentage of Total Protein | Molecular Weight Subunits (kDa) | References |
|------|------------------|-----------------------------|---------------------------------|------------|
| Potato (Solanum tuberosum L.) | Patatins | 45 | 39–45 | [30,77,78] |
| | Kunitz protease inhibitors | | 20 | |
| | Protease inhibitors 1 | | 45 | |
| | Protease inhibitors 2 | | 10 | |
| | Carboxypeptidase inhibitors | | | |
| | Lipoxygenases | | 97 | |
| Soybean (Glycine max L.) | Globulins | | | [79–81] |
| | α-conglycinins | | | |
| | 7S vicilin/β-conglycinins | | 40 | 76 (α), 72 (α'), 52 (β) |
| | γ-conglycinins | | | |
| | 11S legumin/glycinin | | 25 | 56 (G1), 54 (G2), 54 (G3), 64 (G4), 58 (G5) |
| Barley (Hordeum vulgare L.) | Prolamins | | | [68,82] |
| | hordeins | | 35–55 | |
| | B-hordeins | | 15–44 | 30–45 |
| | C-hordeins | | 4–11 | 45–75 |
| | D-hordeins | | | 45 |
| | γ-hordeins | | | |
| Sunflower (Helianthus annuus L.) | Globulins | | | [83–85] |
| | 11S helianthinins | | 38 | 37–43 (α), 31–35 (α'), 21–30 (β) |
| | Albumins | | 2S | 62 | 12–20 |
| Common Bean (Phaseolus vulgaris L.) | Globulins | | | [14,44,86,87] |
| | 7S phaseolins | | 40–50 | |
| | 11S legumin | | 3 | |
| | Lectins | | | phytohemagglutinins |
| | | | | α-amylase inhibitors |
| Oat (Avena sativa L.) | Globulins | | | [71,88,89] |
| | 3S | | 10–55 | 48–52 |
| | 7S | | 48 | 50–70 |
| | 11S | | 60 | |
| | 12S avenalins | | 32–43 (α), 19–25 (β) | |
| | Albumins | | 10–20 | |
| | Prolamins | | 12–14 | |
| | Glutelins | | 23–54 | |
| Pea (Pisum sativum L.) | Globulins | | | [90,91] |
| | 7S vicilins | | 47, 50, 34, 30 | |
| | 11S legumin | | 41 (α), 22 (β), 23 (β') | |
| | convicilins | | 78, 72 | |
| Chickpea (Cicer arietinum L.) | Albumins | | | [89,92] |
| | 2S | | 12 | |
| | Globulins | | 50 | |
| | 7S vicilins | | 40–47 (α), 24–25 (β) | |
| | 11S legumin | | | |
| | Glutelins | | 18.1 | |
| | Prolamins | | 2.8 | |
| Pomegranate (Punica granatum L.) | Globulins | | | [93] |
| | Albumins | | 40.5 | 38–54, 13–18 |
| | Glutelins | | 32.2 | 58–116, 33–46, 15–23 |
| | Prolamins | | 15.6 | 37, 21–23, 14 |
| | | | 9.7 | 15, 20, 24 |
Globulins predominate in dicotyledonous seeds whereas prolamins are the major storage proteins in most cereals. Globulins are located in the embryo and outer aleurone layer of the endosperm and are commonly divided according to their different sedimentation coefficients (7S and 11S). They are very similar to 7S vicilins in legumes and other dicotyledonous plants [5]. In maize (Zea mays L.), globulins are classified as globulin-1, the most abundant storage protein in embryos, and globulin-2. In soybean (Glycine max L.), the seeds contain a considerable amount of globulins, namely β-conglycin (7S globulin) and glycinin (11S globulin). β-conglycin has a trimeric structure composed of α, α′, and β subunits with molecular weights ranging from 50 to 76 kDa. Glycinins consist of six subunits linked by disulfide bonds, but the five major subunits are G1–G5 whose molecular weights range from 54 to 64 kDa. Prolamins are the major proteins in endosperm and they are more variable than globulins. In maize grain, zeins are the most abundant storage proteins and are mainly accumulated in the endosperm between 12 and 40 days after pollination [96]. They are grouped into α (19 and 22 kDa), β (16 kDa), γ (16, 27 and 50 kDa) and δ (10 kDa) zeins [63]. Wheat (Triticum aestivum L.) prolamins, gliadins and glutenins form gluten and are located in grain endosperm. Gliadins are often subdivided into various subtypes in accordance with their electrophoretic mobilities (i.e., α-, β-, γ- and ω-gliadins), whereas glutenin subunits are subdivided according to their molecular weights (i.e., HMW-GS and LMW-GS glutenins). In rice (Oryza sativa L.), glutelins are the major seed storage proteins that contain hexamers of α-polypeptides (35 kDa) and β-polypeptides (22 kDa). Storage proteins are abundant proteins but subtypes are differentially represented in seed/tuber proteomes with relative amounts ranging from 1–4% (δ-zeins, Z. mays) to 72% (7S lentil vicilins, Lens culinaris Medik.), whereas Mr-values range from 10 (δ-zeins, Z. mays) to 130 kDa (HMW-GS glutenins, T. aestivum) (Table 1).

### Table 1. Cont.

| Crop                  | Storage Proteins | Percentage of Total Protein | Molecular Weight Subunits (kDa) | References |
|-----------------------|------------------|-----------------------------|--------------------------------|------------|
| Lentils (Lens culinaris Medik.) | Globulins | 11S legumins | 21 | 38–43 | [94] |
|                        | 7S vicilin/convicilins | 72 | 15–59 | |
|                       | Albumins | 2S | |
| Rapeseed (Brassica napus L.) | Globulins | 12S cruciferins | 20 | 29–33 (α), 21–23 (β) | [95] |
|                       | Albumins | 2S napins | 60 | 4–9 | |

3. Two-Dimensional-Based Reference Maps of Storage Proteins

2-DE can be routinely applied for the separation of highly complex mixtures of proteins from cell, tissue, organ and organism protein extracts in accordance with their isoelectric point (pI) and molecular mass (Mr) in two successive steps: isoelectric focusing (IEF) in the first dimension and sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) to resolve denatured proteins in the second. The introduction of immobilized pH gradients (IPGs) using bifunctional immobiline reagents enabled us to obtain highly stable pH gradients in the first dimension increasing resolution, reproducibility, the detection of lower abundance proteins and the separation of highly acidic and alkaline proteins [97,98]. Many other technical achievements contributed to the optimization of 2-DE, such as more efficient protein extraction methods, the running of multiple gels in parallel, highly sensitive protein stain methods based on fluorescent dyes compatible with subsequent protein identification by MS technologies, and advanced computer software for the analysis of gel images [99–103]. Technical inter-gel variation of protein spots can be reduced using an internal pooled standard in multiplexing methods. Difference gel electrophoresis (DIGE) enables the simultaneous...
running of up to three different samples in a single 2-DE gel using pre-electrophoretic labeling of protein samples with distinct spectrally-resolvable fluorescent CyDyes [101].

Dedicated protein extraction protocols can alleviate in part some of the limitations of the standard 2-DE system, including the analysis of low-abundant proteins and membrane proteins [98,102,104,105]. It is noteworthy that plant tissues contain relatively lower amounts of proteins than other organisms and a large number of biological compounds that interfere notably with the extraction, solubilization and separation of proteins by 2-DE, such as cell walls, lipids, polysaccharides, polyphenols and large quantities of proteases. Therefore, protein extraction is the initial and one of the most critical steps in plant proteomic studies because it determines to a large extent the final quality of 2-DE [99,106–109]. Overall, 2-DE is a laborious and poorly automated technology that requires a great deal of expertise to successfully exploit its potential.

High-resolution 2-DE can successfully separate, detect and quantify up to thousands of proteins simultaneously [99]. It is routinely applied in current proteomics to effectively analyze abundant and soluble proteins with an amount of 1–2 ng per spot expressed at greater than $10^3$ copies/cell, a linear dynamic range about three orders of magnitude, molecular mass ranging from 15 to 150 kDa and pH intervals from 2.5 to 12 [99,104,110]. Accordingly, 2-DE has enough resolving power to separate most of the isoforms of storage proteins. These proteins are soluble and highly abundant, exhibiting a range of $M_r$ and $pI$ within 2-DE resolution limits. By way of illustration, values of $M_r$ over phaseolin and patatin isoforms have a range of variation between 40 and 50 kDa, whereas $pI$-values range from 4.5 to 5.8 [29,30,42,44]. Gel location of storage protein isoforms can be initially established in accordance with their theoretical $M_r$ and $pI$ values and candidate protein spots eventually confirmed by MS for polypeptide/protein identification. 2-DE has the important ability to detect degraded proteins by comparing their $M_r$ values observed on gels to those corresponding theoretical values [109,111].

High-resolution profiles for storage proteins can be achieved by conveniently adjusting the amount of total protein loaded onto IPG strips [29,30,42–44]. Figure 1 shows standard and optimized phaseolin and patatin profiles by loading low amounts of total protein extracts from common bean seeds and potato tubers, respectively. It can be seen that dedicated 2-DE protocols produce good quality gel images with well-focused and separate protein spots corresponding to different phaseolin and patatin isoforms. 2-DE phaseolin and patatin profiles comprise a large number of spots organized in a compact way on the same gel region. Protein storage profiles can also exhibit multiple constellations of spots widely distributed on 2-DE gels (Figure 2). Dedicated 2-DE protocols have the additional advantage that the statistical cost by probability adjustments for multiple hypothesis testing is lower than in protocols addressed to the analysis of total seed proteomes, which leads to an increase in the statistical power of significance tests.
Molecules were obtained from 250 glycinin subunits) obtained by the targeted 2-DE.

Figure 2. Gel image of high-resolution profile of soybean (G. max) storage proteins (β-conglycinin and glycinin subunits) obtained by the targeted 2-DE.

Figure 1. Standard (a) and targeted (b) 2-DE gel images of phaseolin (above) and patatin (below) isoforms from common bean (P. vulgaris) seeds and potato (S. tuberosum) tubers. Standard 2-DE gels were obtained from 250 µg of total seed protein or total tuber protein extracts loaded into 24-cm-long IPG strips of linear pH gradient 4–7 in the first dimension. The second dimension (SDS-PAGE) was run using only 75 µg of total protein extracts. Targeted 2-DE gel images for high-resolution profiles were obtained under the same conditions but using only 75 µg of total protein extracts.

Figure 2. Gel image of high-resolution profile of soybean (G. max) storage proteins (β-conglycinin and glycinin subunits) obtained by the targeted 2-DE.
2-DE is particularly useful for identification of PTMs that change the pI and/or $M_r$ of proteins such as phosphorylations and glycosylations [102,112]. 2-DE-based reference maps of storage proteins can, therefore, be implemented with in-gel detection and mapping of phosphorylated and glycosylated isoforms (Figure 3). The Pro-Q diamond phosphoprotein stain (Pro-Q DPS) is a simple, direct, rapid and commonly used method for in-gel multiplex detection, mapping and quantitation of phosphorylated proteins [113,114]. Recent studies indicate, however, that the phosphoprotein chemical dephosphorylation of seed protein extracts with hydrogen fluoride-pyridine (HF-P) [115] prior to 2-DE is a highly valuable strategy for more accurate in-gel quantitation of phosphorylated storage proteins [29,30]. Phosphorylation levels for 2-DE spots can be directly assessed from volume changes between dephosphorylated and control sample profiles.

![Figure 3](image_url) Gel images of differentially phosphorylated (P) and glycosylated (G) isoforms of phaseolin (above) and patatin (below) obtained by targeted 2-DE.

The analysis of phosphorylated isoforms of storage proteins based on dedicated 2-DE maps has several major advantages in comparison to MS-driven analyses. A standard “bottom up” quantitative phosphoproteomics workflow involves the enzymatic or chemical digestion of a mixture of proteins into peptides to produce MS/MS spectra [33]. The redundancy of peptides and phosphorylation sites over high sequence identity protein isoforms hinders the assignment of specific peptides to a single isoform [112]. It is noteworthy that storage protein isoforms are encoded by gene families that exhibit high sequence identity mainly due to concerted evolution mechanisms of unequal crossing over and gene conversion [116]. For instance, patatin isoforms are encoded by a multigene family constituted by ~10–18 genes per haploid genome [28] and exhibit a sequence homology of at least 90% [58,117]. In addition, many other factors can lead to erroneous conclusions in MS-driven PTM analysis such as the co-elution of peptides, the loss of phosphoryl group during ionization process and phosphate transfer to acceptor residues, a lack of reproducibility and a low number of commonly used biological replicates [96,112,118]. It is noteworthy that some of these methodological constraints apply to powerful MS-based methods used for quantitative proteomics such as stable isotope labeling with amino acids in cell culture (SILAC) and isobaric tags for relative and absolute quantitation (iTRAQ). On the other hand, phosphopeptide enrichment strategies are usually accomplished prior to MS analysis because of the fact that many phosphoproteins/phosphopeptides from biological samples may be present in substoichiometric amounts [33,118]. In the case of storage proteins, the application of enrichment methods is not required because they are abundantly phosphorylated proteins [29,30]. Phosphopeptide enrichment methods such as immobilized metal affinity chromatography (IMAC) and titanium dioxide ($\text{TiO}_2$) impair the evaluation of quantitative changes in the phosphorylation status among storage protein isoforms, although they are very useful for phosphosite identification.
Finally, the detection and quantitation of glycosylated isoforms of storage protein can be assessed by different methods, including the enzymatic deglycosylation of total protein extracts [42], in-gel glycoprotein-specific Pro-Q Emerald fluorescent stain [119] and glycopeptide enrichment using a zwitterionic (ZIC) hydrophilic interaction liquid chromatography (HILIC) column or affinity chromatography on a concanavalin-A-sepharose column [120,121]. Storage protein glycoforms can be identified efficiently by their $M_r$ shifts on gels using targeted 2-DE protocols [30,42]. Glycosylated peptides are often difficult to identify in MS analyses because glycosylations change the hydrophobicity/hydrophilicity of the peptide [110].

4. Advances in the Biology of Storage Proteins

An exhaustive number of studies using 2-DE-based maps have contributed significantly to the characterization of the wide diversity of types, subunits and isoforms of storage proteins, their relative abundance in seeds and tubers, PTMs, targeted mutation effects and both qualitative and quantitative variations within and between wild and cultivated accessions [29–35,39–44]. In addition, 2-DE-based maps have provided valuable information on the complex dynamic changes of storage proteins during seed development and germination.

4.1. Seed Development

The available evidence indicates that storage proteins accumulate following variable patterns during embryo growth and seed filling, depending on the type of storage protein and cultivar. Thus, Gallardo et al. [4] reported that the major storage proteins 11S legumins and 7S vicilins of the model legume Medicago truncatula L. are synthesized in a specific temporal order and accumulated in different relative amounts during seed development. Analysis of protein abundance changes during time course were assessed by 2-DE and protein identification by MALDI-TOF and nano-LC-MS/MS sequencing. Interestingly enough, they also found a parallel evolution in the expression of the plII gene involved in the regulation of the synthesis of the amino acid arginine needed for storage protein synthesis using a transcriptomics dataset. Guo et al. [67] reported that five types of wheat storage proteins (i.e., $\gamma$-gliadins, globulins, avenin-like proteins, triticins and LMW-S glutenin subunits) accumulated differentially during grain development using 2-DE and tandem MALDI-TOF/TOF MS. This study also showed that LMW-S glutelin subunits and triticins exhibited differential abundance in two Chinese wheat cultivars at late seed development stages. In contrast, storage proteins of rapeseed (Brassica napus L.), i.e., naps, cruciferins and oleosins, were found to be accumulated only during the early and middle stages of seed growth by applying histochemical and immunostaining techniques [20,122]. In addition, recent studies have revealed that different isoforms of phaseolin/patatin are differentially accumulated during seed/tuber development within and among cultivars from quantitative analysis of phaseolin/patatin isoforms using dedicated 2-DE protocols and protein identification by MALDI-TOF/TOF MS [29,30,42–44]. Taken together, these observations raise the question of the molecular and biochemical mechanisms responsible for differential accumulation of storage protein isoforms during seed/tuber development, but they also suggest that the differential accumulation of storage proteins and isoforms has a significant meaning for their mobilization in the germination stage.

Reversible phosphorylation is the most ubiquitous and well-studied type of PTM that regulates a huge variety of key biological processes, including cell cycle, metabolism, subcellular localization, apoptosis, and signal transduction pathways [33,123,124]. The analysis of temporal phosphorylation changes in storage proteins is of paramount importance to unraveling their functional role at different stages of seed development. In recent decades the number of reports on the phosphorylation of different SSPs during seed development, dormancy and germination has greatly increased: globulins (7S- and 11S-globulins, 12S cruciferin, 12S triticin, cupin and globulin 3) in Arabidopsis, common bean, rapeseed, rice, Scots pine (Pinus sylvestris L.), sunflower (Helianthus annuus L.) and wheat [29,67,83,125–132]; prolamins in wheat [133,134]; albumins (2S napin) in Scots pine and
Arabidopsis [126,127,130]; and glutelins in rice [135]. Bernal et al. [30] have recently reported the first evidence for the phosphorylation of VSPs in patatin.

Identification and profiling studies of phosphorylated storage proteins based on 2-DE maps combined with various other techniques are listed in Table 2. Phosphoproteomic studies show that storage proteins are abundantly phosphorylated and may play a key role during seed development. Meyer et al. [129] reported a large-scale MS-based study of enriched subproteome of phosphoproteins by the IMAC method at five sequential stages (2–6 weeks after flowering) of seed development in soybean, rapeseed and Arabidopsis. A total of 2001 phosphopeptides and 1026 unambiguous phosphorylation sites were identified across 956 non-redundant proteins, including storage proteins. Interestingly, a considerable fraction (25%) of phosphoproteins consisted of storage proteins that contained the X-S-D-X phosphorylation motif. Targeted 2-DE-based maps coupled to the chemical method of dephosphorylation with HF-P have shown high phosphorylation levels in storage protein isoforms. Phosphorylation rates over phaseolin isoforms in dormant common bean seeds (two cultivars) and patatin isoforms from mature potato tubers (one cultivar) measured by the PR coefficient averaged 46–63% and 34%, respectively [29,30]. Furthermore, in silico phosphopeptide analysis also revealed the occurrence of a putative phosphosite in phaseolin phosphopeptides encompassing sequence X-S-D-X in the phaseolin. This peptide, therefore, appears to be a general target for phosphorylation during seed development.

2-DE-based maps show that the accumulation of phosphorylated storage protein isoforms during seed filling also follows variable patterns. Agrawal and Thelen [125] performed the first comprehensive study aimed at detecting and quantifying phosphoproteins in development seeds. More specifically, phosphoprotein profiling was performed in rapeseed through the same five sequential phases of seed development as Meyer et al. [129] by means of 2-DE-based maps coupled to in-gel phosphoprotein specific staining with Pro-Q DPS fluorescent dye and LC-MS/MS for protein and phosphorylation site identification. The results of the study showed that 40% of phosphorylated cruciferin subunits increased during seed filling process, whereas the remaining phosphorylated subunits generally decreased with seed development. Meyer et al. [129] also reported that some phosphorylated cruciferin subunits were over-represented in the late maturation stage of seed development. Dedicated 2-DE protocols have disclosed that phosphorylation rates (PR) across different phaseolin/patatin isoforms from dormant seed/tuber were in the range of 13–82% and 5–52%, respectively [29,30].

The complex regulatory mechanisms underlying dynamic changes in the phosphorylation status of storage proteins in response to seed development and environmental factors are not yet sufficiently known. However, it is assumed that the interplay of protein kinases, protein phosphatases and phytohormones participates in the signaling and metabolic networks that control the phosphorylation/dephosphorylation levels of storage proteins. The CK2 protein is a Ser/Thr kinase presents in all eukaryotes and has pleiotropic effects; it is also involved in the regulation of multiple plant growth and development processes and ABA signalling [136–138]. Irar et al. [126] used 2-DE-based maps for the phosphoproteome profiling of heat-stable proteins from Arabidopsis dry seeds and phosphoaffinity chromatography for phosphoprotein enrichment. They reported several probable hits of phosphorylation in storage and like-storage proteins, and an increased probability of phosphorylation of serine over threonine residues by CK2, using in silico prediction of phosphorylation sites from MALDI-TOF MS and LC MS/MS data. On the other hand, the ABA-insensitive 1 (ABI1) protein phosphatase is a negative regulator of the ABA signal and interacts with proteins linked to the ubiquitin-proteosome system (UPS) [139,140]. Wan et al. [127] showed that cruciferins of *A. thaliana* may be an in vivo target for ABI1 during seed development and provided evidence that cruciferin phosphorylation levels might be regulated by ABI1 using 2-DE maps coupled to immunological detection against phosphorylated cruciferin. They also found that cruciferins had differential levels of Tyr phosphorylation in mutant *ABII* and wild types, which suggests that Tyr phosphorylation is involved in ABA signaling.
4.2. Seed Germination

2-DE-based proteomic analyses revealed that the accumulation of storage proteins can still proceed in late stages of seed development and the onset of germination. Chibani et al. [141] reported that cruciferin precursors in Arabidopsis are accumulated by de novo synthesis during late stages of seed development leading to dormancy breakage. The accumulation of cruciferin precursors was documented by 2-DE following protein identification by MALDI-TOF MS. Proteomic research on Arabidopsis seed dormancy by 2-DE coupled to MALDI-TOF MS from seeds of the GA-deficient ga1 mutant and wild-type seeds treated with a specific inhibitor of GA biosynthesis suggests that GA is involved in the processing of precursor forms of storage proteins and accumulation of processed forms in mature seeds [142]. The comparison of 2-DE patatin profiles in dormant tubers and the onset of germination led to a better understanding of the metabolic status of storage proteins after the dormancy break. Lehesranta et al. [143] reported temporal differences of patatin abundance throughout the potato tuber lifecycle (cv. Desirée). More specifically, it was found that most patatin isoforms increase during development, are present in high amounts at the onset of sprouting (i.e., sprouts ca. 1 cm long) and remain approximately constant until tubers are fully sprouted (i.e., sprouts ca. 20 cm long) when patatin abundance decreases. Accordingly, analyses on transcripts encoding patatin throughout the potato tuber cycle based on cDNA-AFLP fingerprinting and expressed sequence tag (EST) libraries have shown that patatin transcripts are still expressed at the onset of tuber sprouting [144,145]. Similar results have been reported after chemically (bromoethane) induced cessation of dormancy using microarrays constructed from potato EST libraries [146]. Overall, these studies suggest that the major tuber storage protein encoded by the patatin multigene family is also synthesized after the dormancy break to ensure growth of the developing sprout.

Changes in the abundance or phosphorylation status of storage proteins during seed germination have been monitored using 2-DE-based reference maps [29,128,131,132]. Ghelis et al. [128] reported that the status of Tyr phosphorylation for several cruciferin precursors and cruciferin subunits in Arabidopsis seeds was modulated in response to ABA using 2-DE-based maps and the identification of phosphorylated Tyr residues by means of anti-phosphotyrosine antibodies in western blots. It was found that cruciferins treated with ABA exhibited higher phosphorylation levels than control seeds. In rice, Han et al. [131] detected that the highest level of phosphorylation of cupins coincided with the late stage of germination and protein degradation by means of 2-DE combined with Pro-Q DPS staining and MALDI-TOF/TOF MS. Using DIGE-based maps, Dong et al. [132] detected an increased abundance of phosphorylated wheat globulin 3 at 12 h after imbibition. In common beans, the analysis of targeted 2-DE-based phaseolin profiles coupled to protein dephosphorylation with HF-P revealed changes in the phosphorylation status during dry-to-germinating seed transition [29]. Changes in the phosphorylation status unexplained by parallel variations in the amount of protein are suggestive of their functional role [96]. Importantly, highly phosphorylated phaseolin isoforms were preferentially degraded in germinating seeds. These results support the conclusion that phosphorylation-dependent degradation plays a significant role in the mobilization of phaseolin. It has been suggested that phosphorylation can cause conformational changes in the protein and promote its mobilization during germination [127]. Overall, the molecular pathways, phosphorylation sites and specific kinases/phosphatases governing variations in phosphorylation status are totally unknown.

Protein glycosylation is involved in the modulation of relevant biological processes such as protein folding, protein stability, protein-protein interactions and interaction with membrane components [147–149]. Asparagine (N)-linked glycosylation is the major co- and post-translational modification of proteins in plants [150]. The application of a great diversity of molecular techniques permitted the identification of glycosylated isoforms in many types of storage proteins and species: globulins (7S- and 11S-globulins and convicilin) in adzuki bean (Vigna angularis L.), blue lupins (Lupinus angustifolius L.), cocoa beans (Theobroma cacao L.), common beans, hazelnuts (Corylus avellane L.), lentils, Lotus (Lotus japonicus L.), mung beans (Vigna radiata L.), peas (Pisum sativum L.), peanuts (Arachis hipogea L.), soybeans and white lupin (Lupinus albus L.) [42,120,121,151–179]; prolamin (γ3-hordein)
in barley [180]; albumins (3S albumins) in Inca peanuts (*Plukenetia volubilis* L.) [181]; glutelins in rice [135,182]; VSPs (patatin) in potatoes [30,43,183–186]; and lectins (monocot mannose-binding lectin, phytohemagglutinin) in air potatoes (*Dioscorea bulbifera* L.), common beans and lotus [121,187–193].

Identification and profiling studies of glycosylated storage proteins using 2-DE-based maps together with various other techniques are listed in Table 3. Most of these studies are addressed to the identification of glycosylated isoforms, the assessment of differential degrees of glycosylation and effects in food allergy. The biological role of glycosylated forms remains largely unknown. Interestingly, Santos et al. [177] reported that the glucoside hydrolase β-N-acetylhexosaminidase (β-NAHase) is involved in α-conglutin mobilization in white lupin storage proteins.
| Storage Protein Type | Storage Protein Subtype | Seed Stage | Additional Techniques | Species | References |
|----------------------|-------------------------|------------|-----------------------|---------|------------|
| Globulin             | 12S cruciferin          | Development| Pro-Q DPS LC-MS/MS     | Rapeseed (*Brassica napus* L.) | [113] |
|                     | 12S triticin            | Development| Pro-Q DPS MALDI-TOF MALDI-TOF/TOF | Wheat (*Triticum aestivum* L.) | [67] |
| Globulin 3           | 12S cruciferin          | Dormancy   | 1-DE, Pro-Q DPS immunoblotting LC-MS/MS | *Arabidopsis thaliana* L. | [126,127] |
| 7S phaseolin         | Dormancy/Germination    | Pro-Q DPS, HF-P MALDI-TOF MALDI-TOF/TOF | Common bean (*Phaseolus vulgaris* L.) | [29] |
| 12S cruciferin       | Germination             | Western blotting MALDI-TOF MALDI-TOF/TOF | *Arabidopsis thaliana* L. | [128] |
| Cupin                | Germination             | Pro-Q DPS MALDI-TOF/TOF | Rice (*Oryza sativa* L.) | [131] |
| Globulin 3           | Germination             | Pro-Q DPS LC-MS/MS | Wheat (*Triticum aestivum* L.) | [132] |
| Albumin              | 2S napin                | Dormancy   | 1-DE, Pro-Q DPS immunoblotting LC-MS/MS | *Arabidopsis thaliana* L. | [126,127] |
| Glutelin             | N/A                     | Development| Pro-Q DPS LC-MS/MS     | Rice (*Oryza sativa* L.) | [135] |
| Vegetative           | Patatin                 | Dormancy   | Pro-Q DPS, HF-P MALDI-TOF MALDI-TOF/TOF | Potato (*Solanum tuberosum* L.) | [30] |

N/A, not available.
Table 3. List of 2-DE-based seed glycoproteomic studies including storage proteins.

| Storage Protein Type | Storage Protein Subtype | Additional Techniques | Species                                | References         |
|----------------------|-------------------------|-----------------------|----------------------------------------|--------------------|
| Globulin             | 7S vicilin              | 1-DE, Glycoprotein staining | Cocoa bean (*Theobroma cacao* L.) | [179]              |
|                      | 7S phaseolin            | 1-DE, Fluorography    | Common bean (*Phaseolus vulgaris* L.) | [42,161,162]       |
|                      |                         | Radioactive labelling of sugars, Concanavalin A binding | | |
|                      |                         | Immunoaffinity chromatography | | |
|                      |                         | N-deglycosylation     | Lotus (*Lotus japonicus* L.) | [121]              |
| Glutelin             | N/A                     | Glycoprotein staining, LC-MS/MS | Rice (*Oryza sativa* L.) | [135]              |
| Lectin               | N/A                     | N-deglycosylation     | Lotus (*Lotus japonicus* L.) | [121]              |
| Vegetative           | Patatin                 | N-deglycosylation MALDI-TOF, MALDI-TOF/TOF | Potato (*Solanum tuberosum* L.) | [30,43,184]        |

N/A, not available.
5. Application Areas in Seed Breeding

5.1. Seed Quality

Seed protein quality is an essential trait in seed breeding programs. The nutritional quality of proteins is largely dependent on their essential amino acid (EAA) composition, total protein content and digestibility. Seed proteins are often deficient in specific EAA such as lysine, tryptophan, threonine and methionine. For example, high relative concentrations of lysine can be found in potato tuber but it is a nutritionally limiting EAA in most cereals [194]; whereas soybeans and common beans are deficient in methionine [194,195]. Storage proteins are abundant and determine to a great extent seed protein quality. For example, the relative abundance of prolams in cereals has a key influence for protein quality because of their deficiencies in EAA [73]. In particular, zein is a prolamin that accounts for between 50 and 70% of the total seed protein of maize and is mainly deficient in the content of lysine and tryptophan followed by methionine [61,196,197]. The particular mix of abundant storage proteins can also determine the final quality of seed proteins. For example, glycinin (11S legumin type) and conglycinin (7S vicilin type) are the two major soybean storage proteins, but glycinin harbors three to four times more sulfur-containing amino acids than conglycinin [198].

2-DE-based maps are a very effective tool for screening and selecting varieties containing specific protein storage isoforms linked to high protein quality in plant breeding. This proteomic approach has been addressed in a variety of crops. For instance, wild rice species are a valuable source of genetic resources for improving the nutritional quality of rice by increasing the glutelin content to the detriment of prolams [199,200]. The comparison of 2-DE-based maps between wild rice species and rice cultivars revealed new subunits and precursors of glutelin in wild rice species [199]. 2-DE gels also revealed that the content of gluteins in an ancient Chinese wild rice (Zizania latifolia (Griseb.) Turcz.) was approximately twice as high as that of the Indica rice cultivar [200]. Zarkadas et al. [195] also reported great variability among soybean cultivars for glycinin and β-conglycinin using 2-DE.

In common bean, López-Pedrouso et al. [44] reported that pairwise proteomic distances estimated from wild and domesticated accessions of the major Mesoamerican and Andean gene pools assessed by targeted 2-DE of the phaseolin provide valuable information for identifying outlier cultivars with increased content in methionine.

A number of factors modeling the genetic structure of populations can generate and/or maintain genome-wide non-random associations between alleles at different loci (linkage or gametic disequilibrium) such as founder effects, bottlenecks, inbreeding and selection [201]. These factors or combination of factors often operate in plant breeding. Accordingly, storage proteins encoded by multigene families can be used to detect nonrandom associated quantitative trait loci (QTLs) underlying quality traits. In this regard, the nutritional quality of protein and the starch content and average weight of potato tubers were found to be correlated with patatin content [202,203].

Different types of transgenic-based strategies have been addressed at the improvement of seed protein quality from storage proteins. Some strategies rely on the ectopic expression of transgenes coding for high quality proteins that correct seed deficiencies in the amino acid composition of storage proteins. Shekhar et al. [50] introduced the seed albumin gene AmA1 from Amaranthus hypochondriacus into sweet potato (Ipomoea batatas L.) by Agrobacterium-mediated transformation to assess the behavior of storage proteins in a non-native system. AmA1 is rich in all EAA whereas sweet potato proteins are deficient in tryptophan and sulfur-containing amino acids. Comparative proteomics revealed that 2-DE profiles of transgenic tubers exhibited a higher number of protein spots than wild-type tubers. The results suggest that overexpression of AmA1 in sweet potato tubers seems to have a marked effect on nutrient acquisition, which facilitates an increase in the overall protein and amino acid content. Other alternative transgenics-based approaches are used to overproduce one particular seed protein with higher nutritional quality than the remaining set of storage proteins. For example, the overexpression of glycinin enables an increase in sulfur amino acids in soybean seeds, taking into account that the content of glycinin correlates negatively to the content of β-conglycinin [204].
El-Shemy et al. [198] transformed soybean embryos with a chimeric proglycinin gene encoding a methionine-rich glycinin. The comparison of transgenic and untransformed soybean lines by 2-DE revealed an increased accumulation of glycinin in transgenic soybeans.

5.2. Gluten Disorders and Allergies

Gluten proteins and gluten-like proteins are the main factor triggering coeliac disease (CD), non-coeliac gluten sensitivity and gluten allergies in genetically susceptible individuals [51,205,206]. CD is an autoimmune condition caused by human intolerance to wheat gluten and related proteins from rye (secalins, Secale cereale L.), barley (hordeins) and oat (avenins, Avena sativa L.) that primarily affect the small intestine [72,206]. Gluten is composed of a combination of two toxic prolamins in CD, glutenins and gliadins, but gliadins contain most of the epitopes triggering CD [51,72]. A gluten-free diet is often low in fiber and minerals, high in sucrose and saturated fatty acids, and more expensive [207,208]. A wide-variety of strategies have been applied for the selection and breeding of less toxic varieties. These include obtaining varieties with a lower dose or a different composition of gluten proteins. García-Molina et al. [51] carried out a 2-DE-based proteomic study to evaluate the effects of the strong down-regulation of gliadins on the expression of target and non-target proteins. For this purpose, transgenic wheat lines with downregulation of gliadin expression were obtained by RNA interference (RNAi) technology. As expected, transgenic lines showed a lower abundance of gliadins with respect to control lines. However, the glutelin fraction and other allergen-related wheat proteins increased in low-gliadin lines by a compensation effect. Kawaura et al. [209] obtained aneuploid wheat lines to reduce CD immunotoxicity in breeding programs. An analysis of 2-DE profiles disclosed that α-gliadins containing major CD epitopes were lost in tetrasomic lines. In barley, Tanner et al. [206] obtained an ultra-low gluten variety (hordein content below 5 ppm) by combining three recessive alleles with potential application in the preparation of foods and beverages for CD patients and people who cannot tolerate gluten. Only reduced amounts of the γ-3-hordein protein were observed in the ultra-low gluten variety by 2-DE, in accordance with other protein quantitative determinations. 2-DE also contributed to demonstrating that wheat α-gliadins can be compensated by the addition of avenins to the floor to improve dough quality, taking into account that a minority of CD patients are sensitive to oat avenins [210]. Rizzello et al. [211] showed by in vitro analysis that making bread from flour with an intermediate content of gluten improves its digestibility and nutritional quality without the loss of the chemical, structural and sensory characteristics of traditional breads. 2-DE revealed increased protein degradation in flour with an intermediate content of gluten during fermentation. The authors suggested that this wheat product might be useful to prevent, delay or treat susceptibility to gluten sensitivity, a gluten reaction that does not involve allergic or autoimmune mechanisms.

5.3. Seed Longevity

Dry seed longevity is an essential complex trait for the biodiversity conservation of cultivated plants. Seed longevity and the germination vigor rate slowly decrease during storage ageing, influenced by abiotic and biotic variables, including storage conditions (e.g., temperature and humidity) and genetic factors [212–214]. Compelling evidence indicates that antioxidant systems (antioxidative enzymes and antioxidants) deteriorate during seed ageing leading to the accumulation of reactive oxygen species (ROS) and oxidative damage [49,124,215]. SSPs undergo extensive oxidization (often carbonylation) during long-term seed storage due to their abundance and high affinity to oxidation [49,215–217]. Seed ageing profiling in rice assessed by 2-DE followed by western blotting with antidinitrophenyl hydrazone antibodies revealed that carbonylated SSPs accumulate at the critical node of seed ageing leading to a rapid decline in seed viability [124]. Nguyen et al. [49] proposed that SSPs may be buffers for seed oxidative stress, able to protect relevant proteins for seed germination and seedling development from proteomic profile analysis of Arabidopsis cruciferin mutants based on
2-DE and LC-MS/MS. Dobiesz et al. [214] reported that β- and δ-conglutins may be a useful biomarker of lupin (Lupinus luteus L.) seed viability during long-term storage using 2-DE and LC-MS/MS.

5.4. Other Applications

The analysis of storage proteins by 2-DE-based maps has also contributed to the development of other application areas such as antifungal, antibacterial and insect susceptibility [45–47,218], the identification of allergens [46], drought stress [48,219], wheat cultivar identification in blended flour [220] and the large-scale production of therapeutic proteins [221].

6. General Conclusions and Perspectives

This review shows that the use of 2-DE combined with MS is of vital importance not only to advancing the knowledge of the isoforms of storage proteins and their dynamic changes during seed development and germination in a wide diversity of plants, but also in relevant fields closely connected to seed breeding. Therefore, the employment of 2-DE is expected to follow over the next years due to its high efficiency in the characterization of storage proteins across different biological scenarios. Gel-based and shotgun proteomics are alternative strategies for proteome analysis that have advantages and limitations but complement each other. The joint use of gel-based and gel-free methodologies will probably continue to be necessary in follow-up studies to understand the complex biology of storage proteins. Despite significant progress over the last decades, proteomics faces major challenges in the coming years to unravel the complex molecular puzzle of regulatory networks underlying the activities, functions, and interactions of storage proteins over the lifecycle of seeds. In particular, further experiments are clearly needed to assess the exact role of phosphorylated isoforms and specific phosphorylation sites during seed development and germination. This huge task will probably require the integration of multi-omics data with the help of new bioinformatic tools.

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Abbreviations

1-DE One-dimensional electrophoresis
2-DE Two-dimensional electrophoresis
ABA Abscisic acid
CD Coeliac disease
DIGE Difference gel electrophoresis
EAA Essential amino acid
GA Gibberellic acid
HF-P Hydrogen fluoride-pyridine
IMAC Immobilized metal affinity chromatography
Mr Relative molecular mass
MS Mass spectrometry
pI Isoelectric point
PR Phosphorylation rate
Pro-Q DPS Pro-Q Diamond phosphoprotein stain
PTM Post-translational modification
SSP Seed storage protein
VSP Vegetative storage protein
References

1. Shewry, P.R.; Napier, J.A.; Tatham, A.S. Seed storage proteins: Structures and biosynthesis. *Plant Cell* 1995, 7, 945–956. [CrossRef] [PubMed]
2. Müntz, K. Deposition of storage proteins. *Plant Mol. Biol.* 1998, 38, 77–99. [CrossRef] [PubMed]
3. Shewry, P.R.; Halford, N.G. Cereal seed storage proteins: Structures, properties and role in grain utilization. *J. Exp. Bot.* 2002, 53, 947–958. [CrossRef] [PubMed]
4. Gallardo, K.; Firnhaber, C.; Zuber, H.; Héricher, D.; Belghazi, M.; Henry, C.; Küster, H.; Thompson, R. A combined proteome and transcriptome analysis of developing *Medicago truncatula* seeds evidence for metabolic specialization of maternal and filial tissues. *Mol. Cell. Proteom.* 2007, 6, 2165–2179. [CrossRef] [PubMed]
5. Tan-Wilson, A.L.; Wilson, K.A. Mobilization of seed protein reserves. *Physiol. Plant.* 2012, 145, 140–153. [CrossRef] [PubMed]
6. van Vliet, S.; Burd, N.; van Loon, L. The skeletal muscle anabolic response to plant-versus animal-based protein consumption. *J. Nutr.* 2015, 145, 1981–1991. [CrossRef] [PubMed]
7. Pasiakos, S.; Agarwal, S.; Lieberman, H.; Fulgoni, V. Sources and amounts of animal, dairy, and plant protein intake of US adults in 2007–2010. *Nutrients* 2015, 7, 7058–7069. [CrossRef] [PubMed]
8. FAOSTAT. Statistics Division of the FAO. Available online: http://www.fao.org/faostat/en/ (accessed on 22 June 2018). [CrossRef]
9. World Bank List of Economies. Available online: http://www.worldbank.org// (accessed on 22 June 2018).
10. Aguirrezábal, L.; Martre, P.; Pereyra-Irujo, G.; Echarte, M.M.; Izquierdo, N. Improving grain quality: Ecophysiological and modeling tools to develop management and breeding strategies. In *Crop Physiology, Applications for Genetic Improvement and Agronomy*, 2nd ed.; Sadras, V., Calderini, D., Eds.; Academic Press: London, UK, 2015; pp. 423–465. ISBN 9780124171046.
11. Racusen, D. Lipid acyl hydrolase of patatin. *Can. J. Bot.* 1984, 62, 1640–1644. [CrossRef]
12. Liu, Y.W.; Han, C.H.; Lee, M.H.; Hsu, F.L.; Hou, W.C. Patatin, the tuber storage protein of potato (*Solanum tuberosum* L.), exhibits antioxidant activity in vitro. *J. Agric. Food Chem.* 2003, 51, 4389–4393. [CrossRef] [PubMed]
13. de Souza Cândido, E.; Pinto, M.F.; Pelegrini, P.B.; Lima, T.B.; Silva, O.N.; Pogue, R.; Grossi-de-Sá, M.F.; Franco, O.L. Plant storage proteins with antimicrobial activity: Novel insights into plant defense mechanisms. *FASEB J.* 2011, 25, 3290–3305. [CrossRef] [PubMed]
14. Joshi, J.; Panduranga, S.; Diapari, M.; Marsolais, F. Comparison of Gene Families: Seed Storage and Other Seed Proteins. In *The Common Bean Genome*; de la Vega, M.P., Santalla, M., Marsolais, F., Eds.; Springer: Cham, Switzerland, 2017; pp. 201–219. ISBN 978-3-319-63524-8.
15. Girke, T.; Todd, J.; Ruuska, S.; White, J.; Benning, C.; Ohlrogge, J. Microarray analysis of developing *Arabidopsis* seeds. *Plant Physiol.* 2000, 120, 1570–1581. [CrossRef]
16. Tzafrir, I.; Dickerman, A.; Brazhnik, O.; Nguyen, Q.; McElver, J.; Frye, C.; Patton, D.; Meinke, D. The *Arabidopsis* seed genes project. *Nucleic Acids Res.* 2003, 31, 90–93. [CrossRef] [PubMed]
17. McElver, J.; Tzafrir, I.; Aux, G.; Rogers, R.; Ashby, C.; Smith, K.; Thomas, C.; Schetter, A.; Zhou, Q.; Cushman, M.A.; et al. Insertional mutagenesis of genes required for seed development in *Arabidopsis thaliana*. *Genetics* 2001, 159, 1751–1763. [PubMed]
18. Meinke, D.; Murailla, R.; Sweeney, C.; Dickerman, A. Identifying essential genes in *Arabidopsis thaliana*. *Trends Plant Sci.* 2008, 13, 483–491. [CrossRef] [PubMed]
19. Le, B.H.; Cheng, C.; Bui, A.Q.; Wagmaister, J.A.; Henry, K.F.; Pelletier, J.; Kwong, L.; Belmonte, M.; Kirkbride, R.; Horvath, S.; et al. Global analysis of gene activity during *Arabidopsis* seed development and identification of seed-specific transcription factors. *Proc. Natl. Acad. Sci. USA* 2010, 107, 8063–8070. [CrossRef] [PubMed]
20. Gacek, K.; Bartkowiak-Broda, I.; Batley, J. Genetic and molecular regulation of Seed Storage Proteins (SSPs) to improve protein nutritional value of oilseed rape (*Brassica napus* L.) seeds. *Front. Plant Sci.* 2018, 9, 890. [CrossRef] [PubMed]
21. Rasheed, A.; Xia, X.; Yan, Y.; Appels, R.; Mahmood, T.; He, Z. Wheat seed storage proteins: Advances in molecular genetics, diversity and breeding applications. *J. Cereal Sci.* 2014, 60, 11–24. [CrossRef]
22. Finch-Savage, W.; Leubner-Metzger, G. Seed dormancy and the control of germination. *New Phytol.* 2006, 171, 501–523. [CrossRef] [PubMed]

23. Hirayama, T.; Shinozaki, K. Perception and transduction of abscisic acid signals: Keys to the function of the versatile plant hormone ABA. *Trends Plant Sci.* 2007, 12, 343–351. [CrossRef] [PubMed]

24. Gutierrez, L.; Van Wuystwinkel, O.; Castelain, M.; Bellini, C. Combined networks regulating seed maturation. *Trends Plant Sci.* 2007, 12, 294–300. [CrossRef] [PubMed]

25. Han, C.; Yang, P. Studies on the molecular mechanisms of seed germination. *Proteomics* 2015, 15, 1671–1679. [CrossRef] [PubMed]

26. Né, G.; Kramer, K.; Nakabayashi, K.; Yuan, B.; Xiang, Y.; Miatton, E.; Finkemeier, I.; Soppe, W.J.J. Delay of germination requires PP2C phosphatases of the ABA signalling pathway to control seed dormancy. *Nat. Commun.* 2017, 8, 72. [CrossRef] [PubMed]

27. Kim, H.T.; Choi, U.K.; Ryu, H.S.; Lee, S.J.; Kwon, O.S. Mobilization of storage proteins in soybean seed (*Glycine max* L.) during germination and seedling growth. *Biochim. Biophys. Acta* 2011, 1814, 1178–1187. [CrossRef] [PubMed]

28. The Potato Genome Sequencing Consortium. Genome sequence and analysis of the tuber crop potato. *Nature* 2011, 475, 189–195. [CrossRef] [PubMed]

29. López-Pedrouso, M.; Alonso, J.; Zapata, C. Evidence for phosphorylation of the major seed storage protein of the common bean and its phosphorylation-dependent degradation during germination. *Plant Mol. Biol.* 2014, 84, 415–428. [CrossRef] [PubMed]

30. Bernal, J.; López-Pedrouso, M.; Franco, D.; Bravo, S.; García, L.; Zapata, C. Identification and mapping of phosphorylated isoforms of the major storage protein of potato based on two-dimensional electrophoresis. In *Advances in Seed Biology*; Jimenez-Lopez, J.C., Ed.; InTech: Rijeka, Croatia, 2017; pp. 65–82. ISBN 978-953-51-3621-7.

31. Jorrín, J.V.; Maldonado, A.M.; Castillejo, M.A. Plant proteome analysis: A 2006 update. *Proteomics* 2007, 7, 2947–2962. [CrossRef] [PubMed]

32. Kersten, B.; Agrawal, G.K.; Durek, P.; Neigenfind, J.; Schulze, W.; Walther, D.; Rakwal, R. Plant phosphoproteomics: An update. *Proteomics* 2009, 9, 964–988. [CrossRef] [PubMed]

33. Silva-Sanchez, C.; Li, H.; Chen, S. Recent advances and challenges in plant phosphoproteomics. *Proteomics* 2015, 15, 1127–1141. [CrossRef] [PubMed]

34. Miernyk, J.A.; Hajduch, M. Seed proteomics. *J. Proteom.* 2011, 74, 389–400. [CrossRef] [PubMed]

35. Miernyk, J.A. Seed Proteomics. In *Plant Proteomics*; Jorrin-Novó, J.J., Komatsu, S., Weckwerth, W., Wienkoop, S., Eds.; Humana Press: New York, NY, USA, 2014; pp. 361–379. ISBN 978-1-62703-630-6.

36. Narula, K.; Sinha, A.; Haider, T.; Chakraborty, N.; Chakraborty, S. Seed Proteomics: An Overview. In *Agricultural Proteomics*; Salekdeh, G.H., Ed.; Springer: Cham, Switzerland, 2016; Volume 1, pp. 31–53. ISBN 978-3-319-43273-1.

37. Zargar, S.M.; Mahajan, R.; Nazir, M.; Nagar, P.; Kim, S.T.; Rai, V.; Masi, A.; Ahmad, S.M.; Shah, R.A.; Ganai, N.A.; et al. Common bean proteomics: Present status and future strategies. *J. Proteom.* 2017, 169, 239–248. [CrossRef] [PubMed]

38. O’Farrel, P.H. High resolution two-dimensional electrophoresis of proteins. *J. Biol. Chem.* 1975, 250, 4007–4021.

39. Barbier-Brygoo, H.; Joyard, J. Focus on plant proteomics. *Plant Physiol. Biochem.* 2004, 42, 913–917. [CrossRef] [PubMed]

40. Chen, S.; Harmon, A.C. Advances in plant proteomics. *Proteomics* 2006, 6, 5504–5516. [CrossRef] [PubMed]

41. Ghatak, A.; Chaturvedi, P.; Weckwerth, W. Cereal crop proteomics: Systemic analysis of crop drought stress responses towards marker-assisted selection breeding. *Front. Plant Sci.* 2017, 8, 757. [CrossRef] [PubMed]

42. de la Fuente, M.; López-Pedrouso, M.; Alonso, J.; Santalla, M.; de Ron, A.M.; Alvarez, G.; Zapata, C. In-depth characterization of the phaeoelin protein diversity of common bean (*Phaseolus vulgaris* L.) based on two-dimensional electrophoresis and mass spectrometry. *Food Technol. Biotechnol.* 2012, 50, 315–325.

43. Bárt, J.; Bártová, V.; Záhráhal, Z.; Šedo, O. Cultivar variability of patatin biochemical characteristics: Table versus processing potatoes (*Solanum tuberosum* L.). *J. Agric. Food Chem.* 2012, 60, 4369–4378. [CrossRef] [PubMed]
44. López-Pedrouso, M.; Bernal, J.; Franco, D.; Zapata, C. Evaluating two-dimensional electrophoresis profiles of the protein phaseolin as markers of genetic differentiation and seed protein quality in common bean (Phaseolus vulgaris L.). J. Agric. Food Chem. 2014, 62, 7200–7208. [CrossRef] [PubMed]
45. Flores, T.; Alape-Girón, A.; Flores-Díaz, M.; Flores, H.E. Ocatin. A novel tuber storage protein from the andean tuber crop oca with antibacterial and antifungal activities. Plant Physiol. 2002, 128, 1291–1302. [CrossRef] [PubMed]
46. Palomares, O.; Cuesta-Herranz, J.; Vereda, A.; Sirvent, S.; Villalba, M.; Rodríguez, R. Isolation and identification of an 11S globulin as a new major allergen in mustard seeds. Ann. Allergy Asthma Immunol. 2005, 94, 586–592. [CrossRef]
47. Collins, R.M.; Afzal, M.; Ward, D.A.; Prescott, M.C.; Sait, S.M.; Rees, H.H.; Tomsett, A.B. Differential proteomic analysis of Arabidopsis thaliana genotypes exhibiting resistance or susceptibility to the insect herbivore, Plutella xylostella. PLoS ONE 2010, 5, e10103. [CrossRef] [PubMed]
48. Zhang, Y.F.; Huang, X.W.; Wang, L.L.; Wei, L.; Wu, Z.H.; You, M.S.; Li, B.Y. Proteomic analysis of wheat seed in response to drought stress. J. Integr. Agric. 2014, 13, 919–925. [CrossRef]
49. Nguyen, T.P.; Cueff, G.; Hegedus, D.D.; Rajou, L.; Bentsink, L. A role for seed storage proteins in Arabidopsis seed longevity. J. Exp. Bot. 2015, 66, 6399–6413. [CrossRef] [PubMed]
50. Shekhar, S.; Agrawal, L.; Mishra, D.; Buragohain, A.K.; Unnikrishnan, M.; Mohan, C.; Chakraborty, S.; Chakraborty, N. Ectopic expression of amaranth seed storage albumin modulates photoassimilate transport and nutrient acquisition in sweetpotato. Sci. Rep. 2016, 6, 25384. [CrossRef] [PubMed]
51. García-Molina, M.D.; Muccilli, V.; Saletti, R.; Fosti, S.; Masci, S.; Barro, F. Comparative proteomic analysis of two transgenic low-gliadin wheat lines and non-transgenic wheat cultivars. J. Proteom. 2017, 165, 102–112. [CrossRef] [PubMed]
52. Osborne, T.B. The Vegetable Proteins, 2nd ed.; Longmans, Green and Co.: London, UK, 1924; pp. 1–154.
53. Marla, S.; Bharatiya, D.; Bala, M.; Singh, V.; Kumar, A. Classification of rice seed storage proteins using neural networks. J. Plant Biochem. Biotechnol. 2010, 19, 123–126. [CrossRef]
54. Radhika, V.; Rao, V.S. Computational approaches for the classification of seed storage proteins. J. Food Sci. Technol. 2015, 52, 4246–4255. [CrossRef] [PubMed]
55. Beauchemin, T.; Wetzel, S.; Burgess, D.; Charest, P.J. Characterization of seed storage proteins in Populus and their homology with Populus vegetative storage proteins. Tree Physiol. 1996, 16, 833–840. [CrossRef] [PubMed]
56. Fujiwara, T.; Nambara, E.; Yamagishi, K.; Goto, D.B.; Naito, S. Storage proteins. Arabidopsis Book 2002, 1, e0020. [CrossRef] [PubMed]
57. Pikaard, C.S.; Brusca, J.S.; Hannapel, D.J.; Park, W.D. The two classes of genes for the major potato tuber protein, patatin, are differentially expressed in tubers and roots. Nucleic Acids Res. 1979. [CrossRef]
58. Mignery, G.A.; Pikaard, C.; Park, W. Molecular characterization of the patatin multigene family of potato. Gene 1988, 62, 27–44. [CrossRef]
59. Staswick, P.E. Novel regulation of vegetative storage protein genes. Plant Cell 1990, 2, 1–6. [CrossRef] [PubMed]
60. Consoli, I.; Damerval, C. Quantification of individual zein isoforms resolved by two-dimensional electrophoresis: Genetic variability in 45 maize inbred lines. Electrophoresis 2001, 22, 2983–2989. [CrossRef]
61. Lund, G.; Ciceri, P.; Viotti, A. Maternal-specific demethylation and expression of specific alleles of zein genes in the endosperm of Zea mays L. Plant J. 1995, 8, 571–581. [CrossRef] [PubMed]
62. Pinheiro, C.; Sergeant, K.; Machado, C.M.; Renault, J.; Ricardo, C.P. Two traditional maize inbred lines of contrasting technological abilities are discriminated by the seed flour proteome. J. Proteome Res. 2013, 12, 3152–3165. [CrossRef] [PubMed]
63. Xu, J.H.; Messing, J. Organization of the prolamin gene family provides insights into the evolution of the maize genome and gene duplications in grass species. Proc. Natl. Acad. Sci. USA 2008, 105, 14330–14335. [CrossRef] [PubMed]
64. Ning, F.; Niu, L.; Yang, H.; Wu, X.; Wang, W. Accumulation profiles of embryonic salt-soluble proteins in maize hybrids and parental lines indicate matroclinous inheritance: A proteomic analysis. Front. Plant Sci. 2017, 8, 1824. [CrossRef] [PubMed]
65. Payne, P.I. Genetics of wheat storage proteins and the effect of allelic variation on bread-making quality. Ann. Rev. Plant Physiol. 1987, 38, 141–153. [CrossRef]
66. Shewry, P.R.; Tatham, A.S. The prolamin storage proteins of cereal seeds: Structure and evolution. *Biochem. J.* 1990, 267, 1–12. [CrossRef] [PubMed]
67. Guo, G.; Lv, D.; Yan, X.; Subburaj, S.; Ge, P.; Li, X.; Hu, Y.; Yan, Y. Proteome characterization of developing grains in bread wheat cultivars *(Triticum aestivum)*. *BMC Plant Biol.* 2012, 12, 147. [CrossRef] [PubMed]
68. Malik, A.H. Nutrient uptake, transport and translocation in cereals: Influences of environmental and farming conditions. *Swed. Univ. Agric. Sci.* 2009, 1, 1–46.
69. Zhou, J.; Liu, D.; Deng, X.; Zhen, S.; Wang, Z.; Yan, Y. Effects of water deficit on breadmaking quality and storage protein compositions in bread wheat *(Triticum aestivum)*. *J. Sci. Food Agric.* 2018, 98, 4357–4368. [CrossRef] [PubMed]
70. Xie, Z.; Wang, C.; Wang, K.; Wang, S.; Li, X.; Zhang, Z.; Ma, W.; Yan, Y. Molecular characterization of the celiac disease epitope domains in α-gliadin genes in *Aegilops tauschii* and hexaploid wheats *(Triticum aestivum)*. *Theor. Appl. Genet.* 2010, 121, 1239–1251. [CrossRef] [PubMed]
71. Cavazos, A.; Gonzalez de Mejia, E. Identification of bioactive peptides from cereal storage proteins and their potential role in prevention of chronic diseases. *Compr. Rev. Food Sci. Food Saf.* 2013, 12, 364–380. [CrossRef]
72. Ferranti, P.; Mamone, G.; Picariello, G.; Addeo, F. Mass spectrometry analysis of gliadins in celiac disease. *J. Mass Spectrom.* 2007, 42, 1531–1548. [CrossRef] [PubMed]
73. Yadav, D.; Singh, N. Wheat triticin: A potential target for nutritional quality improvement. *Asian J. Biotechnol.* 2011, 3, 1–21. [CrossRef]
74. Zhang, W.; Sun, J.; Zhao, G.; Wang, J.; Liu, H.; Zheng, H.; Zhao, H.; Zou, D. Association analysis of the glutelin synthesis genes *GluA* and *GluB1* in a *japonica* rice collection. *Mol. Breed.* 2017, 37, 129. [CrossRef]
75. Kim, H.J.; Lee, J.Y.; Yoon, U.H.; Lim, S.H.; Kim, Y.M. Effects of reduced prolamin on seed storage protein composition and the nutritional quality of rice. *Int. J. Mol. Sci.* 2013, 14, 17073–17084. [CrossRef] [PubMed]
76. He, Y.; Wang, S.; Ding, Y. Identification of novel gluten subunits and a comparison of gluten composition between *japonica* and *indica* rice (*Oryza sativa* L.). *J. Cereal Sci.* 2013, 57, 362–371. [CrossRef]
77. Bártová, V.; Bártá, J. Chemical composition and nutritional value of protein concentrates isolated from potato *(Solanum tuberosum)* fruit juice by precipitation with ethanol or ferric chloride. *J. Agric. Food Chem.* 2009, 57, 9028–9034. [CrossRef] [PubMed]
78. Jørgensen, M.; Stensballe, A.; Welinder, K.G. Extensive post-translational processing of potato tuber storage proteins and vacuolar targeting. *FEBS J.* 2011, 278, 4070–4087. [CrossRef] [PubMed]
79. Boehn, J.D.; Nguyen, V.; Tashiro, R.M.; Anderson, D.; Shi, C.; Wu, X.; Woodrow, L.; Yu, K.; Cui, Y.; Li, Z. Genetic mapping and validation of the loci controlling 7S α′ and 11S A-type storage protein subunits in soybean *(Glycine max)* (L.) Merr. *Theor. Appl. Genet.* 2018, 131, 659–671. [CrossRef] [PubMed]
80. Goyal, R.; Sharma, S. Genotypic variability in seed storage protein quality and fatty acid Composition of soybean *(Glycine max)* (L.) Merril. *Legum. Res.* 2015, 38, 297–302. [CrossRef]
81. Friedman, M.; Brandon, D.L. Nutritional and health benefits of soy proteins. *J. Agric. Food Chem.* 2001, 49, 1069–1086. [CrossRef] [PubMed]
82. Silva, F.; Nogueira, L.C.; Gonçalves, C.; Ferreira, A.A.; Ferreira, I.P.L.V.O.; Teixeira, N. Electrophoretic and HPLC methods for comparative study of the protein fractions of malts, worts and beers produced from *Solanum tuberosum* (L.) fruit juice by precipitation with ethanol or ferric chloride. *J. Agric. Food Chem.* 2009, 57, 4070–4087. [CrossRef] [PubMed]
83. Quiroga, I.; Regente, M.; Pagnussat, L.; Maldonado, A.; Jorrín, J.; de la Canal, L. Phosphorylated 11S A-type storage protein subunits in *Glycine max* (L.) Merr. *Theor. Appl. Genet.* 2011, 1239–1251. [CrossRef] [PubMed]
84. Youle, R.J.; Huang, A.H.C. Occurrence of low molecular weight and high cysteine containing albumin storage protein in oil-seeds of diverse species. *Am. J. Bot.* 1981, 68, 44–48. [CrossRef]
85. Žilić, S.; Barać, M.; Pešić, M.; Crevar, M.; Stanojević, S.; Nišavić, A.; Saratić, G.; Tolimir, M. Characterization of sunflower seed and kernel proteins. *Helia* 2010, 33, 103–113. [CrossRef]
86. Montoya, C.A.; Leterme, P.; Victoria, N.F.; Toro, O.; Souffrant, W.B.; Beebe, S.; Lallès, J.P. Susceptibility of phaseolin to in vitro proteolysis is highly variable across common bean varieties *(Phaseolus vulgaris)*. *J. Agric. Food Chem.* 2008, 56, 2183–2191. [CrossRef] [PubMed]
87. D’Amico, L.; Valsasina, B.; Daminati, M.G.; Fabbri, M.S.; Nitti, G.; Bollini, R.; Ceriotti, A.; Vitale, A. Bean homologs of the mammalian glucose regulated proteins: Induction by tunicamycin and interaction with newly synthesized storage proteins in the endoplasmic reticulum. *Plant J.* 1992, 2, 443–455. [CrossRef] [PubMed]
88. Mäkienen, O.E.; Sozer, N.; Ercili-Cura, D.; Poutanen, K. Protein form oat: Structure, processes, functionality, and nutrition. In Sustainable Protein Sources; Nadathur, S.R., Wanasundara, J.P.D., Scanlin, L., Eds.; Academic Press: London, UK, 2017; pp. 105–119. ISBN 978-0-12-802779-3.

89. Chang, Y.W.; Alli, I.; Konishi, Y.; Ziomek, E. Characterization of protein fractions from chickpea (Cicer arietinum L.) and oat (Avena sativa L.) seeds using proteomic techniques. Food Res. Int. 2011, 9, 3049–3014. [CrossRef]

90. Tulbek, M.C.; Lam, R.S.H.; Wang, Y.; Asavajaru, P.; Lam, A. Pea: A sustainbable vegetable protein crop. In Sustainable Protein Sources; Nadathur, S.R., Wanasundara, J.P.D., Scanlin, L., Eds.; Academic Press: London, UK, 2017; pp. 145–164. ISBN 978-0-12-802779-3.

91. Barac, M.; Cabrilo, S.; Pesic, M.; Stanojevic, S.; Zilic, S.; Macej, O.; Ristic, N. Profile and Functional Properties of Seed Proteins from Six Pea (Pisum sativum) Genotypes. Int. J. Mol. Sci. 2010, 11, 4973–4990. [CrossRef] [PubMed]

92. Singh, P.K.; Shrivastava, N.; Chaturvedi, K.; Sharma, B.; Bhagyawant, S.S. Characterization of Seed Storage Proteins from Chickpea Using 2D Electrophoresis Coupled with Mass Spectrometry. Biochem. Res. Int. 2016, 12, 109462. [CrossRef] [PubMed]

93. Elfalleh, W.; Nasri, N.; Sarraï, N.; Guasmi, F.; Triki, T.; Marzougui, N.; Ferchichi, A. Storage protein contents and morphological characters of some Tunisian pomegranate (Punica granatum L.) cultivars. Acta Bot. Gallica 2010, 157, 401–409. [CrossRef]

94. Scippa, G.S.; Rocco, M.; Ialiccico, M.; Trupiano, D.; Viscosi, V.; Di Michele, M.; Arena, S.; Chiatante, D.; Scaloni, A. The proteome of lentil (Lens culinaris Medik.) seeds: Discriminating between landraces. Electrophoresis 2010, 31, 497–506. [CrossRef] [PubMed]

95. Schatzki, J.; Ecke, W.; Becker, H.C.; Möllers, C. Mapping of QTL for the seed storage proteins cruciferin and napin in a winter oilseed rape doubled haploid population and their inheritance in relation to other seed traits. Theor. Appl. Genet. 2014, 127, 1213–1222. [CrossRef] [PubMed]

96. Kim, S.G.; Lee, J.S.; Shin, S.H.; Koo, S.C.; Kim, J.T.; Bae, H.H.; Son, B.Y.; Kim, Y.H.; Kim, S.L.; Baek, S.B.; et al. Profiling of differentially expressed proteins in mature kernels of Korean waxy corn cultivars using proteomic analysis. J. Korean Soc. Appl. Biol. Chem. 2015, 58, 293–303. [CrossRef]

97. Görg, A.; Postel, W.; Günther, S. The current state of two-dimensional electrophoresis with immobilized pH gradients. Electrophoresis 1988, 9, 531–546. [CrossRef] [PubMed]

98. Görg, A.; Drews, O.; Lück, C.; Weiland, F.; Weiss, W. 2-DE with IPGs. Electrophoresis 2009, 30 (Suppl. 1), 1221–1232. [CrossRef]

99. Weiss, W.; Görg, A. Two-dimensional electrophoresis for plant proteomics. Methods Mol. Biol. 2007, 355, 121–143. [PubMed]

100. Wheelock, A.M.; Wheelock, C.E. Bioinformatics in gel-based proteomics. In Plant Proteomics: Technologies, Strategies and Applications; Agrawal, G.K., Rakwal, R., Eds.; John Wiley & Sons, Inc.: Hoboken, NJ, USA, 2008; pp. 1–18. ISBN 978-0-470-06976-9.

101. Chevalier, F. Highlights on the capacities of “Gel-based” proteomics. Proteome Sci. 2010, 8, 23. [CrossRef] [PubMed]

102. Rabilloud, T.; Lelong, C. Two-dimensional gel electrophoresis in proteomics: A tutorial. J. Proteom. 2011, 74, 1829–1841. [CrossRef] [PubMed]

103. Dowsey, A.W.; Morris, J.S.; Gutstein, H.G.; Yang, G.Z. Informatics and statistics for analyzing 2-D gel electrophoresis images. Methods Mol. Biol. 2010, 604, 239–255. [CrossRef] [PubMed]

104. Görg, A.; Weiss, W.; Dunn, M.J. Current two-dimensional electrophoresis technology for proteomics. Proteomics 2004, 4, 3665–3685. [CrossRef] [PubMed]

105. Gupta, R.; Min, C.W.; Wang, Y.; Kim, Y.C.; Agrawal, G.K.; Rakwal, R., Eds.; John Wiley & Sons, Inc.: Hoboken, NJ, USA, 2008; pp. 1–18. ISBN 978-0-470-06976-9.

106. Carpentier, S.C.; Witters, E.; Laukens, K.; Deckers, P.; Swennen, R.; Panis, B. Preparation of protein extracts from recalcitrant plant tissues: An evaluation of different methods for two-dimensional gel electrophoresis analysis. Proteomics 2006, 5, 2497–2507. [CrossRef] [PubMed]
108. Faurobert, M.; Pelpoir, E.; Chaib, J. Phenol extraction of proteins for proteomic studies of recalcitrant plant tissues. Methods Mol. Biol. 2007, 355, 9–14. [CrossRef] [PubMed]

109. de la Fuente, M.; Borrajo, A.; Bermúdez, J.; Lores, M.; Alonso, J.; López, M.; Santalla, M.; de Ron, A.M.; Zapata, C.; Alvarez, G. 2-DE-based proteomic analysis of common bean (Phaseolus vulgaris L.) seeds. J. Proteom. 2011, 74, 262–267. [CrossRef] [PubMed]

110. Rabilloud, T.; Chevallet, M.; Luche, S.; Lelong, C. Two-dimensional gel electrophoresis in proteomics: Past, present and future. J. Proteom. 2010, 73, 2064–2077. [CrossRef] [PubMed]

111. López-Pedrouso, M.; Pérez-Santaescolástica, C.; Franco, D.; Fulladosa, E.; Carballo, J.; Zapata, C.; Lorenzo, J.M. Comparative proteomic profiling of myofibrillar proteins in dry-cured ham with different proteolysis indices and adhesiveness. Food Chem. 2018, 244, 238–245. [CrossRef] [PubMed]

112. Rabilloud, T. How to use 2D gel electrophoresis in plant proteomics. In Plant Proteomics: Methods and Protocols; Jorrin-Novó, J.V., Komatsu, S., Weckwerth, W., Wienkoop, S., Eds.; Humana Press: New York, NY, USA, 2014; pp. 43–50. ISBN 978-1-4939-6029-3.

113. Agrawal, G.K.; Thelen, J.J. Development of a simplified, economical polyacrylamide gel staining protocol for phosphoproteins. Proteomics 2005, 5, 4684–4688. [CrossRef] [PubMed]

114. Han, C.; Yang, P. Two Dimensional Gel Electrophoresis-Based Plant Phosphoproteomics. [CrossRef] [PubMed]

115. Kuyama, H.; Toda, C.; Watanabe, M.; Tanaka, K.; Nishimura, O. An efficient chemical method for isolation and sequence analysis of cDNAs for the Arabidopsis thaliana ABI1 gene. Biochem. Biophys. Res. Commun. 1994, 204, 277–283. [CrossRef] [PubMed]

116. Mignery, G.A.; Pikaard, C.S.; Hannapel, D.J.; Park, W.D. Isolation and sequence analysis of cDNAs for the Arabidopsis ABI1 gene. Biochemistry 1994, 33, 6300–6306. [CrossRef] [PubMed]

117. Mignery, G.A.; Pikaard, C.S.; Hannapel, D.J.; Park, W.D. Isolation and sequence analysis of cDNAs for the Arabidopsis ABI1 gene. Biochemistry 1994, 33, 6300–6306. [CrossRef] [PubMed]

118. Gonzalez-Sanchez, M.B.; Lanucara, F.; Helm, M.; Eyers, C.E. Attempting to rewrite history: Challenges with the analysis of histidine-phosphorylated peptides. Biochem. Soc. Trans. 2013, 41, 1089–1095. [CrossRef] [PubMed]

119. Mehta-D’souza, P. Detection of glycoproteins in polyacrylamide gels using Pro-Q Emerald 300 Dye, a fluorescent periodate Schiff-base stain. Methods Mol. Biol. 2012, 869, 561–566. [CrossRef] [PubMed]

120. Duranti, M.; Scarafoni, A.; Gius, C.; Negri, A.; Faoro, F. Heat-induced synthesis and tunicamycin-sensitive secretion of the putative storage glycoprotein conglutin γ from mature lupin seeds. Eur. J. Biochem. 1994, 222, 387–393. [CrossRef] [PubMed]

121. Dam, S.; Thaysen-Andersen, M.; Stenkjaer, E.; Lorentzen, A.; Roepstorff, P.; Packer, N.H.; Stougaard, J. An efficient chemical method for conservation of protein structure and glycosylation in legumes. J. Proteome Res. 2013, 12, 3383–3392. [CrossRef] [PubMed]

122. Borisjuk, L.; Neuberger, T.; Schwender, J.; Heinzel, N.; Sunderhaus, S.; Fuchs, J.; Hay, J.O.; Tschiernack, H.; Braun, H.P.; Denolf, P.; et al. Seed architecture shapes embryo metabolism in oilseed rape. Plant Cell 2012, 24, 1625–1640. [CrossRef] [PubMed]

123. Friso, G.; van Wijk, K.J. Posttranslational protein modifications in plant metabolism. Plant Physiol. 2015, 169, 1469–1487. [CrossRef] [PubMed]

124. Yin, X.; Wang, X.; Komatsu, S. Phosphoproteomics: Protein phosphorylation in regulation of seed germination and plant growth. Curr. Protein Pept. Sci. 2018, 19, 401–412. [CrossRef] [PubMed]

125. Agrawal, G.K.; Thelen, J.J. Large-scale identification and quantitative profiling of phosphoproteins expressed during seed filling in oilseeds. Mol. Cell Proteom. 2006, 5, 2044–2059. [CrossRef] [PubMed]

126. Irar, S.; Oliveira, E.; Pagès, M.; Goday, A. Towards the identification of late-embryogenic-abundant phosphoproteome in Arabidopsis by 2-DE and MS. Proteomics 2006, 6, 175–185. [CrossRef] [PubMed]

127. Wán, L.; Ross, A.R.S.; Yang, J.; Hegedus, D.D.; Kermode, A.R. Phosphorylation of the 12 S globulin cruciferin in wild-type and ab1-1 mutant Arabidopsis thaliana (thale cress) seeds. Biochem. J. 2007, 404, 247–256. [CrossRef] [PubMed]

128. Ghelis, T.; Boibich, G.; Clodic, G.; Habricot, Y.; Miginiac, E.; Sotta, B.; Jeannette, E. Protein tyrosine kinases and protein tyrosine phosphatases are involved in abscisic acid-dependent processes in Arabidopsis seeds and suspension cells. Plant Physiol. 2008, 148, 1668–1680. [CrossRef] [PubMed]
Vilela, B.; Pagès, M.; Riera, M. Emerging roles of protein kinase CK2 in abscisic acid signaling. *Front. Plant Sci.* **2015**, *6*, 966. [CrossRef] [PubMed]

Mulekar, J.J.; Huq, E. Expanding roles of protein kinase CK2 in regulating plant growth and development. *J. Exp. Bot.* **2014**, *65*, 2883–2893. [CrossRef] [PubMed]

Montenarh, M.; Götz, C. Ecto-protein kinase CK2, the neglected form of CK2 (Review). *Biomed. Rep.* **2018**, *8*, 307–313. [CrossRef] [PubMed]

Gosti, F.; Beaudoin, N.; Serizet, C.; Webb, A.A.; Vartanian, N.; Giraudat, J. ABI1 protein phosphatase 2C is a negative regulator of abscisic acid signaling. *Plant Cell* **1999**, *11*, 1897–1910. [CrossRef] [PubMed]

Ludwików, A. Targeting proteins for proteasomal degradation—A new function of *Arabidopsis* ABI1 protein phosphatase 2C. *Front. Plant Sci.* **2015**, *6*, 310. [CrossRef] [PubMed]

Chibani, K.; Ali-Rachedi, S.; Job, C.; Job, D.; Jullien, M.J.; Grappin, P. Proteomic analysis of seed dormancy in *Arabidopsis*. *Plant Physiol.* **2006**, *142*, 1493–1510. [CrossRef] [PubMed]

Gallardo, K.; Job, C.; Groot, S.P.; Puype, M.; Demol, H.; Vandekerckhove, J.; Job, D. Proteomics of *Arabidopsis* seed germination. A comparative study of wild-type and gibberellin-deficient seeds. *Plant Physiol.* **2002**, *129*, 823–837. [CrossRef] [PubMed]

Lehesranta, S.J.; Davies, H.V.; Shepherd, L.V.T.; Koistinen, K.M.; Massat, N.; Nunnan, N.; McNicol, J.W.; Kärenlampi, S.O. Proteomic analysis of the potato tuber life cycle. *Proteomics* **2006**, *6*, 6042–6052. [CrossRef] [PubMed]

Bachem, C.; Van der Hoeven, R.; Luckner, J.; Oomen, R.; Casarin, E.; Jacobsen, E.; Visser, R. Functional genomic analysis of potato tuber life-cycle. *Potato Res.* **2000**, *43*, 297–312. [CrossRef]

Ronning, C.M.; Stegalkina, S.S.; Ascenzi, R.A.; Bougri, O.; Hart, A.L.; Utterbach, T.R.; Vanaken, S.E.; Riedmuller, S.B.; White, J.A.; Cho, J.; et al. Comparative analyses of potato expressed sequence tag libraries. *Plant Physiol.* **2003**, *131*, 419–429. [CrossRef] [PubMed]

Campbell, M.; Segear, E.; Beers, L.; Knauber, D.; Suttle, J. Dormancy in potato tuber meristems: Chemically induced cessation in dormancy matches the natural process based on transcript profiles. *Funct. Integr. Genom.* **2008**, *8*, 317–328. [CrossRef] [PubMed]

Baginsky, S. Plant proteomics: Concepts, applications, and novel strategies for data interpretation. *Mass Spectrom. Rev.* **2009**, *28*, 93–120. [CrossRef] [PubMed]

Strasser, R. Biological significance of complex N-glycans in plants and their impact on plant physiology. *Front. Plant Sci.* **2014**, *5*, 363. [CrossRef] [PubMed]

Strasser, R. Plant protein glycosylation. *Glycobiology* **2016**, *26*, 926–939. [CrossRef] [PubMed]

Lerouge, P.; Cabanes-Macheteau, M.; Rayon, C.; Fischette-Laine, A.C.; Gomord, V.; Faye, L. N-glycoprotein biosynthesis in plants: Recent developments and future trends. *Plant Mol. Biol.* **1998**, *38*, 31–48. [CrossRef] [PubMed]
Koshiyama, I. Carbohydrate component in 7S protein of soybean casein fraction. Agric. Biol. Chem. 1966, 30, 646–650. [CrossRef]

Ericson, M.C.; Chrispeels, M.J. Isolation and characterization of glucosamine-containing storage glycoproteins from the cotyledons of Phaseolus aureus. Plant Physiol. 1973, 52, 98–104. [CrossRef] [PubMed]

Basha, S.M.M.; Beevers, L. Glycoprotein metabolism in the cotyledons of Pisum sativum during development and germination. Plant. Physiol. 1975, 57, 93–97. [CrossRef] [PubMed]

Hall, T.C.; Mleeester, R.C.; Bliss, F.A. Equal expression of the maternal and paternal alleles for the polypeptide subunits of the major storage protein of the bean Phaseolus vulgaris L. Plant Physiol. 1977, 59, 1122–1124. [CrossRef] [PubMed]

Eaton-Mordas, C.A.; Moore, K.G. Seed glycoproteins of Lupinus angustifolius. Phytochemistry 1978, 17, 619–621. [CrossRef]

Badenoch-Jones, J.; Spencer, D.; Higgins, T.J.V.; Millerd, A. The role of glycosylation in storage-proteins synthesis in developing pea seeds. Planta 1981, 153, 201–209. [CrossRef] [PubMed]

Sengupta, C.; Deluca, V.; Bailey, D.S.; Verma, D.P.S. Post-translational processing of 7S and 11S components of soybean storage proteins. Plant Mol. Biol. 1981, 1, 19–34. [CrossRef] [PubMed]

Weber, E.; Manteuffel, R.; Jakubek, M.; Neumann, D. Comparative studies on protein bodies and storage proteins of Pisum sativum L. and Vicia faba L. Biochem. Physiol. Pflanzen 1981, 176, 342–356. [CrossRef]

Chrispeels, M.J.; Higgins, T.J.V.; Craig, S.; Spencer, D. Role of the endoplasmic reticulum in the synthesis of reserve proteins and the kinetics of their transport to protein bodies in developing pea cotyledons. J. Cell Biol. 1982, 93, 5–14. [CrossRef] [PubMed]

Bollini, R.; Vitale, A.; Chrispeels, M.J. 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. [CrossRef] [PubMed]

Lioi, L.; Bollini, R. Contribution of processing events to the molecular heterogeneity of four banding types of phaseolin, the major storage protein of Phaseolus vulgaris L. Plant Mol. Biol. 1984, 3, 345–353. [CrossRef] [PubMed]

Paaren, H.E.; Slightom, J.L.; Hall, T.C.; Inglis, A.S.; Blagrove, R.J. Purification of a seed glycoprotein: N-terminal and deglycosylation analysis of phaseolin. Phytochemistry 1987, 26, 335–343. [CrossRef]

Sturm, A.; Van Kuik, J.A.; Vliegenthart, J.F.G.; Chrispeels, M.J. Structure, position, and biosynthesis of the high mannose and complex oligosaccharide chains of the bean storage protein phaseolin. J. Biol. Chem. 1987, 262, 13392–13403. [PubMed]

Duranti, M.; Guerrieri, N.; Takajashi, T.; Cerletti, P. The legumin-like storage proteins of Lupinus albus seeds. Phytochemistry 1988, 27, 15–23. [CrossRef]

Duranti, M.; Gorinstein, S.; Cerletti, P. Rapid separation and detection of concanavalin. A reacting glycoproteins: Application to storage proteins of a legume seed. J. Food Biochem. 1990, 14, 327–330. [CrossRef]

Lawrence, M.C.; Suzuki, E.; Varghese, J.N.; Davis, P.C.; Van Donkelaar, A.; Tulloch, P.A.; Colman, P.M. The three-dimensional structure of the seed storage protein phaseolin at 3 Å resolution. EMBO J. 1990, 9, 9–15. [CrossRef] [PubMed]

Duranti, M.; Guerrieri, N.; Cerletti, P.; Vecchio, G. The legumin precursor from white lupin seed. Eur. J. Biochem. 1992, 206, 941–947. [CrossRef] [PubMed]

Duranti, M.; Gius, C.; Sessa, F.; Vecchio, G. The saccharide chain of lupin seed conglutin does not prevent it from being protonated during its degradation by trypsin, but facilitates the refolding of the conglutin to its resistant conformation. Eur. J. Biochem. 1995, 230, 886–891. [CrossRef] [PubMed]

Duranti, M.; Horstmann, C.; Gilroy, J.; Croy, R.R.D. The molecular basis for N-glycosylation in the 11S globulin (legumin) of lupin seed. J. Protein Chem. 1995, 14, 107–110. [CrossRef] [PubMed]

Kolarich, D.; Altmann, F. N-glycan analysis by matrix-assisted laser desorption/ionization mass spectrometry of electrophoretically separated nonmammalian proteins: Application to peanut allergen Ara h 1 and olive pollen allergen Ole e 1. Anal. Biochem. 2000, 285, 64–75. [CrossRef] [PubMed]

López-Torrejón, G.; Salcedo, G.; Martín-Esteban, M.; Díaz-Perales, A.; Pascual, C.Y.; Sánchez-Monge, R. Len c 1, a major allergen and vicilin from lentil seeds: Protein isolation and cDNA cloning. J. Allergy Clin. Immunol. 2003, 112, 1208–1215. [CrossRef] [PubMed]
173. Lauer, I.; Foetisch, K.; Kolarich, D.; Ballmer-Weber, B.K.; Conti, A.; Altmann, F.; Vieths, S.; Scheurer, S. Hazelnut (*Corylus avellana*) vicilin Cor a 11: Molecular characterization of a glycoprotein and its allergenic activity. *Biochem. J.* 2004, 383, 327–334. [CrossRef] [PubMed]

177. Santos, C.N.; Alves, M.; Oliveira, A.; Ferreira, R.B. Physicochemical properties of native adzuki bean (*Vigna angularis*) 7S globulin and the molecular cloning of its cDNA isoforms. *J. Agric. Food Chem.* 2007, 55, 3667–3674. [CrossRef] [PubMed]

180. Sn

183. Racusen, D.; Foote, M.A. A major soluble glycoprotein from potato tubers. *Biochem. J.* 2004, 378, 23–34. [CrossRef] [PubMed]

184. Kishimoto, T.; Watanabe, M.; Mitsui, T.; Mori, H. Glutelin basic subunits have a mammalian mucin type Attachment in the Golgi apparatus and removal in protein bodies. *Eur. J. Biochem.* 1984, 141, 33–41. [CrossRef] [PubMed]

186. Lattová, E.; Brabcová, A.; Bártová, V.; Potšila, D.; Bártá, J.; Zdral, Z. O-linked disaccharide side chain. *Biochim. Biophys. Acta* 2004, 1555, 271–277. [CrossRef] [PubMed]

187. Allen, L.W.; Svenson, R.H.; Yachnin, S. Purification of mitogenic proteins derived from *Phaseolus vulgaris*: Isolation of potent and weak phytohemagglutinins possessing mitogenic activity. *Proc. Natl. Acad. Sci. USA* 1969, 63, 334–341. [CrossRef] [PubMed]

188. Miller, J.B.; Hsu, R.; Heinrikson, R.; Yachnin, S. Extensive homology between the subunits of the phytohemagglutinin mitogenic proteins derived from *Phaseolus vulgaris*. *Proc. Natl. Acad. Sci. USA* 1975, 72, 1388–1391. [CrossRef] [PubMed]

189. Vitale, A.; Chrispeels, M.J. Transient N-acetylglucosamine in the biosynthesis of phytohemagglutinin: Attachment in the Golgi apparatus and removal in protein bodies. *J. Cell Biol.* 1984, 99, 133–140. [CrossRef] [PubMed]

190. Faye, L.; Sturm, A.; Bollini, R.; Vitale, A.; Chrispeels, M.J. The position of the oligosaccharide side-chains of phytohemagglutinin and their accessibility to glycosidases determines their subsequent processing in the Golgi. *Eur. J. Biochem.* 1986, 158, 655–661. [CrossRef] [PubMed]
191. Sturm, A.; Chrispeels, M.J. The high mannose oligosaccharide of phytohemagglutinin is attached to asparagine 12 and the modified oligosaccharide to asparagine 60. *Plant Physiol.* **1986**, *80*, 320–322. [CrossRef]

192. Sturm, A.; Bergwerff, A.A.; Vliegenthart, J.F.G. H-NMR structural determination of the N-linked carbohydrate chains on glycopeptides obtained from the bean lectin phytohemagglutinin. *Eur. J. Biochem.* **1992**, *204*, 313–316. [CrossRef] [PubMed]

193. Sharma, M.; Vishwanathreddy, H.; Sindhuara, B.R.; Kamalanathan, A.S.; Swamy, B.M.; Inamdar, S.R. Purification, characterization and biological significance of mannosse binding lectin from *Dioscorea bulbifera* bulbs. *Int. J. Biol. Macromol.* **2017**, *102*, 1146–1155. [CrossRef] [PubMed]

194. Ogawa, T.; Tayama, E.; Kitamura, K.; Kaizuma, N. Genetic improvement of seed storage proteins using three variant alleles of 7S globulin subunits in soybean (*Glycine max* L.) Merr. cultivars using amino acid analysis and two-dimensional electrophoresis. *Food Res. Int.* **2007**, *40*, 129–146. [CrossRef]

195. Kirihara, J.A.; Hunsperger, J.P.; Mahoney, W.C.; Messing, J.W. Differential expression of a gene for a methionine-rich storage protein in maize. *Mol. Gen. Genet.* **1988**, *211*, 477–484. [CrossRef] [PubMed]

196. Gibbon, B.C.; Wang, X.; Larkins, B.A. Altered starch structure is associated with endosperm modification in Quality Protein Maize. *Proc. Natl. Acad. Sci. USA* **2003**, *100*, 15329–15334. [CrossRef] [PubMed]

197. Jiang, C.; Cheng, Z.; Zhang, C.; Yu, T.; Zhong, Q.; Shen, J.; Huang, X. Proteomic analysis of seed storage proteins in wild rice species of the *Oryza* genus. *Proteome Sci.* **2014**, *12*, 51. [CrossRef] [PubMed]

198. El-Shemy, H.A.; Khalafalla, M.M.; Fujita, K.; Ishimoto, M. Improvement of protein quality in transgenic soybean plants. *Biol. Plant.* **2007**, *51*, 277–284. [CrossRef]

199. Jiang, C.; Cheng, Z.; Zhang, C.; Yu, T.; Zhong, Q.; Shen, J.; Huang, X. Proteomic analysis of seed storage proteins in wild rice species of the *Oryza* genus. *Proteome Sci.* **2014**, *12*, 51. [CrossRef] [PubMed]

200. Waglay, A.; Karboune, S.; Alli, I. Potato protein isolates: Recovery and characterization of their properties. *Czech J. Food Sci.* **2008**, *26*, 347–359. [CrossRef]

201. Sturm, A.; Bergwerff, A.A.; Vliegenthart, J.F.G. H-NMR structural determination of the N-linked carbohydrate chains on glycopeptides obtained from the bean lectin phytohemagglutinin. *Eur. J. Biochem.* **1992**, *204*, 313–316. [CrossRef] [PubMed]

202. Sturm, A.; Bergwerff, A.A.; Vliegenthart, J.F.G. H-NMR structural determination of the N-linked carbohydrate chains on glycopeptides obtained from the bean lectin phytohemagglutinin. *Eur. J. Biochem.* **1992**, *204*, 313–316. [CrossRef] [PubMed]

203. Gobbetti, M.; Giuseppe Rizzello, C.; Di Cagno, R.; De Angelis, M. Sourdough lactobacilli and celiac disease. *Food Chem.* **2014**, *142*, 373–382. [CrossRef] [PubMed]

204. Gobbetti, M.; Giuseppe Rizzello, C.; Di Cagno, R.; De Angelis, M. Sourdough lactobacilli and celiac disease. *Food Chem.* **2014**, *142*, 373–382. [CrossRef] [PubMed]

205. El-Shemy, H.A.; Khalafalla, M.M.; Fujita, K.; Ishimoto, M. Improvement of protein quality in transgenic soybean plants. *Biol. Plant.* **2007**, *51*, 277–284. [CrossRef]

206. Tanner, G.J.; Blundell, M.J.; Colgrave, M.L.; Howitt, C.A. Creation of the first ultra-low gluten barley (*Hordeum vulgare* L.) tubers, and its occurrence as genotype effect: Processing versus table potatoes. *Czech J. Food Sci.* **2008**, *26*, 347–359. [CrossRef]

207. Wild, D.; Robins, G.G.; Burley, V.J.; Howdle, P.D. Evidence of high sugar intake, and low fibre and mineral intake, in the gluten-free diet. *Aliment. Pharmacol. Ther.* **2010**, *32*, 573–581. [CrossRef] [PubMed]

208. Øhlund, K.; Olsson, C.; Hernell, O.; Øhlund, I. Dietary shortcomings in children on a gluten-free diet. *J. Hum. Nutr. Diet.* **2010**, *23*, 294–300. [CrossRef] [PubMed]

209. Kawaura, K.; Miura, M.; Kamei, Y.; Ikeda, T.M.; Oghara, Y. Molecular characterization of gladiins of Chinese Spring wheat in relation to celiac disease elicitors. *Genes Genet. Syst.* **2018**. [CrossRef] [PubMed]

210. van den Broeck, H.C.; Gilissen, L.J.W.J.; Smulders, M.J.M.; van der Meer, I.M.; Hamer, R.J. Dough quality of bread wheat lacking α-gliadins with celiac disease epitopes and addition of celiac-safe avenins to improve dough quality. *J. Cereal Sci.* **2011**, *53*, 206–216. [CrossRef]

211. Rizzello, C.G.; Curiel, J.A.; Nionelli, L; Vincentini, O.; Di Cagno, R.; Silano, M.; Gobbetti, M.; Coda, R. Use of fungal proteases and selected sourdough lactic acid bacteria for making wheat bread with an intermediate content of gluten. *Food Microbiol.* **2014**, *37*, 59–68. [CrossRef] [PubMed]

212. Bewley, J.D.; Black, M. *Seeds: Physiology of Development and Germination*, 2nd ed.; Plenum Press: New York, NY, USA, 1994; pp. 377–416. ISBN 978-1-4899-1002-8.
213. Sugliani, M.; Rajjou, L.; Clerkx, E.J.M.; Koornneef, M.; Soppe, W.J.J. Natural modifiers of seed longevity in the Arabidopsis mutants abscisic acid insensitive3-5 (abi3-5) and leafy cotyledon1-3 (lec1-3). New Phytol. 2009, 184, 898–908. [CrossRef] [PubMed]

214. Dobiesz, M.; Piotrowicz-Cieślak, A.I.; Michalczyk, D.J. Physiological and biochemical parameters of lupin seed subjected to 29 years of storage. Crop Sci. 2017, 57, 2149–2159. [CrossRef]

215. Rajjou, L.; Lovigny, Y.; Groot, S.P.C.; Belghazi, M.; Job, C.; Job, D. Proteome-wide characterization of seed aging in Arabidopsis: A comparison between artificial and natural aging protocols. Plant Physiol. 2008, 148, 620–641. [CrossRef] [PubMed]

216. Sano, N.; Rajjou, L.; North, H.M.; Debeaujon, I.; Marion-Poll, A.; Seo, M. Staying alive: Molecular aspects of seed longevity. Plant Cell Physiol. 2015, 57, 660–674. [CrossRef] [PubMed]

217. Kalemba, E.M.; Pukacka, S. Carbonylated proteins accumulated as vitality decreases during long-term storage of beech (Fagus sylvatica L.) seeds. Trees 2014, 28, 503–515. [CrossRef]

218. Senakoon, W.; Nuchadomrong, S.; Chiou, R.Y.Y.; Senawong, G.; Jogloy, S.; Songsri, P.; Patanothai, A. Identification of peanut seed prolamins with an antifungal role by 2D-GE and drought treatment. Biosci. Biotechnol. Biochem. 2015, 79, 1771–1778. [CrossRef] [PubMed]

219. Hajheidari, M.; Eivazi, A.; Buchanan, B.B.; Wong, J.H.; Majidi, I.; Salekdeh, G.H. Proteomics uncovers a role for redox in drought tolerance in wheat. J. Proteome Res. 2007, 6, 1451–1460. [CrossRef] [PubMed]

220. Yahata, E.; Maruyama-Funatsuki, W.; Nishio, Z.; Tabiki, T.; Takata, K.; Yamamoto, Y.; Tanida, M.; Saruyama, H. Wheat cultivar-specific proteins in grain revealed by 2-DE and their application to cultivar identification of flour. Proteomics 2005, 5, 3942–3953. [CrossRef] [PubMed]

221. Kim, Y.S.; Lee, Y.H.; Kim, H.S.; Kim, M.S.; Hahn, K.W.; Ko, J.H.; Joung, H.; Jeon, J.H. Development of patatin knockdown potato tubers using RNA interference (RNAi) technology, for the production of human-therapeutic glycoproteins. BMC Biotechnol. 2008, 8, 36. [CrossRef] [PubMed]

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