A Proteomic Analysis of Seed Development in *Brassica campestri* L

Wenlan Li¹,², Yi Gao¹, Hong Xu¹, Yu Zhang¹, Jianbo Wang¹

¹ State Key Laboratory of Hybrid Rice, College of Life Sciences, Wuhan University, Wuhan, China, ² College of Chemistry and Bioengineering, Guilin University of Technology, Guilin, China

### Abstract

To gain insights into the protein dynamics during seed development, a proteomic study on the developing *Brassica campestri* L. seeds with embryos in different embryogenesis stages was carried out. The seed proteins at 10, 16, 20, 25 and 35 DAP (days after pollination), respectively, were separated using two-dimensional gel electrophoresis and identities of 209 spots with altered abundance were determined by matrix-assisted laser desorption ionization time-of-flight/time-of-flight mass spectrometry (MALDI-TOF/TOF MS). These proteins were classified into 16 groups according to their functions. The most abundant proteins were related to primary metabolism, indicating the heavy demand of materials for rapid embryo growth. Besides, the high amount of proteins involved in protein processing and destination indicated importance of protein renewal during seed development. The remaining were those participated in oxidation/detoxification, energy, defense, transcription, protein synthesis, transporter, cell structure, signal transduction, secondary metabolism, transposition, DNA repair, storage and so on. Protein abundance profiles of each functional class were generated and hierarchical cluster analysis established 8 groups of dynamic patterns. Our results revealed novel characters of protein dynamics in seed development in *Brassica campestri* L. and provided valuable information about the complex process of seed development in plants.

### Introduction

Plant seed is an important organ that is evolutionarily advantageous for plant survival and contributes so much to animal and human life [1]. Seed development goes through three overlapping phases, i.e. morphogenesis, seed filling and seed desiccation, which involve coordinated growth of three seed components, seed coat, endosperm and embryo [2].

Seed development involves highly dynamic processes of cell division, differentiation, growth, pattern formation and macromolecule production [3,4], elucidating the underlying mechanisms will provide insight into the complex system coordinating plant development and metabolism. In recent years, genetic and molecular analyses have identified critical players in the process of seed development [1]. DNA microarray and RNA-seq technique are also advantageous by large-scale genome-wide study of the mRNA level [5–7]. However, mRNA level doesn’t always reflect protein abundance [8], and genomic tools can’t provide precise information on protein levels [9], limiting our understanding on those metabolic and molecular networks. Proteomics provides more powerful tool to understand the complex protein dynamics and the underlying regulatory mechanisms during seed development [10–12]. By examining temporal patterns and simultaneous changes in protein accumulation, extensive proteomic studies have been carried out in legumes [13,14], Arabidopsis [15,16], rapeseed [17,18], rice [19], wheat [20,21] and many other species [10] to profile protein dynamics during seed development. The most popular proteins are those participating in central metabolism, followed by those related to cellular structure, and many previously unknown proteins are indicated important roles in embryo development [12]. In addition, proteome studies also reveal some important characters of seed proteins. For example, a proteome study on *Medicago truncatula* reveals a remarkable compartmentalization of enzymes involved in methionine biosynthesis between the seed tissues, therefore regulating the availability of sulfur-containing amino acids for embryo protein synthesis during seed filling [22]; in tomato seed, the most abundant proteins in both the embryo and endosperm were found to be seed storage proteins, such as legumins, vicilins and albumin [23]. These proteomic applications have greatly expanded our knowledge on seed development.

Plant embryo development, also known as embryogenesis, defines an important development process in higher plant life cycle [24]. Embryo development starts from a double fertilization event in which two sperm nuclei fuse with the egg cell and central cell nuclei respectively, then the zygote undergoes a series of cell divisions and differentiation events to initiate embryo development, going through a globular embryo stage, then a heart-stage, a torpedo-stage and a bended-cotyledon-stage embryo consecutively to produce the mature embryo [25–27]. Therefore, embryogenesis covers part of the processes of morphogenesis and seed filling during seed development. There are gaps in our understanding on
the complete seed development process, as current proteomic studies mainly focus on the protein dynamics during the seed filling or seed dessication. A systematic view of the seed development process encompassing complete embryo development stages is necessary for integrity of our knowledge of full seed development. This is especially meaningful for most dicot plants, because in the mature seed of different species, the relative content of endosperm and embryo is variable. The embryo in dicots is normally the major part of the mature seed, such as in species of Arabidopsis thaliana and Brassica napus, and the endosperm is almost completely absent in the mature seed, whereas in monocots such as wheat, maize, and rice, endosperm tissues possess the majority of the whole seed mass.

To this end, oilseed (Brassica campestri L.) takes its advantage for its relatively larger embryo compared to the model plant Arabidopsis and ease to be accurately differentiated from embryo developmental stages [28]. B. campestri belongs to the mustard family (Brassicaceae), and like most dicotyledonous plants, its embryo development goes through morphologically defined globular, heart, torpedo, and bended cotyledon stages to produce the mature embryo [25,26,29]. Here, using B. campestri seeds with embryos in five sequential development stages of embryogenesis, we carried out a proteomic study on protein dynamics aiming at understanding seed development of oilseed.

**Results**

**High-resolution Proteomes of the Developing B. campestri Seeds**

To isolate proteins of seeds at different stages, developing B. campestri seeds were harvested at precisely 10, 16, 20, 25 and 35 DAP when their embryos were in the globular embryo stage, heart stage, torpedo stage, bended-cotyledon stage and C-shaped mature embryo, respectively (Figure 1). The whole proteins were resolved and detected using high-resolution two-dimensional electrophoresis (2-DE) followed by colloidal Coomassie brilliant blue staining. Initial analyses were performed with immobilized pH gradient (IPG) strips that ranged from pH 3 to 10. It was observed that the region from pH 4 to 7 was a highly dense area on the proteome map, so analyses with pH 4 to 7 IPG strips were further performed to attain high resolution proteome maps. The 2-DE maps showed a highly dynamic proteome during B. campestri seed development (Figure 1). Using the ImageMaster 2D Platinum software 6.0 (GE Healthcare), more than 800 CBB R250-stained protein spots were reproducibly detected from at least three independent 2-D gels, suggesting they were involved in the seed development.

**Identification of Dynamically Accumulated Seed Proteins of B. campestri**

To select protein differentially accumulated over five developmental stages, their proteome profiles were compared using ImageMaster software and 260 spots with at least a two-fold change in statistical analysis (P≤0.05) in combination with manual validation and quantification. Then they were excised from the 2-DE gels and identified by MALDI-TOF/TOF-MS MASCOT and MASCOT database searching. Identities of a total of 209 proteins with altered accumulation were established (Table 1 and Figure 2). G/O analysis was carried out on the base of protein function and these proteins were classified into 16 groups, as depicted in Table 1, including primary metabolism, protein processing and destination, oxidation and detoxification, energy, transcription, protein synthesis, cell structure, signal transduction, defense, secondary metabolism, DNA repair and storage, suggesting these proteins should be involved in a wide range of cellular activities during seed development. Those proteins related to primary metabolism could be further classified into the TCA cycle, carbohydrate metabolism, fatty acid metabolism, nitrogen metabolism, amino acid metabolism, and others (Table 1). Importantly, these spots represented 147 non-redundant proteins, for example, five spots were identified to be enolase (127, 139, 248, 452, 132) and a total of 8 spots were found to be triosephosphate isomerase (591, 592, 557, 583, 338, 350, 573, 589), indicating some of the selected spots are isoforms or modified (Table 1). By calculating the relative proportions, it was found the most abundant proteins participated in the primary metabolism (32.1%), highlighting the dynamic requirement for the growing seed. The second group is related to protein processing/destination (23.4%) followed by those in energy production (14.1%), oxidation/and detoxification (6.7%), disease and defense (5.7%). The other processes these proteins got involved were protein synthesis (4.3%), signal transduction (3.8%), secondary metabolism (3.8%), cell structure (1.9%), transcription (1.9%), DNA repair (1.9%), transposon (1.4%), storage (1.4%), transporter (1.4%), unclear classification (1.0%) and unknown (1.0%) (Figure 3).

**Protein Abundance Profiles of Each Functional Class**

To characterize global abundance kinetics of proteins involved in different processes, composite expression profiles were generated by summing protein abundance, expressed as relative volume [17,30,31], of each functional class over the five developmental stages. As shown in Figure 4, relative abundances of metabolic proteins fluctuated along the experimental period, reflecting different metabolic activity during the embryo maturation. Abundance of those responsible for protein synthesis, destination and second metabolism decreased during early seed growth, but increased and reached the top at the 25 DAP before a second reduction. Disease- and defense-related proteins were highly abundant at the late stage of seed development, and those involved in energy production, oxidation and detoxification, signal transduction, transposition, storage and transportation shared very similar patterns which increased and reached the summit at 25 DAP, whereas proteins related to cell structure, transcription, DNA repair and continued to accumulate and had the highest abundance at 20 DAP (Figure 4). Generally, it’s very interesting to find most of the protein groups possessed relatively higher abundance at a stage from 20 DAP to 25 DAP, reflecting extensive cellular activities during the processes of seed development.

**Hierarchical Clustering Analyses of Seed Proteins**

To further improve the understanding of the identified proteins, abundance profiles were analyzed by hierarchical clustering. Finally, we generated a total of eight cluster groups (c1, c2, c3, c4, c5, c6, c7 and c8) that displayed similar dynamics (Table 2 and Figure 5), suggesting complicated regulatory patterns of these identified proteins during the seed development. The largest group contained 64 proteins (c8), expression of which increased from the early stage of seed development and reached the top at the 25 DAP but decreased at the late stage (35 DAP). The second group included 46 proteins (c7), and most of them were not detected until 16 DAP and were highly accumulated even at 35 DAP, different from those in the group of c1. The smallest cluster, c6, had only four proteins which displayed U-type expression profiles (Figure 5). Clusters c1 and c3 consisted of 28 and 30 proteins, which had the highest abundance at 16 DAP and 20 DAP, respectively. Seventeen proteins were grouped into the c8, and their abundance remained reducing along the seed growth. Notably, most of the
Figure 1. Two-dimensional gel electrophoresis analysis of proteins isolated from developing *Brassica campestris* L. seeds. Seed samples were collected at 10 (B), 16 (C), 20 (D), 25 (E) and 35 (F) days after pollination (DAP) respectively (A). Seed development were monitored by observing their embryos that were at globular stage (g), heart stage (h), torpedo stage (t), bended cotyledon stage (b), and mature embryo stage (m), respectively (A). Total seed proteins were separated by 2-DGE with IEF (pH 4–7) and detected by Coomassie Brilliant Blue.
doi:10.1371/journal.pone.0050290.g001
Table 1. List of total 209 seed proteins differentially accumulated over sequential seed development stages in *Brassica campestri* L.

| Spot Number | Description                                      | Species ID          | MW     | PI    | MOWSE Score | Relative Protein Abundance |
|-------------|--------------------------------------------------|---------------------|--------|-------|-------------|---------------------------|
| 347         | malate dehydrogenase                             | Arabidopsis thaliana| gi|15219721 | 35483.3   | 6.11 415 | 0.074 0.291 0.147 1.71 0.061 |
| 350         | cytosolic malate dehydrogenase                   | Arabidopsis thaliana| gi|21593565 | 35639.4   | 7 161   | 0.132 0.152 0.107 0.457 0.027 |
| 355         | malate dehydrogenase                             | Arabidopsis thaliana| gi|15219721 | 35483.3   | 6.11 234 | 1.438 0.493 0.083 0.077 0.044 |
| 428         | mitochondrial NAD-dependent malate dehydrogenase | Arabidopsis thaliana| gi|21592905 | 35753.8   | 8.54 381 | 0.624 0.227 0.023 0.319 0.143 |
| 429         | mitochondrial NAD-dependent malate dehydrogenase | Arabidopsis thaliana| gi|21592905 | 35753.8   | 8.54 358 | 0.119 0.088 0.046 0.2 0.059 |
| 423         | chloroplast malate dehydrogenase                 | Brassica napus      | gi|207667274| 42292.2   | 8.51 773 | 0.795 0.237 0.031 0.324 1.279 |
| 446         | chloroplast malate dehydrogenase                 | Brassica rapa       | gi|207667274| 42292.2   | 8.51 108 | 0 0 0.125 0.067 0.078 |
| 333         | fructose-bisphosphate aldolase                   | Arabidopsis thaliana| gi|15231715 | 38515.9   | 6.05 190 | 0.059 0.358 0.133 0.037 0.038 |
| 345         | fructose-bisphosphate aldolase                   | Arabidopsis thaliana| gi|15231715 | 38515.9   | 6.05 211 | 0.165 0.154 0.294 0.807 0.234 |
| 348         | fructose-bisphosphate aldolase                   | Arabidopsis thaliana| gi|15231715 | 38515.9   | 6.05 128 | 0.314 0.146 0.114 0.26 0.073 |
| 7           | 2,3-bisphosphoglycerate-independent phosphoglycerate mutase | Arabidopsis thaliana| gi|21537260 | 60661.9   | 5.51 329 | 1.82 0.153 0.112 0.14 0.043 |
| 66          | 2,3-bisphosphoglycerate-independent phosphoglycerate mutase | Arabidopsis thaliana| gi|18391066 | 60541.7   | 5.32 80  | 2.519 0.062 0.676 0.085 2.278 |
| 127         | enolase                                           | Brassica napus      | gi|34597330 | 47346     | 5.46 247 | 0.237 0.155 0.116 2.24 0.126 |
| 139         | enolase                                           | Brassica rapa       | gi|34597330 | 47346     | 5.46 154 | 0.56 0.324 0.461 0.393 0.051 |
| 248         | enolase                                           | Brassica rapa       | gi|34597330 | 47346     | 5.46 216 | 0.075 0.273 0.12 0.127 0.082 |
| 452         | enolase                                           | Brassica rapa       | gi|34597330 | 47346     | 5.46 138 | 0 0.01 0.07 0.52 0.065 |
| 132         | Enolase (LOS2)                                    | Arabidopsis thaliana| gi|15227987 | 47689.3   | 5.54 162 | 0.273 0.216 0.412 0.918 0.044 |
| 536         | glyoxalase II                                     | Arabidopsis thaliana| gi|1644427 | 27770     | 5.58 321 | 0 0 0.128 0.216 0.361 |
| 346         | glyceraldehyde-3-phosphate dehydrogenase C       | Arabidopsis thaliana| gi|21593240 | 36966.1   | 6.62 48  | 0.053 0.174 0.127 0.289 0.105 |
| 360         | glyceraldehyde-3-phosphate dehydrogenase C2      | Arabidopsis thaliana| gi|145323882| 33884.5   | 6.67 40  | 0.356 0.144 0.125 0.101 0.061 |
| 332         | phosphoglycerate kinase                           | Arabidopsis thaliana| gi|21536853 | 42121.4   | 5.49 345 | 0.053 0.565 0.234 0.068 0.037 |
Table 1. Cont.

| Spot Number | Description                                      | Species ID                        | Species                        | MW      | PI      | Days After Pollination (DAP) | MOWSE Score | PI      | Relative Protein Abundance |
|-------------|--------------------------------------------------|-----------------------------------|--------------------------------|---------|---------|-----------------------------|-------------|---------|---------------------------|
| 336         | phosphoglycerate kinase                          | Arabidopsis thaliana             | gi|21536853 | 42121.4 | 5.49 | 630 | 0.089 | 0.673 | 0.23 | 0.131 | 0.063 |
| 591         | triosephosphate isomerase                        | Oryza sativa                     | gi|553107   | 27588.3 | 6.6  | 222 | 0       | 0.09  | 0.356 | 1.905 |
| 592         | triosephosphate isomerase                        | Arabidopsis thaliana             | gi|414550   | 27138   | 5.24 | 188 | 0       | 0.243 | 0.187 | 0.182 |
| 557         | triosephosphate isomerase-like protein type II   | Dimocarpus longan                | gi|262410515| 27261.3 | 6.13 | 56  | 0       | 0.072 | 0.42  | 0.127 |
| 583         | triosephosphate isomerase                        | Arabidopsis thaliana             | gi|15226479 | 33325.1 | 7.67 | 287 | 0 | 0.234 | 0.352 | 0.055 |
| 588         | cytosolic triosephosphate isomerase              | Arabidopsis thaliana             | gi|414550   | 27138   | 5.24 | 205 | 0       | 0.079 | 0.687 | 0.161 |
| 560         | triosephosphate isomerase                        | Oryza sativa                     | gi|553107   | 27588.3 | 6.6  | 210 | 0       | 0.11  | 0.395 | 0.069 |
| 573         | cytosolic triosephosphate isomerase              | Arabidopsis thaliana             | gi|414550   | 27138   | 5.24 | 150 | 0 | 0.064 | 0.42  | 0.127 |
| 589         | cytosolic triosephosphate isomerase              | Arabidopsis thaliana             | gi|414550   | 27138   | 5.24 | 221 | 0 | 0.077 | 0.416 | 0.128 |
| 201         | UTP-glucose-1-phosphate uridylytransferase       | Arabidopsis thaliana             | gi|15237947 | 51887.2 | 5.73 | 129 | 0.071 | 0.184 | 0.304 | 0.397 | 0.087 |
| 199         | UTP-glucose-1-phosphate uridylytransferase       | Arabidopsis thaliana             | gi|15237947 | 51887.2 | 5.73 | 295 | 0.236 | 0.473 | 0.122 | 0.089 | 0.094 |
| 436         | mannose 6-phosphate reductase                    | Arabidopsis thaliana             | gi|15226489 | 34988   | 6.16 | 141 | 0 | 0.011 | 0.084 | 0.123 |
| 135         | alanine aminotransferase                         | Arabidopsis thaliana             | gi|21954071 | 59496   | 5.91 | 101 | 0.123 | 0.206 | 0.426 | 0.151 | 0.097 |
| 413         | beta-glucosidase, putative                       | Ricinus communis                 | gi|255542147| 42630.9 | 5.58 | 58  | 0.138 | 0.367 | 0.301 | 1.008 | 0.072 |
| 251         | UDP-D-apiose/UDP-D-xylose synthetase              | Gossypium hirsutum                | gi|211906520| 43382.2 | 6.11 | 271 | 0.19  | 0.147 | 0.121 | 0.105 | 0.084 |
| 257         | 3-isopropylmalate dehydrogenase                  | Arabidopsis thaliana             | gi|21553584 | 43272.7 | 5.8  | 61  | 0.187 | 0.757 | 0.436 | 0.156 | 0.14  |
| 261         | 3-isopropylmalate dehydrogenase                  | Ricinus communis                 | gi|255579212| 43454.4 | 5.48 | 68  | 0.103 | 0.194 | 0.388 | 0.123 | 0.06  |
| 531         | Trehalose-phosphatase family protein             | Oryza sativa                     | gi|77555881 | 24791.3 | 5.58 | 47  | 0 | 0.114 | 0.285 | 0.425 |
| 1.3 Fatty acid metabolism                          |                                   |                                   |               |         |         |         |               |         |         |         |
| 405         | enoyl-(acyl-carrier-protein) reductase            | Brassica napus                   | gi|14422259 | 40683.8 | 8.53 | 375 | 0.51  | 0.963 | 0.657 | 0.495 | 0.15  |
| 386         | enoyl reductase                                  | Brassica napus                   | gi|1769966  | 40831.1 | 9.25 | 237 | 0.504 | 0.256 | 0.183 | 0.154 | 0.131 |
| 401         | enoyl reductase                                  | Brassica napus                   | gi|14422257 | 40865.2 | 9.27 | 193 | 0.43  | 0.388 | 0.052 | 0.148 | 0.151 |
| 193         | 3-ketoacyl-acyl carrier protein synthase1 (KAS1) | Arabidopsis thaliana             | gi|79329956 | 44701.8 | 8.73 | 223 | 0.111 | 0.133 | 0.188 | 0.147 | 0.053 |
| 195         | KAS1                                             | Arabidopsis thaliana             | gi|79329956 | 44701.8 | 8.73 | 364 | 0.182 | 0.21  | 0.325 | 0.286 | 0.018 |
| 670         | 3-ketoacyl-ACP dehydratase                       | Brassica napus                   | gi|14334124 | 24621.8 | 9.19 | 82  | 0 | 0.028 | 0.194 | 0.13  |
| 1.4 Nitrogen metabolism                            |                                   |                                   |               |         |         |         |               |         |         |         |
| 256         | Glutamine synthetase                             | Brassica napus                   | gi|166406194| 47315.8 | 5.84 | 515  | 0.291 | 1.074 | 0.159 | 0.101 | 0.029 |
| 315         | glutamine synthetase                             | Raphanus sativus                 | gi|1526562  | 38494.2 | 5.93 | 383 | 0.255 | 0.636 | 0.357 | 0.24  | 0.109 |
| 1.5 Amino acid metabolism                          |                                   |                                   |               |         |         |         |               |         |         |         |
| 138         | Imidazole glycerol-phosphate synthase            | Arabidopsis thaliana             | gi|222424719| 64152.9 | 6.31 | 274 | 0.086 | 0.17  | 0.275 | 0.045 | 0.04  |
| 213         | IAA amidohydrolase                               | Arabidopsis thaliana             | gi|18129692 | 47986.3 | 5.81 | 244 | 0.1   | 0.201 | 0.096 | 0.085 | 0.037 |
| 235         | Fumarylacetocetase                               | Arabidopsis thaliana             | gi|22329501 | 46066.1 | 5.31 | 117 | 1.112 | 0.609 | 0.181 | 0.136 | 0.052 |
| 237         | fumarylacetocetase                               | Arabidopsis thaliana             | gi|22329501 | 46066.1 | 5.31 | 96  | 0.138 | 0.163 | 0.446 | 0.153 | 0.045 |
| Spot Number | Description                                             | Species ID          | MW (Da) | PI | MOWSE Score | Relative Protein Abundance |
|-------------|----------------------------------------------------------|---------------------|---------|----|-------------|---------------------------|
| 276         | acetylomithine deacetylase                               | gi|89257686 | 48003.3 | 5.45 | 401         | 0.158 0.593 0.247 0.178   |
| 581         | indole-3-glycerol phosphate synthase                     | gi|21592587 | 44595.5 | 6.99 | 59          | 0 0 0.082 0.228 0.747     |
| 629         | indole-3-glycerol phosphate synthase                     | gi|21592587 | 44595.5 | 6.99 | 215         | 0 0 0.045 0.092 0.154     |
| 271         | o-acetylsine lyase isoform A1 (OASA1)                    | gi|22331257 | 19625.2 | 7.71 | 80          | 0 0 0.109 0.097 0.062     |
| 668         | D-galacturonic acid reductase                            | gi|284437941 | 34701.8 | 6.67 | 53          | 0 0 0.062 0.39 0.3        |
| 236         | MAT3 (methylene adenosyltransferase 3(SAMhecheng)        | gi|15228048 | 42470.6 | 5.76 | 272         | 0.134 0.615 0.125 0.087 0.058 |
| 231         | SAM-2                                                   | gi|15234354 | 43227.8 | 5.67 | 554         | 0.063 0.77 0.187 0.144 0.048 |
| 537         | GLYR1 (glyoxylate reductase 1)                           | gi|79313434 | 29500   | 6.22 | 42          | 0 0 0.25 0.5 0.24        |
| 601         | translation initiation factor eIF3                      | gi|17528988 | 66697.4 | 5.54 | 53          | 0 0 0.08 0.147 0.182     |
| 407         | elongation factor                                        | gi|23397287 | 74184.4 | 7.12 | 168         | 1.62 0.393 0.053 0.462 0.048 |
| 539         | putative cysteinyl-tRNA synthetase                       | gi|22758381 | 95090.9 | 6.2  | 58          | 0 0 0.065 0.189 0.113    |
| 308         | small ribosomal protein 4                                | gi|70516247 | 21815.8 | 10.54| 44          | 0.221 0.173 0.056 1.258 0.045 |
| 681         | small ribosomal protein 4                                | gi|16565378 | 21708   | 9.94 | 55          | 0 0 0.054 0.688 0.181    |
| 489         | nascent polypeptide-associated complex subunit alpha-like protein 3 | gi|240256288 | 22044  | 4.41 | 248         | 0 0 0.049 0.094 0.389    |
| 515         | nascent polypeptide-associated complex subunit alpha-like protein 3 | gi|240256288 | 22044  | 4.41 | 215         | 0 0 0.043 0.267 0.489    |
| 470         | nascent polypeptide-associated complex subunit alpha-like protein 3 | gi|240256288 | 22044  | 4.41 | 190         | 0 0 0.059 0.217 0.145    |
| 288         | nascent polypeptide-associated complex subunit alpha-like protein 3 | gi|240256288 | 22044  | 4.41 | 48          | 0.542 0.145 0.09 0.14 0.17 |
| 42          | heat shock protein 70                                   | gi|6746592  | 77058.6 | 5.13 | 295         | 0.248 0.224 0.499 0.304 0.223 |
| 46          | chloroplast HSP70                                        | gi|124245039 | 75350  | 5.18 | 455         | 0.105 0.304 0.48 0.22 0.107 |
| 49          | BIP2                                                    | gi|30693962 | 73151.9 | 5.11 | 428         | 0.542 1.33 0.438 0.277 0.176 |
| 53          | BIP2                                                    | gi|30693962 | 73151.9 | 5.11 | 519         | 1.492 0.418 0.307 0.203 0.111 |
| 73          | protein disulfide isomerase                              | gi|77999357 | 55706.2 | 133  | 133         | 0.049 0.227 0.361 0.588 0.038 |
| 77          | ATPDIL1-2                                               | gi|152223975 | 56329.4 | 4.9  | 87          | 0.763 0.458 0.388 0.226 0.043 |
| 82          | ATPDIL1-2                                               | gi|152223975 | 56329.4 | 4.9  | 120         | 0.241 0.34 0.546 1.26 0.123 |
| 87          | ATPDIL1-1                                               | gi|30687521 | 54125.4 | 4.97 | 115         | 1.687 0.96 0.47 0.324 0.23  |
| Spot Number | Description                                      | Species ID          | MW     | PI    | MOWSE Score | Relative Protein Abundance | Days After Pollination(DAP) |
|------------|--------------------------------------------------|---------------------|--------|-------|-------------|---------------------------|-----------------------------|
| 90         | ATPDIL1-1                                        | Arabidopsis thaliana | 54125.4| 4.97  | 0.715       | 0.084                     | 0.298                       | 0.541                       | 0.064                       |
| 91         | CPN60b                                           | Arabidopsis thaliana | 63769.6| 6.21  | 0.483       | 0.117                     | 0.094                       | 0.085                       | 0.704                       |
| 112        | BIP2                                             | Arabidopsis thaliana | 73515.9| 5.11  | 0.141       | 0.127                     | 0.131                       | 0.545                       | 0.193                       |
| 216        | RPT3 (regulatory particle triple-A ATPase 3)     | Arabidopsis thaliana | 45722.6| 5.42  | 0.246       | 0.079                     | 0.079                       | 0.704                       | 0.09                        |
| 328        | protein disulphide isomerase                     | Brassica napus      | 25754.3| 6.45  | 0.321       | 0.179                     | 0.411                       | 0.16                        | 0.065                       |
| 344        | UNE5 (unfertilized embryo sac5)                  | Arabidopsis thaliana | 36514.8| 5.49  | 2.46        | 0.151                     | 0.151                       | 0.864                       | 0.039                       |
| 353        | UNE5 (unfertilized embryo sac5)-disulfite isomerase | Arabidopsis thaliana | 39472.4| 5.11  | 0.156       | 0.692                     | 0.101                       | 0.105                       | 0.079                       |
| 455        | PAF2                                             | Arabidopsis thaliana | 30391  | 4.97  | 0.056       | 0.094                     | 0.134                       |                            |                            |
| 527        | peptidyl-prolyl cis-trans isomerase-like protein  | Arabidopsis thaliana | 27457.9| 6.6   | 0.069       | 0.251                     | 0.168                       |                            |                            |
| 576        | OUT-like cysteine protease family protein        | Arabidopsis thaliana | 23410.6| 4.98  | 0.23        | 0.42                      | 0.64                        |                            |                            |
| 592        | PAE2-20S PROTEASOME ALPHA SUBUNIT E2            | Arabidopsis thaliana | 25960.9| 4.7   | 0.243       | 0.227                     | 0.238                       |                            |                            |
| 619        | chaperonin 10                                    | Arabidopsis thaliana | 26912.5| 8.66  | 0.33        | 0.33                      | 0.67                        |                            |                            |
| 626        | multicatalytic endopeptidase complex, proteasome precursor, beta subunit | Arabidopsis thaliana | 24016.1| 5.7   | 0.037       | 0.089                     | 0.127                       |                            |                            |
| 527        | peptidyl-prolyl cis-trans isomerase-like protein  | Arabidopsis thaliana | 32917  | 7.66  | 0.012       | 0.214                     | 0.115                       |                            |                            |
| 635        | peptidylprolyl isomerase ROC4                    | Arabidopsis thaliana | 28178.1| 8.83  | 0.148       | 0.087                     | 0.067                       |                            |                            |
| 641        | translationally-controlled tumor protein          | Zea mays            | 18730.4| 4.53  | 0.107       | 0.203                     | 0.234                       |                            |                            |
| 645        | VFB1 (VIER F-BOX PROTEINE 1)                     | Arabidopsis thaliana | 56459.9| 8.93  | 0.057       | 0.06                      | 0.316                       |                            |                            |
| 84         | ATPDIL1-1 (PDI-LIKE 1-1)                         | Arabidopsis thaliana | 51425.4| 4.97  | 0.067       | 0.514                     | 0.096                       | 0.75                        | 0.122                       |
| 93         | chaperonin HSP60                                 | Arabidopsis thaliana | 55218.9| 5.3   | 0.138       | 0.375                     | 0.087                       | 0.09                        | 0.325                       |
| 96         | chaperonin HSP60                                 | Arabidopsis thaliana | 61312.3| 5.66  | 0.648       | 0.296                     | 0.166                       | 0.1                         | 0.072                       |
| 123        | HSP60-3A                                         | Arabidopsis thaliana | 60428.8| 8.56  | 0.206       | 1.3                       | 0.37                        | 0.078                       | 0.031                       |
| 214        | Tat binding protein like protein                 | Brassica rapa       | 47448.3| 4.91  | 0.462       | 0.273                     | 0.165                       | 0.104                       | 0.045                       |
| 542        | senescence-associated cysteine protease          | Brassica oleracea    | 39309.3| 5.47  | 0.047       | 0.174                     | 0.175                       |                            |                            |
| 566        | PAA2 (20S PROTEASOME SUBUNIT PAA2)               | Arabidopsis thaliana | 27332.8| 5.75  | 0.125       | 0.148                     | 0.225                       |                            |                            |
| Spot Number | Description                                                                 | Species ID                                                                 | MW     | PI    | MOWSE Score | Relative Protein Abundance |
|-------------|------------------------------------------------------------------------------|----------------------------------------------------------------------------|--------|-------|-------------|---------------------------|
| 569         | PAA2 (20S PROTEASOME SUBUNIT PAA2)                                          | Arabidopsis thaliana                                                      | g|15224993 | 27332.8 | 5.75 | 384 | 0 | 0 | 0.06 | 0.165 | 0.164 |
| 586         | PAB1 (PROTEASOME SUBUNIT PAB1)                                              | Arabidopsis thaliana                                                      | g|15219257 | 25685.3 | 5.53 | 249 | 0 | 0 | 0.093 | 0.805 | 0.232 |
| 590         | chaperonin 10                                                               | Arabidopsis thaliana                                                      | g|3057150  | 26912.5 | 8.86 | 158 | 0 | 0 | 0.06 | 0.543 | 0.118 |
| 597         | endopeptidase                                                                | Arabidopsis thaliana                                                      | g|15231824 | 26960.9 | 4.7  | 34  | 0 | 0 | 0.065 | 0.116 | 0.342 |
| 600         | chaperonin 10                                                               | Arabidopsis thaliana                                                      | g|3057150  | 26912.5 | 8.86 | 245 | 0 | 0 | 0.034 | 0.34  | 0.233 |
| 502         | putative proteasome 20S beta1 subunit                                        | Brassica napus                                                             | g|41352683 | 18942.6 | 7.71 | 625 | 0 | 0 | 0.109 | 0.4  | 0.157 |
| 706         | RPP3A (60S acidic ribosomal protein P3)                                      | Arabidopsis thaliana                                                      | g|15236029 | 11841.6 | 4.42 | 70  | 0 | 0 | 0.109 | 0.4  | 0.157 |
| 699         | immunophilin                                                                 | Arabidopsis thaliana                                                      | g|1272408  | 17675.9 | 4.9  | 71  | 0 | 0 | 0.303 | 0.354 | 0.379 |
| 406         | cyclase family protein                                                        | Arabidopsis thaliana                                                      | g|18418598 | 29968.6 | 5.64 | 160 | 0.066 | 0.403 | 0.306 | 0.234 | 0.158 |
| 608         | putative chloroplast nucleoid DNA-binding protein                            | Arabidopsis thaliana                                                      | g|19424106 | 53186.5 | 5.26 | 98  | 0 | 0 | 0.268 | 0.245 | 0.27  |
| 675         | peotidylprolyl isomerase ROC4                                                | Arabidopsis thaliana                                                      | g|21555831 | 28178.1 | 8.83 | 144 | 0 | 0 | 0.275 | 0.395 | 0   |

4. Energy

| Spot Number | Description                                                                 | Species ID                                                                 | MW     | PI    | MOWSE Score | Relative Protein Abundance |
|-------------|------------------------------------------------------------------------------|----------------------------------------------------------------------------|--------|-------|-------------|---------------------------|
| 131         | Ribulose-1,5-bisphosphate carboxylase/oxygenase                              | Arabidopsis thaliana                                                      | g|211573299 | 51767  | 6.04 | 140 | 0.134 | 0.629 | 0.145 | 0.741 | 0.041 |
| 219         | Ribulose-1,5-bisphosphate carboxylase activase                               | Arabidopsis thaliana                                                      | g|30687999 | 48469.4 | 7.55 | 64  | 0.094 | 0.147 | 0.612 | 0.078 | 0.055 |
| 330         | Ribulose-1,5-bisphosphate carboxylase activase                               | Hevea brasiliensis                                                        | g|168997361| 20732.2 | 8.44 | 61  | 0.288 | 0.89  | 0.808 | 0.249 | 0.045 |
| 304         | chloroplast rubisco activase                                                 | Cucumis sativus                                                           | g|239837354 | 66941  | 4.49 | 315 | 0.134 | 0.169 | 0.054 | 0.345 | 1.247 |
| 490         | oxygen-evolving complex (OEC)                                                 | Arabidopsis thaliana                                                      | g|21593220 | 35136.6 | 5.55 | 543 | 0 | 0 | 0.09 | 1.44  | 0.876 |
| 609         | adenosine kinase 2 (ADK2)                                                    | Arabidopsis thaliana                                                      | g|15242717 | 37821.8 | 5.14 | 118 | 0 | 0 | 0.087 | 0.314 | 0.078 |
| 281         | adenosine kinase 2 (ADK2)                                                    | Arabidopsis thaliana                                                      | g|15242717 | 37821.8 | 5.14 | 465 | 0.147 | 0.275 | 0.052 | 0.247 | 0.27  |
| 612         | oxygen-evolving complex (OEC)                                                 | Arabidopsis thaliana                                                      | g|10765373 | 1433.7  | 9.71 | 68  | 0 | 0 | 0.303 | 0.103 | 0.09  |
| 633         | PSBP-1                                                                        | Arabidopsis thaliana                                                      | g|186478207 | 23744.2 | 7.71 | 112 | 0 | 0 | 0.144 | 0.364 | 0.598 |
| 677         | water-soluble chlorophyll protein                                             | Brassica oloracea                                                         | g|27530881 | 22720.9 | 7.83 | 66  | 0 | 0 | 0.059 | 0.337 | 0.09  |
| 682         | water-soluble chlorophyll protein                                             | Brassica oloracea                                                         | g|27530881 | 22720.9 | 7.83 | 237 | 0 | 0 | 0.079 | 0.177 | 0.138 |
| 159         | ATPase subunit 1                                                             | Brassica napus                                                             | g|112253900| 55096.8 | 6.01 | 275 | 0.083 | 0.233 | 0.551 | 0.224 | 0.034 |
| 529         | PSBO2                                                                        | Arabidopsis thaliana                                                      | g|15230324 | 34997.7 | 5.92 | 371 | 0 | 0 | 0.244 | 0.244 | 0.269 |
| 631         | PSBP-1                                                                        | Arabidopsis thaliana                                                      | g|186478207 | 23744.2 | 7.71 | 112 | 0 | 0 | 0.163 | 0.486 | 0.185 |
| 627         | OEE2                                                                          | Arabidopsis thaliana                                                      | g|10765373 | 1433.7  | 9.71 | 96  | 0 | 0 | 0.469 | 0.613 | 0.762 |
| 280         | adenosine kinase 2 (ADK2)                                                    | Arabidopsis thaliana                                                      | g|15242717 | 37821.8 | 5.14 | 198 | 0.248 | 0.45  | 0.167 | 0.144 | 0.107 |

5. Oxidation/Detoxification

| Spot Number | Description                                                                 | Species ID                                                                 | MW     | PI    | MOWSE Score | Relative Protein Abundance |
|-------------|------------------------------------------------------------------------------|----------------------------------------------------------------------------|--------|-------|-------------|---------------------------|
| 324         | peroxidase POA1                                                              | Capsicum annuum                                                           | g|72534134 | 31852.9 | 8.43 | 76  | 0.245 | 0.493 | 0.881 | 0.067 | 0.106 |
### Table 1. Cont.

| Spot Number | Description                  | Species ID          | MW     | PI     | MOWSE Score | Relative Protein Abundance |
|-------------|------------------------------|---------------------|--------|--------|-------------|---------------------------|
| 339         | peroxidase POA1              | Capsicum annuum     | 31852.9 | 8.43   | 0.064       | 0.126                     |
| 393         | peroxidase 27 (PER27)        | Arabidopsis thaliana| 34927.9 | 9.16   | 0.0            | 0.8                       |
| 705         | glutathione peroxidase, putative | Ricinus communis  | 18546.4 | 6.58   | 0.0288       | 0.311                     |
| 446         | disulfide oxidoreductase, putative | Ricinus communis  | 39320.1 | 8.2    | 0.216        | 0.113                     |
| 719         | copper/zinc superoxide dismutase | Arabidopsis thaliana| 22161.2 | 6.28   | 0.0           | 0.28                      |
| 688         | CCH (COPPER CHAPERONE)       | Arabidopsis thaliana| 12962.6 | 4.91   | 0.0           | 0.189                     |
| 605         | GSH-dependent dehydroascorbate reductase 1 | Arabidopsis thaliana| 23406.4 | 6      | 0.019        | 0.314                     |
| 652         | (R)-limonene synthase, putative | Ricinus communis  | 67886   | 5.87   | 0.0044       | 0.063                     |
| 657         | (R)-limonene synthase, putative | Ricinus communis  | 67886   | 5.87   | 0.0045       | 0.382                     |
| 671         | (R)-limonene synthase, putative | Ricinus communis  | 67886   | 5.87   | 0.0062       | 0.552                     |
| 301         | CAD9 (CINNAMYL ALCOHOL DEHYDROGENASE 9) | Arabidopsis thaliana| 38908.6 | 6.21   | 0.417        | 0.917                     |
| 545         | caffeoyl-CoA 3-O-methyltransferase | Brassica rapa | 29005.8 | 5.21   | 0.0033       | 0.128                     |
| 454         | zeta-carotene desaturase     | Zea mays           | 63050.5 | 7.53   | 0.0129       | 0.136                     |
| 713         | phenylalanine amonnia lyase  | Populus trichocarpa| 7744.7  | 5.83    | 0.124        | 0.682                     |
| 223         | reverse transcriptase        | Oryza sativa       | 161394.6 | 6.84  | 0.131        | 0.236                     |
| 502         | myb family transcription factor | Arabidopsis thaliana| 23097.5 | 6.92   | 0.0072       | 0.098                     |
| 607         | transcription factor APF1    | Arabidopsis thaliana| 30034.5 | 6.24   | 0.0164       | 0.262                     |
| 271         | histone acetyltransferase    | Arabidopsis thaliana| 63084.2 | 6.01    | 0.185        | 0.265                     |
| 560         | ATSAR1B (SECRETION-ASSOCIATED RAS 1 B) | Arabidopsis thaliana| 21972.4 | 6.52    | 0.0101       | 0.395                     |
| 476         | AKT2/3 (arabidopsis potassium transport 2/3) | Arabidopsis thaliana| 91249.9 | 6.09    | 0.0018       | 0.07                      |
| 241         | actin-1                      | Diospyros kaki     | 35616.1 | 5.57   | 0.121        | 0.268                     |
| 519         | kinesin motor domain containing protein | Oryza sativa | 31642.4 | 5  | 0.0044       | 0.346                     |
| 318         | RGP4 (REVERSIBLY GLYCOSYLATED POLYPEPTIDE 4) | Arabidopsis thaliana| 41839  | 6.56    | 0.189        | 1.56                      |

**6. Secondary metabolism**

**7. Transcription**

**8. Transportors**

**9. Cell structure**
### Table 1. Cont.

| Spot Number | Description                        | Species                  | ID          | MW        | PI         | MOWSE Score | Relative Protein Abundance | Days After Pollination(DAP) |
|-------------|------------------------------------|--------------------------|-------------|-----------|------------|-------------|---------------------------|-------------------------------|
| 252         | actin1                             | Actinidia deliciosa      | gi|149938964 | 41637     | 5.31        | 0.589        | 0.228                       | 0.504                         | 0.276                         | 0.118                         |
| 10. Signal transduction                             |                                    |                          |             |           |            |             |                           |                               |                               |                               |
| 541         | annexin                            | Arabidopsis thaliana     | gi|1429207    | 35757.2   | 5.2         | 625         | 0.057          | 0.874                        | 0.161                         |
| 88          | calreticulin 2 (CRT2/1)            | Arabidopsis thaliana     | gi|15217459   | 48127.1   | 4.37        | 109         | 0.649          | 0.221                        | 0.12                          | 0.07                          |
| 424         | receptor protein kinase CLAVATA1   | Ricitus communis         | gi|255565085  | 105782.5  | 7.53        | 65          | 0.22           | 0.331                        | 0.415                         | 0.839                         | 1.092                         |
| 478         | pas/lov protein B (PLPB)           | Arabidopsis thaliana     | gi|30678020   | 44660.4   | 6.68        | 80          | 0.237          | 0.871                        | 0.345                         |
| 574         | phosphoprotein phosphatase 2A      | Arabidopsis thaliana     | gi|62321445   | 36064     | 5.32        | 76          | 0.049          | 0.7                          | 0.109                         |
| 674         | calcineurin B-like protein 5 variant | Oryza sativa         | gi|226731839  | 22525.4   | 5.48        | 59          | 0.125          | 0.394                        | 0.599                         |
| 95          | calreticulin                       | Arabidopsis thaliana     | gi|1009712    | 46554     | 4.37        | 57          | 0.057          | 0.871                        | 0.345                         |
| 699         | calcium-dependent protein kinase    | Arabidopsis thaliana     | gi|15229002   | 64506.4   | 8.84        | 56          | 0.39           | 0.303                        | 0.341                         |
| 11. Disease and defense                             |                                    |                          |             |           |            |             |                           |                               |                               |                               |
| 138         | imidazoleglycerol-phosphate synthase | Arabidopsis thaliana   | gi|15236905   | 64152.9   | 6.31        | 274         | 0.03           | 0.17                         | 0.277                         | 0.046                         | 0.041                         |
| 392         | lesion initiation 2 (LIN2)         | Arabidopsis thaliana     | gi|240254000  | 43768.7   | 6.24        | 487         | 1.52           | 0.595                        | 0.271                         | 0.156                         | 1.483                         |
| 394         | putative gag-pol precursor         | Oryza sativa            | gi|16905189   | 77155.9   | 8.74        | 78          | 0.402          | 0.136                        | 0.205                         | 0.335                         | 0.1                           |
| 512         | metacaspase 2 (ATMC2)              | Arabidopsis thaliana     | gi|42567134   | 45781.1   | 5.37        | 56          | 0.083          | 0.48                         | 0.056                         |
| 568         | NHO1 (nonhost resistance to P.s.phaseolicola 1) | Arabidopsis thaliana | gi|79321536   | 52449.7   | 6.11        | 64          | 0.143          | 0.432                        | 0.25                          |
| 665         | NHO1 (nonhost resistance to P.s.phaseolicola 1) | Arabidopsis thaliana | gi|79321536   | 52449.7   | 6.11        | 79          | 0.07           | 0.446                        | 0.209                         |
| 792         | putative blight resistance protein | Oryza sativa            | gi|57891996   | 138160.7  | 6.78        | 83          | 0.093          | 0.591                        | 0.114                         | 0.082                         | 0                              |
| 586         | At2G37660                          | Arabidopsis thaliana     | gi|227204455  | 26279.8   | 5.29        | 250         | 0.093          | 0.806                        | 0.169                         |
| 662         | heat stress-induced protein        | Brassica oleracea       | gi|3319646   | 23474.3   | 8.37        | 139         | 0.049          | 0.166                        | 0.82                          |
| 192         | heat stress-induced protein        | Brassica oleracea       | gi|3319646   | 23474.3   | 8.37        | 224         | 0.529          | 0.213                        | 0.17                          | 0.115                         | 0.051                         |
| 659         | nonhost resistance to P.s. phaseolicola1 (NHO1) | Arabidopsis thaliana | gi|79321536   | 52449.7   | 6.11        | 78          | 0.071          | 0.496                        | 0.414                         |
| 171         | aldehyde dehydrogenase 2B4 (ALDH2B4) | Arabidopsis thaliana | gi|15228319  | 58552.1   | 7.11        | 275         | 0.177          | 0.317                        | 0.057                         |
| 12. Transposon                                      |                                    |                          |             |           |            |             |                           |                               |                               |                               |
| 554         | retrotransposon protein,putative   | Oryza sativa             | gi|110288989  | 112022.1  | 8.57        | 48          | 0.035          | 0.34                         | 0.074                         |
| 349         | retrotransposon protein,putative   | Oryza sativa             | gi|77552111   | 225611    | 8.48        | 60          | 0.261          | 0.068                        | 0.095                         | 0.26                          |
| 637         | retrotransposon protein, putative, unclassified | Oryza sativa | gi|108709588  | 253746.6  | 7.62        | 65          | 0.212          | 0.117                        | 0.065                         |
| 13. DNA repair                                      |                                    |                          |             |           |            |             |                           |                               |                               |                               |
| 223         | reverse transcriptase               | Oryza sativa             | gi|62733278  | 161394.6  | 6.84        | 90          | 0.131          | 0.146                        | 0.859                         | 0.209                         | 0.055                         |
| 158         | DNA repair protein RAD23,putative   | Arabidopsis thaliana     | gi|15240922   | 40041.1   | 4.58        | 190         | 0.106          | 0.272                        | 1.157                         | 0.181                         | 0.045                         |
| 160         | DNA repair protein RAD23,putative   | Arabidopsis thaliana     | gi|45334669  | 34690.5   | 4.85        | 175         | 0.135          | 0.459                        | 0.189                         | 0.167                         | 0.258                         |
| 147         | RAD23-like protein                  | Arabidopsis thaliana     | gi|30409726   | 36198.2   | 4.66        | 110         | 0.181          | 0.173                        | 0.041                         | 0.195                         | 0.128                         |
| 14. Storage                                         |                                    |                          |             |           |            |             |                           |                               |                               |                               |
proteins involved in primary metabolism, energy production, protein destination and oxidation were included into the e4 group (Figure 5 and Table 2), suggesting these cellular activities are essential for the early-stage seed development.

Discussion
Proteins Associated with Metabolism and Protein Renewal are Prevalent in the Developing Seed
Currently, a large number of proteomic studies have been carried out in different species to understand seed development [11,32]. Most of these studies, both in embryo-dominant seeds and endosperm dominant seeds, identify the largest group of proteins

![Proteomic Analysis of B campestri Seed Development](https://example.com/proteomic-analysis.png)

**Figure 2.** A representative image showing spot identification and localization of proteins from developing *Brassica campestri* L. seeds. Those spots indicated by arrows were excised from 2-D gels and analyzed by MALDI-TOF MS/MS. 209 proteins spots that have been identified are indexed as numbered, and the complete list of proteins is in the Table 1.

doi:10.1371/journal.pone.0050290.g002
involved in metabolism, which is consistent with the rapid and complicated metabolic changes during seed development [12]. Our analysis revealed similar character that a proportion of 32.1% total identified proteins participated in primary metabolism (Figure 3). For example, for the enzymes involved in the glycolytic pathway, 7 of them were identified as 15 protein spots. Besides, five enzymes of TCA pathway and three enzymes in lipid biosynthesis were identified (Table 1). An obvious pattern shared by these enzymes is their accumulation remained increasing over five stages of seed development (Figure 3), indicating these

Figure 3. Functional classification of identified seed proteins of Brassica campestri L. The changed protein spots were identified by MALDI-TOF/TOF MS, and classified into 16 groups according to their functions using the NCBI database. Out of a total of 209 identified proteins (red), 147 had non-redundant function (blue). The number above the bar indicates the proportion of each group of protein. doi:10.1371/journal.pone.0050290.g003

Figure 4. Composite protein abundance profiles of each functional categories. The combined accumulation profiles were calculated as the sum of expression value of all the proteins in each category (y axis) at each developmental stage (x axis). doi:10.1371/journal.pone.0050290.g004
metabolic pathways were increasingly required for the seed development. Many proteins with altered expression in our analysis were related to other metabolic events, like amino acid metabolism. In addition, proteins associating with energy and metabolism, defense, oxidation/detoxification were prevalent in the developing seed (Table 1). Interestingly, one transcript study on Arabidopsis embryo development indicates that transition from globular to torpedo stage is associated with up-regulation of genes involved in energetics and metabolism [33], which is consistent to our proteomic study. Abundance of these proteins probably suggests that their activities defined the basal requirement during seed development.

Our data revealed 23.4% total proteins (25.9% of nonredundant proteins) were involved in protein processing and destination (Figure 3). They were those molecular chaperons that helped protein folding of newly synthesized proteins (spot 42, 53, 73), those isomerases that functioned in changing protein conformation (spot 353, 328, 406 and 675), the ubiquitin proteasome group including 20S, 26S proteasome subunits (spot 91, 464, 592, and 1477), the ubiquitin proteasome group that increased its abundance all over the five stages (Figure 5), suggesting that cysteine proteinase also played an important role in maturation and senescence of seed growth.

Altered accumulation of these proteins indicated active protein degradation pathway plays an important role in various aspects of plant growth and development [34]. Polyubiquitination of substrates is achieved through the action of three enzymes: E1, ubiquitin-activating enzyme, E2, ubiquitin-conjugating enzyme, and E3, ubiquitin ligase that determines the specificity of the substrate. The modified protein is then processed by the 20S proteasome, which consists of a core 20S protease capped at each of its ends by a regulatory 19S complex [35]. In our analysis, four isoforms of E1 and eight proteasome components were observed (Table 1). Folding of nascent polypeptides into functional proteins is controlled by a number of molecular chaperones and protein-folding catalysts. Our analysis revealed 6 different isoforms of protein disulfide isomerase, an endoplasmic reticulum-located protein that catalyzes the formation, isomerization, and reduction/oxidation of disulfide bonds [36]. Seven chaperonins or chaperones were also observed, including the plant homolog of the immunoglobulin heavy-chain binding protein (BiP), which is an endoplasmic reticulum-localized member of the heat shock 70 family. BiP has been proposed to play a role in protein body assembly within the endoplasmic reticulum [37,38].

These proteins displayed different accumulation patterns in the process of seed development. For example, spot 73 was identified as a protein disulfide isomerase that continued to accumulate and reached the highest at 20 DAP. Consistent with this in the transcript level, our gene expression analysis also revealed disulfide isomerase can be detected at the late stage of embryogenesis [39]. Plant cysteine proteases are important for organ senescence, plant defense and nutrient mobilization during seed germination [40], and previous studies reveal cysteine proteinases are up-regulated in various senescing plants, such as Arabidopsis, B. napus, and Nicotiana tabacum [41]. In this study, we identified spot 542 as senescence-associated cysteine protease, and spot 576 as another cysteine protease that increased its abundance all over the five stages (Figure 5), suggesting that cysteine protease also played an important role in maturation and senescence of seed growth.

Altered accumulation of these proteins indicated active protein production and elimination occurred in the process of seed development, which might serve as a monitoring mechanism over those intricate processes of metabolism and energy production. It’s also highly likely that the accumulation of these proteins may be used during rapid cell division and cell structure construction. Despite of these, preponderance of these proteins seemed to be particular of our study, because few of previous reports has indicated so many proteins with similar function [13,42–44], which make us underestimate the importance of protein self-renewal. Therefore, protein renewal could be an essential regulatory mechanism for seed development.

Table 2. Hierarchical clusters of differentially accumulated seed proteins and distribution of the proteins belonging to each category in different clusters.

| Categories                          | C1 | C2 | C3 | C4 | C5 | C6 | C7 | C8 | Total |
|-------------------------------------|----|----|----|----|----|----|----|----|-------|
| 1. Primary Metabolism               | 15 | 2  | 10 | 16 | 5  | 2  | 10 | 7  | 67    |
| 2. Protein processing/destination  | 6  | 3  | 3  | 13 | 3  | 1  | 14 | 6  | 49    |
| 3. Energy                           | 2  | 2  | 3  | 6  | 0  | 0  | 4  | 0  | 17    |
| 4. Oxidation and Detoxification     | 2  | 1  | 3  | 5  | 0  | 0  | 3  | 0  | 14    |
| 5. Disease and Defense              | 1  | 0  | 1  | 5  | 1  | 1  | 2  | 1  | 12    |
| 6. Protein synthesis                | 0  | 0  | 0  | 4  | 1  | 0  | 3  | 1  | 9     |
| 7. Signal transduction              | 0  | 0  | 0  | 4  | 0  | 0  | 3  | 1  | 8     |
| 8. Secondary metabolism             | 0  | 0  | 2  | 4  | 0  | 0  | 2  | 0  | 8     |
| 9. Cell structure                   | 1  | 0  | 1  | 1  | 1  | 0  | 0  | 0  | 4     |
| 10. Transcription                   | 0  | 0  | 2  | 1  | 0  | 0  | 1  | 0  | 4     |
| 11. DNA repair                      | 1  | 0  | 3  | 0  | 0  | 0  | 0  | 0  | 4     |
| 12. Transposon                      | 0  | 1  | 1  | 1  | 0  | 0  | 0  | 0  | 3     |
| 13. Storage                         | 0  | 0  | 0  | 2  | 0  | 0  | 1  | 0  | 3     |
| 14. Transporter                     | 0  | 0  | 0  | 1  | 0  | 0  | 1  | 0  | 2     |
| 15. Unclear classification          | 0  | 0  | 1  | 0  | 0  | 0  | 1  | 1  | 3     |
| 16. Unknown                         | 0  | 0  | 0  | 1  | 0  | 0  | 1  | 0  | 2     |
| Total                               | 28 | 9  | 30 | 64 | 11 | 4  | 46 | 17 | 209   |

doi:10.1371/journal.pone.0050290.t002
Carbon Assimilation During Seed Development

The developing oilseeds take up sugars and amino acids from the surrounding endosome liquid and synthesize large quantities of triacylglycerol storage proteins. Previous work characterizes carbon assimilation during seed filling in *Brassica napus* and castor, both of which are oil plants [17,45]. It's interesting to examine this important metabolism pathway in the seed development. It has been demonstrated that glycolysis supplies most carbon to fatty acid synthesis (FAS) in rapeseed developing embryos in culture [46], suggesting glycolysis is essential for carbon assimilation in the developing seeds, but relatively little is known about its regulation and control, and due to the parallel pathways operated in both the cytosol and plastids, it becomes more complex in plants [47,48].

Our study revealed a large number of protein spots corresponding to numerous different glycolytic enzymes both in the cytosol and plastids. FBA catalyzes the aldol cleavage of Fru-1,6-bisP to glyceraldehydes-3-P (GAP) and dihydroxyacetone phosphate(DHAP), and three cytosolic FBA spots were identified in this study (spot 333, 345 and 348) and their expression profiles are different from each other during the development (Figure 6). Triose-P isomerase (TPI) catalyzes the interconversion of GAP and DHAP, and eight TPI (spot 588, 589, 573, 583, 560, 591, 592 and 557), with three in cytosol and five in plastid, were identified (Figure 6). Interestingly, their accumulation was relatively higher at the late stages of seed development. Glyceraldehyde 3-P dehydrogenase (GAPDH) reversibly catalyzes the conversion of GAP into 1,3-

![Figure 5. Abundance profiles of eight hierarchical clusters (c1–c8).](image-url)
bisPGA, and two cytosolic spots (spot 346 and 360) of GAPDH with different expression were identified (Figure 6). Two cytosolic forms of 2,3-bisphosphoglycerate-independent phosphoglycerate mutase (iPGAM) (spot 66 and 71) and two phosphoglycerate kinase spots (PGK) (spot 332 and 336) were separately identified (Figure 6). In addition, five enolases (spot 452, 248, 139, 127 and 132) and malate dehydrogenase (MDH) (spot 347, 350, 355, 423 and 446) and most of them are cytosolic (Figure 6). It is notable that their expression peaked at different seed growth stages, suggesting their importance at different development time. Consistent with a proteomic analysis of seed filling in *B. napus* which suggests that sugar mobilization from glucose to coenzyme A and its acyl derivative is a collaboration between the cytosol and plastids, and temporal control of enzymes and pathways extends beyond transcription [17], the detection of multiple isoelectric species for cytosolic and plastidial glycolytic enzymes indicated balanced dual control of glycolysis in developing *B. nap* seed.
coordination between cytosolic and plastidial glycolysis during seed development.

Possible Seed Development-specific Proteins Indicated by This Study

In our analysis, four spots were identified to be proteins related to cell structure, taking a proportion of only 1.9% of total proteins (Figure 3). This is obviously lower than previous studies on seed development in Arabidopsis [16] or B. napus [18], which often identifies similar proteins of about 20%. Protein level of spot 318, RGP4, continued increasing from the beginning of seed development until at 16 DAP, and could be hardly detected at the late stage (Figure 3), suggesting it might be a novel protein associating with seed development. This is consistent with recent report that expression of RGP4 was restricted in seed and important for development [49]. Such proteins as Actin1 (spot 241, 252) has been found to be highly dynamic in nearly every development stages of seed development, highlighting their importance. Despite these studies use different materials of different development stages, the relative low proportion of proteins contributing to cell structure in this report may suggest another novel character specific to B. campestris seed development.

Our analysis identified only two storage proteins: napin (spot 558) and cruciferin (spot 461 and 540), which are the two major storage proteins in rape seed (B. napus), and constitute 20% and 60% of the total protein in mature seeds [50]. As has been reported that their biological synthesis begins early from the expansion phase of embryo development [51]. Consistent with their roles as “molecular marker” of late embryogenesis, both proteins were found to continually accumulate over the five embryo development stages and reach the highest level in the last stage (Figure 5). In our parallel gene expression analysis, napin gene was found to be up-regulated obviously during embryogenesis and expressed highly only in late embryo stage, but it was not detected in the globular embryo stage [39]. Interestingly, few of previous embryo-related proteomic studies have reported storage protein of napin, in contrast to that cruciferin is frequently detected. Expression of a novel protein, AKT2/3 (spot 476), increased in the early stage of seed development and began to decrease after 20 DAP (Figure 5). In Arabidopsis, AKT2/3 encodes photosynthetic-light-dependent inward rectifying potassium channel with unique gating properties that are regulated by phosphorylation [52,53]. Therefore, identification of AKT2/3 suggested its novel role in seed development. Another interesting finding comes from an unclassified protein TSTJ1 (spot 795), which has been indicated as stem-specific and found in gene chip data [54]. During seed development, it was very highly expressed early at 10 DAP, but after 20 DAP, its protein significantly decreased (Table 2 and Figure 5), therefore, our analysis indicates it may be important for early seed development, which remains to be determined by further experiment.

An investigation on seed development should significantly enrich our knowledge on the molecular and physiological events in whole seed growth process. In this study, we explored the protein dynamics over five stages during B. campestris seed development using a proteomic approach. A total of 209 proteins were identified by mass spectrometry to be differentially seed development and they could be classified into 16 functional groups. It was found that proteins participating in metabolism, energy production, oxidation/detoxification as well as stress/defense were highly dynamic in abundance. However, expressed during functional assignment of these altered proteins uncovers unexpected abundance of proteins related to protein processing and destination, highlighting the importance of protein renewal in seed development, and proportion of those associated to cell structure was rather low compared to previous proteomic analysis of seed development. Our study provides important information to better understanding the seed development in oil plant.

Materials and Methods

Plant Materials and Sample Collection

Brassica campestris L. (cv. Jianghuangzhong) plants were grown in soil-based compost under natural conditions (Wuhan, China). Before flowering, nylon nettings were used to prevent pollen contamination. For sampling seeds in different developmental stages, flowers were tagged immediately after opening of buds, and development of seeds was monitored by checking the embryos under a dissecting microscope. Harvesting the developing seeds was performed at precisely 10, 16, 20, 25 and 35 days after pollination (DAP) when their embryos were at the globular embryo stage, heart stage, torpedo stage, bended-cotyledon stage and C-shaped mature embryo, respectively. Five grams of seeds in each stage were sampled. Then they were frozen in liquid nitrogen and stored at −80°C for use.

Protein Extraction

One gram of seed samples were grounded with mortar and pestle into fine powder in liquid nitrogen, then they were immediately homogenized with ice-cold extraction buffer (8 M Urea, 2 M Thiourea, 4% w/v CHAPS, 40 mM Tris-HCl, pH 8.0) containing protease inhibitors (1 mM PMSF, 10 mM DTT). The supernatant was collected by centrifugation at 20000 g for 30 min at 4°C. Then the pellet was resuspended in ice-cold lysis buffer and centrifuged as described above. After the oil above the supernatant was removed, proteins in the supernatant were precipitated with five volumes of ice-cold trichloroacetic acid-acetone (12.5% trichloroacetic acid in 100% acetone) at −20°C for 2 h and then collected by centrifugation at 20000 g for 30 min. The pellet proteins were resuspended in 80% ice-cold acetone containing 20 mM DTT and centrifuged as above for two times before they were dried by vacuum. The obtained proteins were dissolved in lysis buffer (8 M urea, 4% CHAPS, 10 mM DTT, and 2% pharmalyte 4–7) at room temperature, then vortexed vigorously and centrifuged. The final supernatants were transferred to fresh tubes. The protein concentration was quantified according to the Bradford method [55] using UV-2000 UV-visible spectrophotometry (UNICO) with bovine serum albumin (BSA) as the protein concentration standard. The final protein samples were stored at −70°C for two-dimensional gel electrophoresis (2-DE).

Two-dimensional Electrophoresis (2-DE)

For protein identification, one milligram protein samples in 450 μl rehydration solution containing 8 M Urea, 4% (w/v) CHAPS, 0.5% (v/v) IPG buffer (pH 4–7) (GE Healthcare), 20 mM DTT and 0.002% w/v bromphenol blue were loaded onto 24 cm IPG strips (pH 4–7) (GE Healthcare) after brief sonication and centrifugation. Isoelectric focusing (IEF) was performed at 100 V (1 h), 300 V (1 h), 500 V (1 h), 1000 V (1 h) and 8000 V (12 h) using the Ettan™ IPGphor III™ Isoelectric Focusing System (GE Healthcare). Before the second dimension, strips were equilibrated in the buffer containing 6 M urea, 75 mM Tris-HCl (pH 8.8), 30% (v/v) glyceral, 2% (v/v) SDS, 0.002% (w/v) bromphenol blue and 10 mg/ml DTT for 15 min, then in 25 mg/ml iodoacetamide (15 min) for a second equilibration step. For the second dimension, proteins were separated on 12.5% SDS acrylamide gels (26×20×0.1 cm) at 2.5 W/gel for 45 min, then at 15W/gel for 5 h using the Ettan DALT six System (GE...
Protein Identification by MALDI-TOF/TOF MS

Dynamically accumulated protein spots among five developmental stages were manually excised from 2-D identification gels and digested with trypsin (Promega). Each dried peptide mixture was dissolved into a volume of 50% ACN/0.1% TFA according to its relative abundance in the gel. Then the salts and detergents were removed using Millipore C18 ZipTips (Millipore). Bound peptides were eluted from ZipTip with approximately 3 μl 60% methanol/3% formic acid. 0.5 μl sample solution or calibration standard was then mixed with equal volume of CHCA (α-cyano-4-hydroxycinnamic acid) matrix (10 mg/ml CHCA in 50% ACN/0.1% TFA) and spotted onto a freshly cleaned target plate. After air drying, the crystallized spots were analyzed by MALDI-TOF/TOF (4800 Plus Analyzer, Applied Biosystems). Parent mass peaks were scanned in 1000 laser shots with a mass range of 800~1000 Da after calibration. The minimum signal to noise ratio was 10. Five parent mass peaks with most intensity were picked out for tandem TOF/TOF analysis, each with 1500 laser shots. The searching parameters were set as follows: carbamidomethylation (C) and oxidation (M) as variable modifications, up to one missed cleavage, precursor ion tolerance at 200 ppm, and fragment ion tolerance at 0.3 Da and peptide charge of 1+. Protein hits with protein scores C.I.% (confident identification percentage, based on combined mass and mass/mass spectra) over 95 were reserved. Most identified proteins also have total ion score C.1% (based on mass/mass spectra) over 95. Spectra combined mass and mass/mass were searched against an NCBI protein database, taxonomy Viridiplantae (Green Plants) by GPS Explorer Workstation (Applied Biosystems).

Hierarchical Cluster Analysis

Gene Cluster 3.0/TreeView software was used to do the clustering based on the mean relative volume of each protein spot. Clustering is based on visual inspection of relative similarities or differences between different cluster ranges and the number of clusters was chosen when the dynamics of functional categories between clusters possesses the most significant difference.

Author Contributions

Conceived and designed the experiments: WL JW. Performed the experiments: WL YG HX YZ. Analyzed the data: WL. Wrote the paper: WL JW.

References

1. Santos-Mendoza M, Dubreux B, Baud S, Parry F, Caboche M, et al. (2008) Deciphering gene regulatory networks that control seed development and maturation in Arabidopsis. Plant J 54: 608–620.
2. Chaudhury AM, Koltunow A, Payne T, Luo M, Tucker MR, et al. (2001) Control of early seed development. Annu Rev Cell Dev Biol 17: 677–699.
3. Mansfield SG, Briarty LG (1991) Early embryogenesis in Arabidopsis thaliana. II. The developing embryo. Can J Bot 69: 461–470.
4. Mayer U, Jurgens G (1998) Pattern formation in plant embryogenesis: A reassessment. Sem Cell Dev Biol 9: 187–193.
5. Girke T, Todd J, Kouska S, White J, Benning C, et al. (2000) Microarray analysis of developing Arabidopsis seeds. Plant Physiol 124: 1570–1581.
6. Xue LJ, Zhang JJ, Xue HW (2012) Genome-wide analysis of the complex transcriptional networks of rice developing seeds. Plos One 7: e30646.
7. Rhee SY, Cho SH, Park C, Yang BR, Lee SY, et al. (2010) Genome-wide identification of protein-protein interaction partners in rice embryo. Proteomics 10: 2955–2964.
8. Gygi SP, Rochon Y, France BR, Aebersold R (1999) Correlation between protein and mRNA abundance in yeast. Mol Cell Biol 19: 1720–1730.
9. Anderson NL, Anderson NG (1998) Proteome and proteomics: new technologies, new concepts, and new words. Electrophoresis 19: 1853–1861.
10. Chen SX, Harmon AC (2006) Advances in plant proteomics. Proteomics 6: 5504–5516.
11. Hochholdinger F, Sauer M, Dembinsky D, Hoekker N, Mutreith N, et al. (2006) Proteomic dissection of plant development. Proteomics 6: 4076–4083.
12. Miernyk JA, Hajduch M (2011) Seed proteomics. J Proteome Res 9: 5715–5726.
13. Choudhury AM, Craig S, Dennis ES, Peacock WJ (1998) Ovule and embryo development of metabolic isozymes using high-resolution two-dimensional gel electrophoresis. Plant Physiol 141: 32–46.
14. Agrawal GK, Hajduch M, Graham K, Thelen JJ (2008) In-depth investigation of the soybean seed-filling proteome and comparison with a parallel study of rapeseed. Plant Physiol 148: 504–518.
15. Kim ST, Wang YM, Kang Y, Kim SG, Rakwal R, et al. (2009) Developing rice embryo proteomics reveals essential role for embryonic proteins in regulation of seed germination. J Proteome Res 8: 3598–3605.
16. Xue LJ, Zhang JJ, Xue HW (2012) Genome-wide analysis of the complex transcriptional networks of rice developing seeds. Plos One 7: e31081.
17. Xu H, Gao Y, Wang JB (2012) Transcriptomic analysis of rice (Oryza sativa) developing embryos using the RNA-Seq technique. Plos One 7: e30646.
18. Gallardo K, Le Signor C, Vandekerckhove J, Thompson RD, Burstin J (2003) Proteomic analysis of wheat embryos with 2-DE and liquid-phase chromatography (ProteomeLab PF-2D) - A wider perspective of the proteome. J Proteomics 73: 1707–1721.
19. Guo HX, Zhang HZ, Li YC, Ren JP, Wang X, et al. (2011) Identification of changes in wheat (Triticum aestivum L.) seeds proteome in response to anti-irx s gene. Plos One 6: e22255.
20. Gallardo K, Le Signor C, Vandekerckhove J, Thompson RD, Burstin J (2003) Proteomics of Medicago truncatula seed development establishes the time frame of diverse metabolic processes related to reserve accumulation. Plant Physiol 133: 664–682.
21. Sheoran IS, Ross ARS, Olson DJH, Sashey NK (2007) Proteomic analysis of tomato (Lycopersicon esculentum) pollen. J Exp Bot 58: 3525–3535.
22. Harada J (1999) Signalling in plant embryogenesis. Curr Opin Plant Biol 2: 23–27.
23. Chaudhury AM, Craig S, Dennis ES, Peacock WJ (1998) Ovule and embryo development, apomixis and fertilization. Curr Opin Plant Biol 1: 26–31.
24. Goldberg RB, Depaiva G, Yadegari R (1994) Plant embryogenesis - zygote to seed. Science 266: 605–614.
25. West MAL, Harada J (1999) Embryogenesis in higher plants - an overview. Plant Cell 5: 1361–1369.
26. Dong JZ, Keller WA, Yan W, Georges F (2004) Gene expression at early stages of soybean seed development as revealed by transcript profiling of seed-abundant cDNAs. Planta 218: 485–491.
27. Jurgens G (2001) Apical-basal pattern formation in Arabidopsis embryogenesis. EMBO J 20: 3609–3616.
28. Dong JZ, Keller WA, Yan W, Georges F (2004) Gene expression at early stages of soybean seed development as revealed by transcript profiling of seed-abundant cDNAs. Planta 218: 485–491.
29. Jurgens G (2001) Apical-basal pattern formation in Arabidopsis embryogenesis. EMBO J 20: 3609–3616.
30. Mechin V, Thevenot C, Le Guilloux M, Prioul JL, Damerval C (2007) Developmental analysis of maize endosperm proteome suggests a pivotal role for pyruvate orthophosphate dikinase. Plant Physiol 143: 1203–1219.

31. Xu SB, Li T, Deng ZY, Chong K, Xue YB, et al. (2008) Dynamic proteomic analysis reveals a switch between central carbon metabolism and alcoholic fermentation in rice filling grains. Plant Physiol 148: 908–925.

32. Hajduch M, Matsuova R, Houston NL, Thelen JJ (2011) Comparative proteomics of seed maturation in oilseeds reveals differences in intermediary metabolism. Proteomics 11: 1619–1629.

33. Spencer MW, Casson SA, Lindsey K (2007) Transcriptional profiling of the Arabidopsis embryo. Plant Physiol 143: 924–940.

34. Trujillo M, Shirasu K (2010) Ubiquitination in plant immunity. Curr Opin Plant Biol 13: 402–408.

35. Vierstra RD (2009) The ubiquitin-26S proteasome system at the nexus of plant biology. Nat Rev Mol Cell Biol 10: 385–397.

36. Houston NL, Fan CZ, Xiang QY, Schulze JM, Jung R, et al. (2005) Phylogenetic analyses identify 10 classes of the protein disulfide isomerase family in plants, including single-domain protein disulfide isomerase-related proteins. Plant Physiol 137: 762–778.

37. Vitale A, Ceriotti A (2004) Protein quality control mechanisms and protein storage in the endoplasmic reticulum. A conflict of interests? Plant Physiol 136: 3420–3426.

38. Fontes EBP, Shank BB, Wrobel RL, Moose SP, Obrian GR, et al. (1991) Characterization of an immunoglobulin-binding protein homolog in the maize floury-2 endosperm mutant. Plant Cell 3: 483–496.

39. Li WL, Zhao Q, Tao P, Wang JB (2012) Identification of genes differentially expressed during embryogenesis in Brassica campestris L. Acta Physiol Plant 34: 669–681.

40. Beers EP, Jones AM, Dickerman AW (1991) Characterization of an immunoglobulin-binding protein homolog in the maize floury-2 endosperm mutant. Plant Cell 3: 483–496.

41. Chen GH, Huang LT, Yap MN, Lee RH, Huang YJ, et al. (2002) Molecular characterization of a senescence-associated gene encoding cysteine proteinase and its gene expression during leaf senescence in sweet potatoes. Plant Cell Physiol 43: 984–991.

42. Kim ST, Kang SY, Wong W, Kim SG, Hwang DH, et al. (2008) Analysis of embryonic proteome modulation by GA and ABA from germinating rice seeds. Proteomics 8: 3577–3587.

43. Liu H, Liu YJ, Yang MF, Shen SH (2009) A comparative analysis of embryo and endosperm proteome from seeds of Jatropha curcas. J Integr Plant Biol 51: 850–857.

44. Cordewener J, van der Wal F, Jossen R, Boutilier K, America T (2009) Proteomics in rapeseed microspore embryogenesis. Adv Haploid Production Higher Plants: 135–146.

45. Houston NL, Hajduch M, Thelen JJ (2009) Quantitative proteomics of seed filling in castor: comparison with soybean and rapeseed reveals differences between photosynthetic and nonphotosynthetic seed metabolism. Plant Physiol 151: 837–868.

46. Schwender J, Ohlrogge JB, Shachar-Hill Y (2003) A flux model of glycolysis and the oxidative pentosephosphate pathway in developing Brassica napus embryos. J Biol Chem 278: 29442–29453.

47. Plaxton WC (1996) The organization and regulation of plant glycolysis. Annu Rev Plant Physiol Plant Mol Biol 47: 183–214.

48. Fernie AR, Carrari F, Swederse LJ (2004) Respiratory metabolism: glycolysis, the TCA cycle and mitochondrial electron transport. Curr Opin Plant Biol 7: 254–261.

49. Rautengarten C, Ebert B, Herter T, Petzold CJ, Ishii T, et al. (2011) The interconversion of UDP-Arabinosyranose and UDP-Arabinoferanose is indispensable for plant development in Arabidopsis. Plant Cell 23: 1373–1390.

50. Dalgalarrondo M, Robin JM, Azanza JL (1986) Subunit composition of the globulin fraction of rapeseed (Brassica napus L.). Plant Sci 43: 115–124.

51. Hoglund AS, Rodin J, Larsson E, Raak L (1992) Distribution of napin and cruciferin in developing rape seed embryos. Plant Physiol 98: 509–515.

52. Ivanishkina N, Decken R, Fischer S, Ape C, Hedrich R (2005) AKT2/3 subunits render guard cell K⁺ channels Ca²⁺ sensitive. J Gen Physiol 125: 483–492.

53. Cherel I, Michard E, Platet N, Mouline K, Alcon C, et al. (2002) Physical and functional interaction of the Arabidopsis K⁺ channel AKT2 and phosphatase ATP2CA. Plant Cell 14: 1133–1146.

54. Chua YL, Most E, Brown ACP, MacLean D, Gray JC (2004) Microarray analysis of chromatin-immunoprecipitated DNA identifies specific regions of tobacco genes associated with acetylated histones. Plant J 37: 789–800.

55. Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72: 248–254.

56. Candiano G, Bruschi M, Musante L, Santucci I, Ghiggeri GM, et al. (2004) Blue silver: a very sensitive colloidal Coomassie G-250 staining for proteome analysis. Electrophoresis 25: 1327–1333.