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DATASET BRIEF

A Tandem Mass Tags (TMTs) labeling approach highlights differences between the shoot proteome of two *Arabidopsis thaliana* ecotypes, Col-0 and Ws

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Abstract

Arabidopsis has become a powerful model to study morphogenesis, plant growth, development but also plant response to environmental conditions. Over 1000 *Arabidopsis* genomes are available and show natural genetic variations. Among them, the main reference accessions Wassilewskija (Ws) and Columbia (Col-0), originally growing at contrasted altitudes and temperatures, are widely studied, but data contributing to their molecular phenotyping are still scarce. A global quantitative proteomics approach using isobaric stable isotope labeling (Tandem Mass Tags, TMT) was performed on Ws and Col-0. Plants have been hydroponically grown at 16 h/8 h (light/dark cycle) at 23°C day/19°C night for three weeks. A TMT labeling of the proteins extracted from their shoots has been performed and showed a differential pattern of protein abundance between them. These results have allowed identifying several proteins families possibly involved in the differential responses observed for Ws and Col-0 during plant development and upon environmental changes. In particular, Ws and Col-0 mainly differ in photosynthesis, cell wall-related proteins, plant defense/stress, ROS scavenging enzymes/redox homeostasis and DNA/RNA binding/transcription/translation/protein folding.

KEYWORDS

Arabidopsis thaliana, Columbia Col-0 ecotype, shoot, TMT labeling, Wassilewskija ecotype, quantitative proteomics

Arabidopsis thaliana is an important model organism with a robust network of resources that is of paramount importance to the plant science research community. Researchers have used the natural variations between *A. thaliana* accessions or ecotypes for both molecular and genetic studies to uncover complex genetic interactions, such as those underlying plant responses to environmental clues [1, 2]. These accessions are quite different in terms of morphology, development

and physiology. Two of the most popular *A. thaliana* ecotypes are Col-0 and Ws [3]. They have been the chosen genetic background for the majority of *Arabidopsis* T-DNA insertion mutant collections [4]. The Col-0 ecotype originates from Poland where it was adapted to high temperature and low altitude, whereas the Ws ecotype originally comes from Vasljevic in Belarus where it was adapted to low temperature and high altitude (<http://vseed.nottingham.ac.uk>).

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Few studies showing differences between Col-0 and Ws have been performed at the molecular or phenotypic levels. It has been shown that Ws contains a deletion in the phytochrome D gene, *PHYD*, with consequences on morphological traits including longer hypocotyl under light, reduction in cotyledon expansion and accumulation of anthocyanin in seedlings stem [5]. Other studies report that the flowering response is induced earlier in Ws than in Col-0 [5, 6]. The root length is less important in Ws than in Col-0 [7, 8]. Plant growth homeostasis and defense responses are regulated by *BONZAI1* (*BON1*) [9]. Its deletion in Col-0 leads to a dwarf phenotype while a wild-type phenotype is observed in Ws, indicating the presence of a *bon1* suppressor in Ws. *BON1* is a negative regulator of the resistance gene *SNC1* encoding a TIR-NB-LRR receptor-like which mediates defense response and is specific to Col-0 [9]. In addition, the two repressors of plant defense MKP1 (MAP Kinase Phosphatase 1) and PTP1 (Protein Tyrosine Phosphatase 1) are active in Col-0 [10]. Besides, the sensitivity of Col-0 to pathogens, as shown after a treatment with flagellin, is characterized by the induction of an oxidative burst, callose deposition and growth inhibition which is not observed in Ws [11].

It has been shown that protein levels change in the same direction that transcripts abundance changes but not necessary of comparable degree [12]. A recent study also showed that expression patterns for individual genes are often consistent with transcript, protein and sometimes with phosphorylation site level [13]. However, significant differences were shown in the expression of genes encoding cell wall proteins, genes that might participate in cell elongation (transcriptomic data) and the presence of the proteins (proteomic data) in different tissues at different developmental stages. This data revealed the importance of posttranscriptional mechanisms for the regulation of expression of genes and that transcriptomic and proteomic approaches are complementary [14, 15].

To our knowledge there is no data reporting the characterization of the proteomes of Col-0 and Ws, as a molecular phenotype. In this study, a comparative quantitative proteomic analysis has been performed to investigate the differences between the protein profiles of Col-0 and Ws. Total soluble proteins from the shoot of 3 week-old plants were extracted, identified and quantified using a TMTs-based labeling technology [16]. To identify Differentially Accumulated Proteins (DAPs), the results were classified according to the ratio of amount of proteins in Ws versus Col-0. Altogether, we could identify 6383 proteins. Among them, 235 and 30 were under- and over-accumulated, respectively, in Ws as compared to Col-0.

A. thaliana seeds were surface-sterilized with ethanol, sown on seed holder containing 0.65% agar. The plants were grown hydroponically using the Araponics system (Araponics, Liège, Belgium) and commercial media Tripack Floraseries (FloraGrow, 0.5 mL/L; FloraBloom, 0.5 mL/L; and FloraMicro, 0.5 mL/L; GHE, Fleurance, France) adapted from [17]. The seeds were stratified at 4°C for 2 days and grown under controlled environmental conditions (16 h/8 h light/dark cycle, 120 μmol photons/m²/s, 23°C day/19°C night). The hydroponic solution was oxygenated by air bubbling and changed once a week. The shoots were harvested after 21 days. Samples were immediately placed in liquid nitrogen, ground to a fine powder in a ball mill and stored at -80°C until

Statement of significance

Arabidopsis has become a powerful model to study morphogenesis, plant growth and development. The main reference accessions Wassilewskija (Ws) and Columbia (Col-0) are widely studied but data reporting the characterization of the proteomes of Col-0 and Ws are not available, as a molecular phenotype. A global TMTs-based quantitative proteomics approach was performed on the shoot of these two ecotypes. The work sheds new light on the differential pattern of protein abundance between Col-0 and Ws. We show for the first time that Ws and Col-0 mainly differ in five major protein families, possibly involved in the differential responses observed for these two ecotypes during their development and upon environmental changes. Our data reveal that the natural variants Col-0 and Ws have probably different phenotypic adaptation or plasticity to their habitat and environment.

This manuscript represents advancement in the field of developmental biology and opens the way towards future proteomic studies of *Arabidopsis* natural variants complementary to molecular and genetic analyses.

use. This process was repeated for three biologically and temporally independent replicates per genotype. Soluble proteins were extracted using a protocol adapted from [18]. The proteins were quantified using the Bradford method [19]. Twenty-five micrograms of proteins of each sample were digested and labeled with TMT10-plex reagents (ThermoFisher Scientific, San Jose, CA, USA), mixed in equimolar amounts. The tags used were 126 (126.12772) and 129C (129.13779) for Ws Col-0 samples, respectively. A fractionation was purchased using the High pH Reversed-Phase Peptide Fractionation Kit (Pierce, San Jose, CA, USA) according to manufacturer's recommendations. The tryptic peptide solutions were dried under vacuum and reconstituted in 20 μL water/1% formic acid (v/v) each. The LC-MS/MS platform consisted of an Ultimate 3000 RSLC UPLC system coupled to an Orbitrap Fusion mass spectrometer (MS) (ThermoFisher Scientific) with a nano-trap column (Acclaim PepMap 100 Å C18, 5 μm, 100 μm i.d. x 2 cm length, ThermoFisher Scientific) and an Easy-Spray column (Acclaim PepMap 100 Å C18, 2 μm, 75 μm i.d. x 50 cm length, ThermoFisher Scientific). Ten fractions of the TMT-labeled digest were separated by on-line nanoLC and analyzed by nano-electrospray tandem mass spectrometry. The overall workflow of the analysis is presented in Figure 1. The peptide mixtures were injected onto a nano-trap column with a flow of 5 μL/min and subsequently gradient eluted with a flow of 300 nL/min, from 4 to 30% acetonitrile (v/v) during 140 min. Each fraction was analyzed on an Orbitrap Fusion MS using synchronous precursor selection (SPS) MS3 quantitation. The full scan was performed in the range of 375–2000 *m/z* at nominal resolution of 120,000 at 200 *m/z* and AGC set to 4.10⁵, followed by selection of the most intense ions

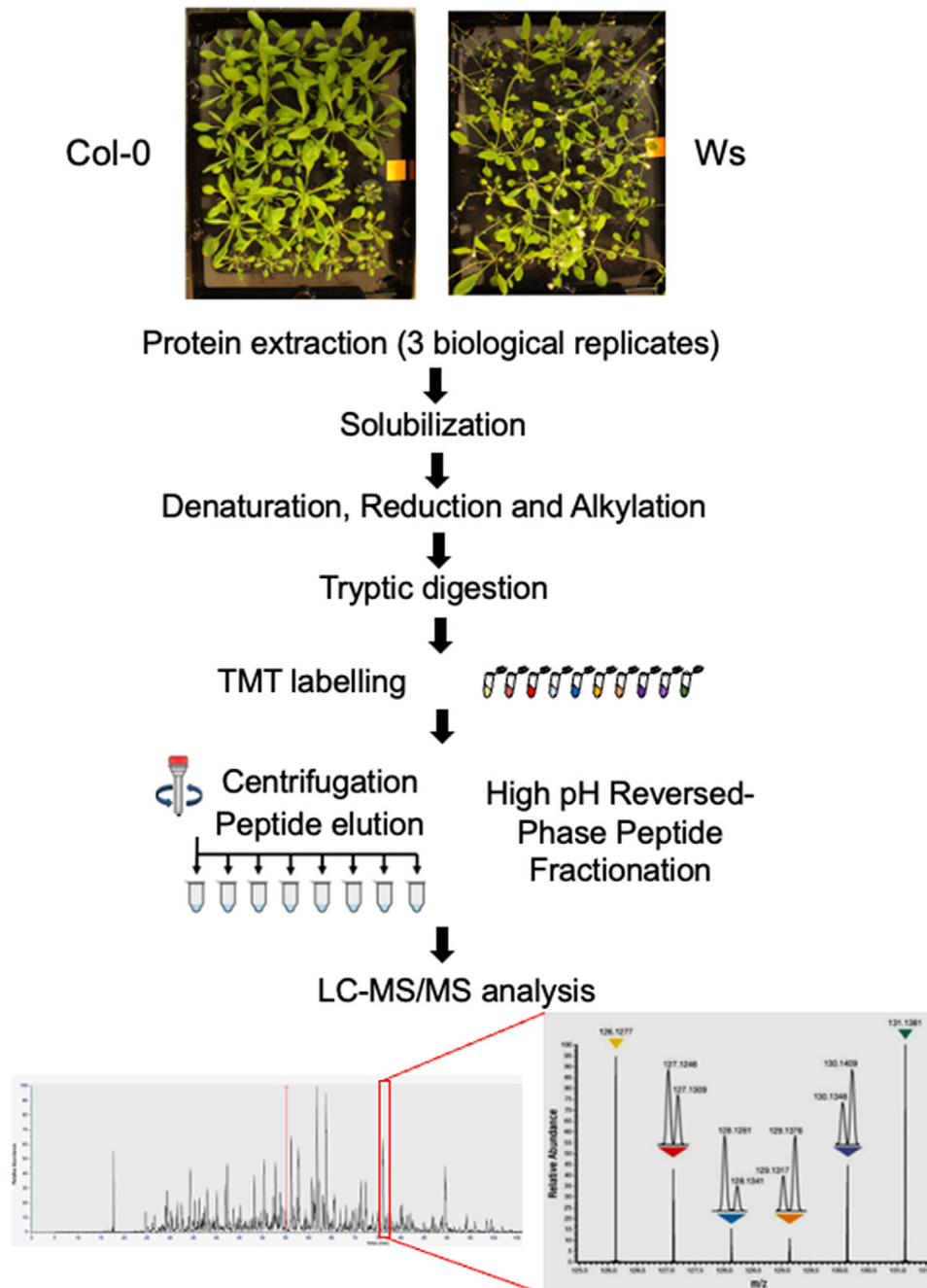


FIGURE 1 General workflow of the experiment for TMTs-based proteomic analysis of the shoot of two ecotypes of *A. thaliana*, Ws, and Col-0

above an intensity threshold of 5000 for collision-induced dissociation (CID)-MS2 fragmentation in the linear ion trap with 35% normalized collision energy. The isolation width for the frontal cortex samples was set to 0.7 m/z with no offset. The top 10 fragment ions for each peptide MS2 were notched out with an isolation width of 2 m/z and co-fragmented to produce MS3 scans analyzed in the MS at a nominal resolution of 50,000 after higher-energy collision dissociation (HCD) fragmentation at a normalized collision energy of 65%. Raw data files from the Orbitrap Fusion were processed using Proteome Discoverer (version 2.4). Protein identification was achieved by comparing the MS data to the TAIR database (www.arabidopsis.com). Parameters

were specified as follows: trypsin enzyme, two miscleavages allowed, minimum peptide length of six amino acids, TMT tags on lysine residues and peptide N-termini (+229.162932 Da), carbamidomethylation of cysteine residues (+57.021 Da) as fixed modifications, oxidation of methionine residues (+15.995 Da) and acetylation of protein N-termini (+42.011 Da) as variable modifications, precursor mass tolerance of 10 ppm, and a fragment mass tolerance of 0.6 Da. Peptide spectral match (PSM) error rates were determined using the target-decoy strategy coupled to Percolator modeling of true and false matches [20]. Reporter ions were quantified from MS3 scans using an integration tolerance of 20 ppm with the most confident centroid setting. An

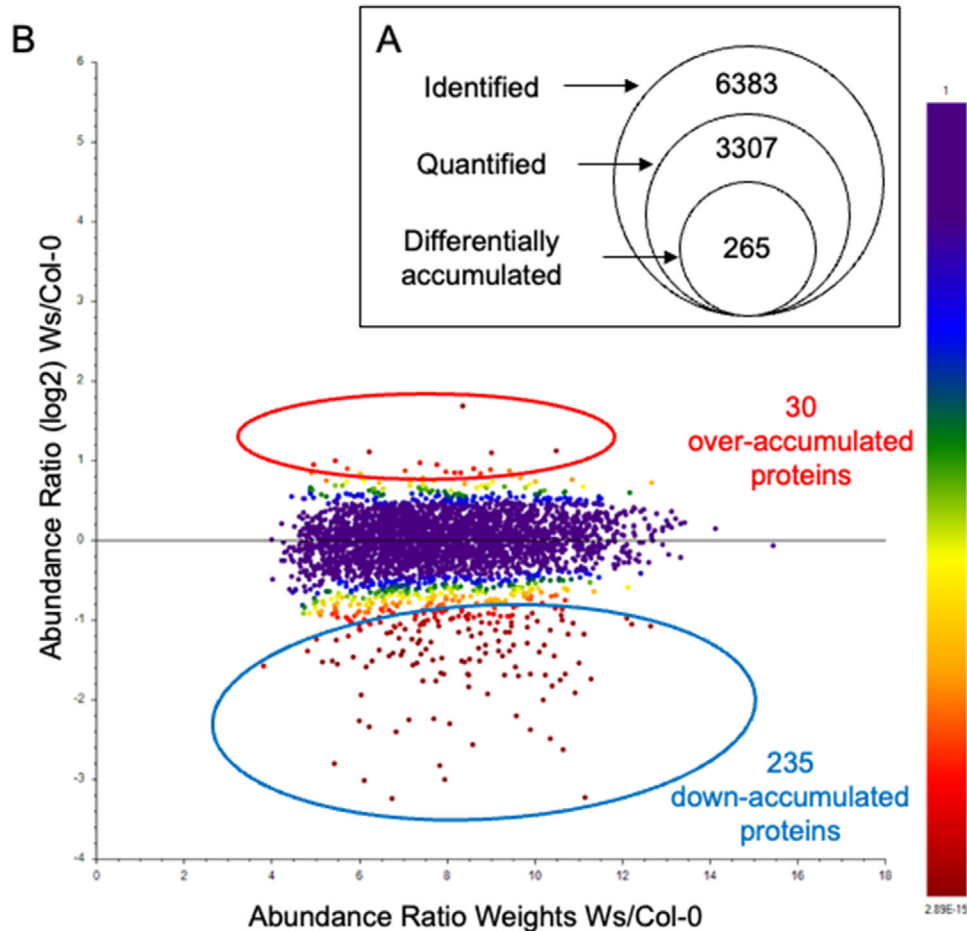


FIGURE 2 Overview of the proteomics results showing (A) the number of identified, quantified and differentially accumulated proteins, and (B) abundance ratio weights versus log₂ of protein abundance with the adjusted *p*-values at the z-axis. DAPs between Ws and Col-0 are indicated with red dots (low *p*-values), and proteins accumulated at similar levels in Ws and Col-0 are indicated with purple/blue dots (*p*-values close to 1)

MS2 spectral assignment false discovery rate (FDR) of less than 1% was achieved by applying the target-decoy strategy. Following spectral assignment, peptides were assembled into proteins and were further filtered, based on the combined probabilities of their constituent peptides to a final FDR of 1%. In addition, we only validated the proteins that were present in the three biological and technical replicates. In cases of redundancy, shared peptides were assigned to the protein sequence with the most matching peptides; thus, adhering to principles of parsimony. The UniProt as well as the *ProtAnnDB* databases were used to annotate the proteins (<https://www.uniprot.org/>, <http://www.polebio.lrsv.ups-tlse.fr/ProtAnnDB/index.php>) [21]. *ProtAnnDB* gives access to multiple bioinformatic programs to predict the sub-cellular localization of proteins and functional domains. The DAPs were identified based on *t* test with *p*-value less than 0.05 and with a fold change >1.7 or <0.6. The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium (<http://www.proteomexchange.org/>) via the PRIDE [22] partner repository with the dataset identifier PXD022441 an <https://doi.org/10.6019/PXD022441>.

Altogether, 6383 non-redundant proteins were identified (Table S1): 3307 could be quantified in Col-0 and Ws and 265 were DAPs

between Ws and Col-0 (Figure 2A). Among them, only 30 proteins were over-accumulated in Ws as compared to Col-0, whereas 235 were under-accumulated (Figure 2B). The DAPs were distributed into 14 functional categories according to their functions or putative functions (Figure 3 and Tables S2 and S3). The number of functional categories for the over-accumulated proteins was smaller (11 DAPs) than for under-accumulated proteins (14 DAPs). Thus, proteins involved in protein degradation, cytoskeleton, and as yet unknown function, were only observed among the under-accumulated proteins. The numbers of proteins in each functional class were very different for over- and under-accumulated proteins. Functional classes as metabolism, cell wall-related proteins, and DNA/RNA binding/transcription/translation/protein folding were common in both over- and -under accumulated proteins while photosynthesis, plant defense/stress, ROS scavenging enzymes/redox homeostasis were specific to under-accumulated proteins (Figure 3). These differences could be related to the genetic background leading to different morphological traits of the 3 week-old plants. For example, genetic variation for photosynthetic traits is commonly observed specifically in *A. thaliana* [23, 24]. Moreover, acclimation responses of *A. thaliana* can be linked to temperature during the evolutionary history of the ecotypes and

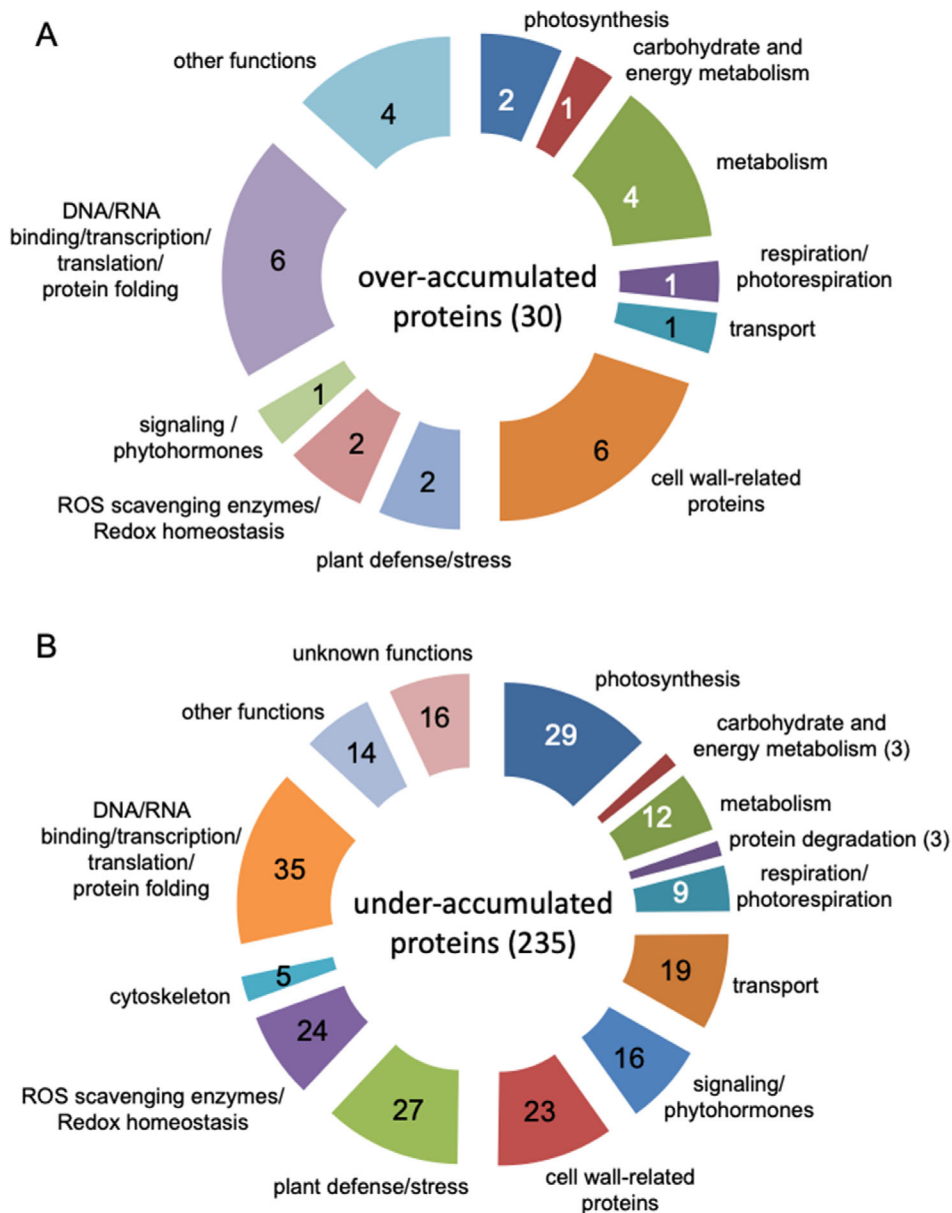


FIGURE 3 Functional categories of proteins differentially expressed in the shoot of Ws versus Col-0. The protein families are assigned based on the information available in the Uniprot and Gene Ontology databases and reclassified according to literature information

to the latitude of origin [25]. Differences observed for photosynthesis DAPs between Col-0 and Ws (Tables S1 and S2) could also be explained by the differences between their developmental stages, the Ws rosettes were smaller than those of Col-0 and Ws plants were starting to develop their floral stem [3]. Cell-wall related proteins also exhibited great differences between Ws and Col-0 (Figure 3 and Tables S2 and S3). These results can be explained by the fact that Col-0 and Ws initially grew at high and low temperature, respectively. Cell wall plays a major role to modify the cell and plant shapes during development [26] and in response to environmental changes [27]. Moreover, recent study showed that temperature and organs have an important impact on the cell wall plasticity in particular on cell wall polysaccharides, cell wall-related gene transcripts and cell wall proteins [28].

Great differences were observed for plant defense/stress DAPs between Ws and Col-0 with 27 proteins under-accumulated and two proteins over-accumulated in WS versus Col-0 (Tables S1 and S2). *A. thaliana* ecotypes can exhibit a large range of defense mechanisms against abiotic and biotic stress [29, 30]. Some ecotypes, like Shahdara (Sha), have been considered to be more tolerant to various stresses, these results being consistent with their native habitat [31, 32], whereas Col-0 is more sensitive to pathogens than Ws [11]. Moreover, a transcriptomic analysis of non-infested leaves of Col-0 and Ws showed differences in gene expression that might explain the differences observed upon aphid attack between these two ecotypes [33]. Thus, the differences observed in plant defense/stress DAPs between the two ecotypes suggest that the ecotype notion should be taken into account to better understand plant defense reactions.

Our comparative proteomic study has also revealed that ROS scavenging enzymes/redox homeostasis proteins like thioredoxins, proteins involved in superoxide dismutation, glutathione-ascorbate cycle, glutathione peroxidase pathway are over-accumulated in Col-0 shoots (Figure 3, Tables S1 and S2). ROS are crucial signaling molecules that can change the activity of many target proteins [34, 35] and affect various developmental processes [36]. Moreover, ROS homeostasis is governed by diverse antioxidant factors like thioredoxins [37, 38]. Recently, *DDC1*, encoding a functional thioredoxin, was demonstrated to be a major determinant of the natural variation in shoot regeneration among *A. thaliana* ecotypes [38]. Abiotic and biotic stresses cause oxidative stress via rapid and excessive production of ROS which can lead to oxidative damages. The Sha ecotype exhibited increased salt tolerance when compared with Landsberg erecta (Ler) and Col-0 [30]. Ler, the most sensitive ecotype, accumulated a higher level of ROS and showed the lowest survival rate. This pattern of ROS scavenging enzymes/redox homeostasis proteins accumulation in Ws and Col-0 may imply a modulation in their response during developmental processes and upon stresses.

DNA/RNA binding/transcription/translation/protein folding DAPs were more numerous in Col-0 shoots than in those of Ws (Figure 3 and Table S1 and S2). All these proteins probably play roles in plant developmental specific responses as well as in stress responses [39]. A recent study has shown that 15% of the ~50,000 studied regulatory DNA sites in *A. thaliana*, varied in accessibility in different ecotypes [40]. Although the authors have found little influence in gene expression levels, they stress that the most variable sites should be considered to predict phenotypic consequences [41]. The RNA-binding proteins are diverse in *A. thaliana* and they play a role in cellular metabolic processes and abiotic stress responses [41–43].

Altogether, our results suggest that Ws and Col-0 must have different phenotypic adaptation or plasticity in response to environmental changes. By using the TMTs labeling approach, we could identify proteins that could be synthesized upon different environmental conditions; thus, allowing a better understanding of adaptation mechanism of natural population in its habitat.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

C.R., K.P., M.I., P.M., and M.L. designed the experiments. M.L. and P.M. performed most of the experiments. P.M., E.J., and H.S.C. anal-

ysed and organized the proteomics data. K.P., P.M., and E.J. wrote the manuscript. All the authors have read and approved the manuscript.

DATA AVAILABILITY STATEMENT

The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium (<http://www.proteomexchange.org/>) via the PRIDE partner repository with the the dataset identifier PXD022441 and 10.6019/PXD022441.

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SUPPORTING INFORMATION

Additional supporting information may be found online <https://doi.org/10.1002/pmic.202000293> in the Supporting Information section at the end of the article.

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