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### ► To cite this version:

Amélie Morin, Benoit Porcheron, Gatepe Cedoine Kodjovi, Bouziane Moumen, Cécile Vriet, et al.. Genome-wide transcriptional responses to water deficit during seed development in *Pisum sativum* , focusing on sugar transport and metabolism. *Physiologia Plantarum*, 2023, 175 (6), pp.e14062. 10.1111/ppl.14062 . hal-04398337

**HAL Id: hal-04398337**

**<https://hal.science/hal-04398337v1>**

Submitted on 14 Oct 2024

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## ORIGINAL RESEARCH

# Genome-wide transcriptional responses to water deficit during seed development in *Pisum sativum*, focusing on sugar transport and metabolism

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**Funding information**

Centre National de la Recherche Scientifique; European Regional Development Fund; Grand Poitiers; Université de Poitiers; Ministère de l'Enseignement Supérieur et de la Recherche Scientifique

Edited by Alisdair Fernie

**Abstract**

Agriculture is particularly impacted by global changes, drought being a main limiting factor of crop production. Here, we focus on pea (*Pisum sativum*), a model legume cultivated for its seed nutritional value. A water deficit (WD) was applied during its early reproductive phase, harvesting plant organs at two key developmental stages, either at the embryonic or the seed-filling stages. We combined phenotypic, physiological and transcriptome analyses to better understand the adaptive response to drought. First, we showed that apical growth arrest is a major phenotypic indicator of water stress. Sugar content was also greatly impacted, especially leaf fructose and starch contents. Our RNA-seq analysis identified 2001 genes regulated by WD in leaf, 3684 genes in root and 2273 genes in embryonic seed, while only 80 genes were regulated during seed-filling. Hence, a large transcriptional reprogramming occurred in response to WD in seeds during early embryonic stage, but no longer during the later stage of nutritional filling. Biological processes involved in transcriptional regulation, carbon transport and metabolism were greatly regulated by WD in both source and sink organs, as illustrated by the expression of genes encoding transcription factors, sugar transporters and enzymes of the starch synthesis pathway. We then looked at the transcriptomic changes during seed development, highlighting a transition from monosaccharide utilization at the embryonic stage to sucrose transport feeding the starch synthesis pathway at the seed-filling stage. Altogether, our study presents an integrative picture of sugar transport and metabolism in response to drought and during seed development at a genome-wide level.

**1 | INTRODUCTION**

Crop production is particularly affected by climate changes with global projections of more frequent and severe episodes of water stress (Hirabayashi et al., 2008). In this context, drought is one of the most

important constraints limiting plant growth and yield stability in field peas, especially when a water deficit occurs at the beginning of the reproductive phase (Ney et al., 1993; Guilioni et al., 2003; Henriot et al., 2019). Water deficit applied during flowering and seed development can lead to severe yield decrease (Baigorri et al., 1999;

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Sousa-Majer et al., 2004), linked to the abortion of flowers, pods, and seeds (Salter, 1963; Maurer et al., 1968; Pumphrey and Schwanke, 1974), the reduction of seed weight and the cessation of new phyto-mer development (Ney et al., 1993; Fougereux et al., 1997; Guilioni et al., 2003; Henriët et al., 2019). Although the phenotypic traits impacted by drought have been widely described in legume crops (Ney et al., 1993; Farooq et al., 2017; Cuellar-Ortiz et al., 2008; Morales-Elias et al., 2022), the molecular mechanisms underpinning this yield reduction during seed development have not yet been deciphered.

Pea seed development occurs through a sequence of events divided into three major steps: (1) embryogenesis, (2) the transition and (3) maturation phases (Wang and Hedley, 1993; Weber et al., 2005). During embryogenesis, the embryo develops mainly through maternal control exerted from the seed coat. At this early stage of seed development, sucrose is degraded by the catalytic action of invertases (INV) to sustain the embryonic growth through cell division (Weber et al., 1996; Ruan et al., 2010; Morin et al., 2022a). Thus, embryogenesis is characterized by a high hexose state and the endosperm is a major source of sugars for the embryo (Weber et al., 2005; Melkus et al., 2009). Then, during the transition phase, embryonic transfer cells develop a membrane surface enriched with sugar transporters (Offler et al., 2003), and the embryo switches to a high sucrose stage (Borisjuk et al., 2002; Weber et al., 2005). This transition is characterized by a switch from mitotic cell division to a growth driven by cell expansion (Weber et al., 2005) and once this transitional milestone is passed, the seed can no longer abort (Duthion and Pigeaire, 1991). After the transition phase, seed-filling begins with the induction of storage-associated genes (Weber et al., 1996). This developmental stage is marked by the increased strength of the seed sink, which drives nutrient remobilization and carbon fluxes through long-distance transport (Jeuffroy and Warembourg, 1991; Morin et al., 2022b).

Throughout seed development, reproductive success and yield are controlled by a cohort of transport events (Pegler et al., 2023). When carbon supply is limited, for instance by environmental factors, competition for carbon can occur between sinks, like roots and seeds. Competition for assimilates also varies depending on the seed developmental stage (Jeuffroy and Warembourg, 1991). We recently reviewed how environmental stress impacted growth and productivity through carbon fluxes in pea crops (Morin et al., 2022b). Carbon fluxes rely on sugar transport from source leaf to sink organs mediated by sugar transporters of the sucrose transporter (SUT), monosaccharide transporter (MST) and sugar will eventually be exported transporter (SWEET) families (Lemoine et al., 2013; Doidy et al., 2019). In apoplastic phloem-loading species, such as Arabidopsis and pea (Van Bel and Gamalei, 1992), sucrose is loaded into the phloem by SWEET (Chen et al., 2012) and SUT transporters (Lalonde et al., 2004; Lu et al., 2019). After being transported to sinks, sucrose can be unloaded by the apoplastic or the symplastic pathway (Patrick, 1997). In starch-storing organs such as pea seeds, sucrose is remobilized to provide a substrate for starch synthesis (Zeeman et al., 2010). In pea seeds, sucrose is converted into glucose-6-phosphate, the only substrate imported into the amyloplast to ensure starch synthesis (Wang et al., 1998). Through the sequential

action of several enzymes (Yu et al., 2021), amylose and amylopectin are then synthesized. The genes involved in starch biosynthesis in seed amyloplast code for the following enzymes: UDP-Glc pyrophosphorylase (UGPase), phosphoglucomutase (PGM), ADP-Glc pyrophosphorylase (AGPase), granule bound starch synthase (GBSS), soluble starch synthase (SS), starch branching enzyme (SBE) and starch debranching enzymes of the isoamylase (ISA) and pullulanase (PUL) families. Recently, these genes have been annotated in the pea genome (Yu et al., 2021), which will help to gain a better understanding of the regulation of starch synthesis during seed development or in response to environmental factors.

Although field pea represents a common crop in agriculture and a model species in biology, limited genome-wide approaches are currently available. Such omics approaches are now facilitated thanks to the recent release of a reference genome by the Pea Genome Consortium sequencing in 2019 (Kreplak et al., 2019). As a result, transcriptomic approaches are now being developed in this model plant (Malovichko et al., 2020; Mazurier et al., 2022; Smolikova et al., 2022) but only a single RNA-seq experiment in drought conditions (combined with sulfur stress) has been published to date (Henriët et al., 2019). Moreover, transcriptomic analyses performed until now on pea were often sampled from entire plants or seedlings (Mazurier et al., 2022; Smolikova et al., 2022) or specific organs (Henriët et al., 2019), which give crucial information on molecular regulation but does not allow to study the interconnection between organs at the plant level.

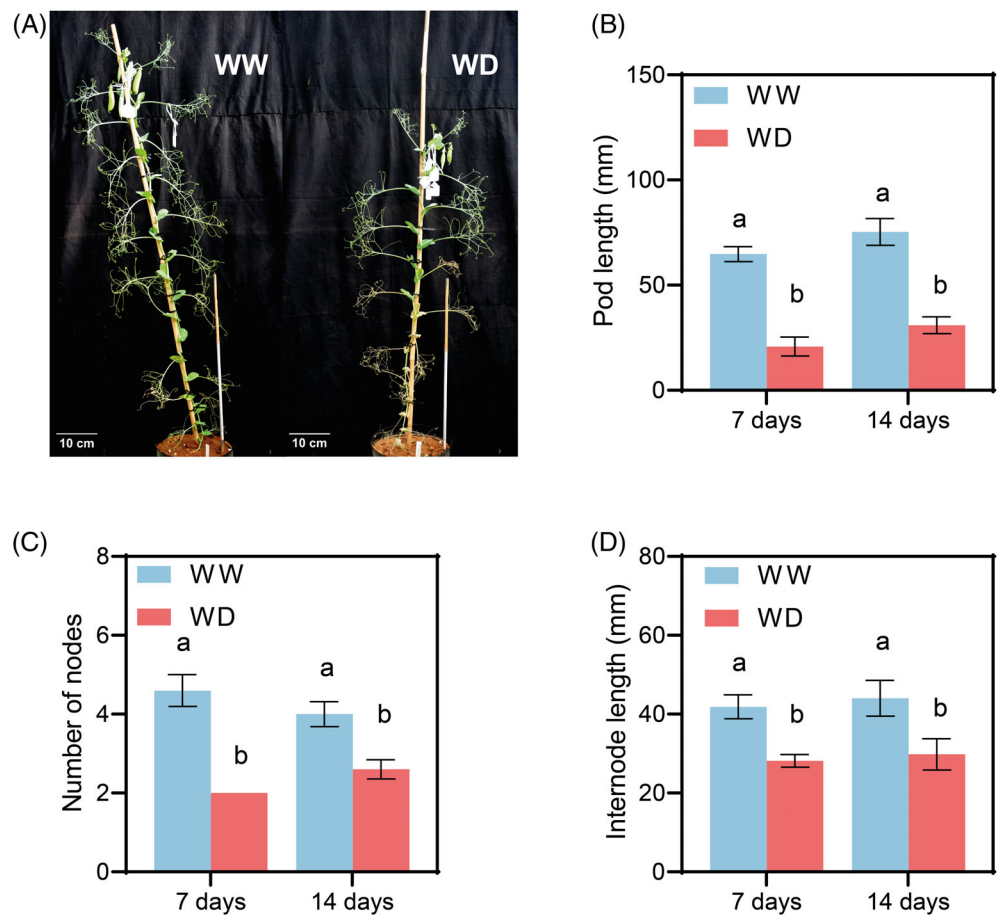
Here, we combined phenotypic, physiological, and transcriptomic approaches to decipher the integrative changes occurring in response to water deficit and during seed development. First, we applied a water deficit at the beginning of the reproductive stage in *P. sativum*. Then, we monitored phenotypic indicators, starch and sugar content modification, and transcriptome-wide changes triggered by this stress. We also looked at the transcriptomic changes taking place during seed development, highlighting major transcriptional reprogramming of sugar transport and starch metabolism pathways during the developmental transition from embryogenesis to seed-filling stages.

## 2 | MATERIALS AND METHODS

### 2.1 | Plant material and experimental design

Seeds of *Pisum sativum* (Safran genotype, a yellow *afila* spring variety provided by UNISIGMA; Figure 1A) were pre-germinated for 6 days at 22°C in the dark. The germinated seeds were transferred individually in 2.5 L pots containing a mixture of loamy soil and sand (2:1 v:v). Plants were positioned as split-plot randomized blocks and grown in a greenhouse with a 14 h photoperiod (sunrise at 7 am and sunset at 9 pm) complemented with artificial lighting (120  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) when natural light was below 150  $\text{W m}^{-2}$ . The determination of soil water content was made at the beginning of the experimentation by saturating the substrate with water (Colman, 1947) and the soil was oven-dried for 48 hours at 105°C. Seedlings were then irrigated to 80% of the water-holding capacity every two days with tap water, and 50 mL

**FIGURE 1** Phenotypic analysis of plants subjected to water deficit. (A) Photographs of pea plants (Safran variety) subjected to a water deficit (WD) of 14 days following its first flower pollination compared to well-watered (WW) control. (B-D) Phenotypic measurements of (B) the pod length, (C) the number of flowering nodes, and (D) the internode length of WD compared to WW plants ( $n = 5$ ). No error bar is shown for the number of nodes at 7 dap WD since the standard error is equal to zero. Letters represent significant differences determined by a Two/K sample permutation test ( $p < 0.05$ ).



of low nitrate nutrient solution (pH 6.5) was supplied once a week (Zuber et al., 2013). Water deficit was applied during floral development by withdrawing water as soon as the first flower was pollinated and until the soil water content reached 40% of the field capacity for Water-Deficit (WD) plants, while Well-Watered (WW) plants were maintained at 80% of the field capacity. Approximately two days were needed for the soil of the WD plants to decrease its water content to 40% of the soil water-holding capacity, which coincides with the pollination of the first flower on the second flowering node. Water deficit was maintained until the harvest of the plants, 7 or 14 days after the pollination of this flower (dap). All harvests were performed at 11:00 am by sampling the last source leaf formed during vegetative development at 14 dap, the entire root system at 14 dap and seeds of the second flowering node at the embryogenesis (7 dap) and the early seed-filling (14 dap) stages. All samples were immediately frozen in liquid nitrogen and stored at  $-80^{\circ}\text{C}$ .

## 2.2 | Phenotypic and physiological trait measurements

At harvest, the internode length of the flowering nodes as well as stem and pod lengths were measured using Image J, and the number of flowering nodes was counted. The evapotranspiration of the plants was assessed daily by weighing the pots on a scale (Radwag<sup>®</sup>). We

calculated evapotranspiration as the daily water loss of each plant at 7 or 14 dap ( $n = 5$ ). The stomatal conductance was measured with a porometer (SC-1, METER) on the last leaf that developed during vegetative development.

Pod development was monitored in a non-destructive manner using a pea pod sizer (Arvalis, Institut du Végétal) that enables the determination of the date at which the seed has passed the final stage in seed abortion (FSSA) (diameter  $>0.7$  cm, Duthion and Pigeaire, 1991; Munier-Jolain et al., 2010).

## 2.3 | Sugar quantification

Sucrose, glucose and fructose were extracted from 8 to 12 mg of lyophilized tissues of the last leaf developed during vegetative development sampled on plants bearing 14 dap seeds ( $n = 5$ ). After three washings (1.5 mL and twice 0.5 mL) in methanol:chloroform:water (12:5:3, v:v:v), supernatants containing soluble sugars were pooled, mixed with 0.6 volume of water and centrifuged. Then, the upper aqueous phase was evaporated at  $50^{\circ}\text{C}$  using a concentrator (MiVac Quattro; Genevac). Soluble sugars were resuspended with 0.5 mL of water and quantified using the Suc/Fru/D-Glc Assay kit (Megazyme). Starch content was measured from the pellets obtained after the last washing of methanol:chloroform:water (12:5:3, v:v:v) using the Total Starch HK Assay kit (Megazyme).

## 2.4 | RNA-seq library preparation

Total RNA from biological replicates ( $n = 3$ ) was extracted using CTAB extraction method modified from Chang et al. (1993) in which an isopropanol precipitation of RNA followed the second step of chloroform:IAA. The pellet was then washed with ethanol (80%) and resuspended in water for a subsequent purification step using the RNeasy Mini Kit (Qiagen). RNA samples were treated with DNase I (Promega) followed by overnight precipitation using LiCl<sub>2</sub> (4 M, v:v). Then, the purified RNA samples were quantified using a Qubit 4.0 Fluorometer (Life Technologies) and RNA integrity was verified with an RNA Kit on Agilent 5300 Fragment Analyzer (Agilent Technologies). RNA-seq libraries were prepared by the company Genewiz (Azenta Life Sciences, Leipzig, Germany) using the NEBNext Ultra RNA Library Prep Kit for Illumina following manufacturer's instructions (NEB). Briefly, mRNAs were first enriched with Oligo(dT) beads and fragmented for 15 min at 94 °C. First-strand and second-strand cDNAs were subsequently synthesized. cDNA fragments were end-repaired, adenylated at 3'ends, and universal adapters were ligated to cDNA fragments, followed by index addition and library enrichment by limited-cycle PCR. RNA sequencing was performed on an Illumina NovaSeq 6000 with a 2x150 pair-end configuration v1.5. Image analysis and base calling were conducted by the NovaSeq Control Software v1.7 on the NovaSeq instrument. Raw sequence data (.bcl files) generated from Illumina NovaSeq were converted into fastq files and de-multiplexed using Illumina bcl2fastq program v2.20. One mismatch was allowed for index sequence identification.

## 2.5 | Analysis of RNA-seq data

After investigating the quality of the raw data, reads were trimmed to remove adapter sequences and nucleotides with poor quality using Trimmomatic v.0.36 (Bolger 2014). The trimmed reads were mapped to the *P. sativum* reference genome v1 (Kreplak et al., 2019) available at URGI (<https://urgi.versailles.inra.fr/Species/Pisum/Pea-Genome-project>) using the STAR aligner v.2.5.2b. As a result of this step, BAM files were generated for each library and mapping statistics of the reads to the reference genome were verified (Table S1). Unique gene hit counts were calculated by using feature Counts from the Subread package v.1.5.2. Only unique reads that fell within exon regions were counted. After the extraction of gene hit counts, the gene hit counts table was used for downstream differential expression analysis. Using DESeq2, a comparison of gene expression between the groups of samples was performed. Four comparisons (root WW vs WD, leaf WW vs WD, seed at 7 dap WW vs WD and seed at 14 dap WW vs WD) were performed to identify genes differentially expressed between the WW and WD conditions and one comparison (seed at 7 dap vs seed at 14 dap) was performed to identify genes differentially expressed between seed developmental stages. The Wald test was used to generate p-values and log<sub>2</sub> fold changes. Genes with adjusted p-values <0.05 and absolute log<sub>2</sub> fold changes >1 were called as differentially expressed genes for each comparison (Tables S2-S7).

Validation of RNA-seq data was performed by RT-qPCR on sugar transport genes (*PsSUT1.1* Psat6g188160; *PsSUT2* Psat4g175520;

*PsSUT1.4* Psat1g098880; *PsSUT4.1* Psat2g063400 and *PsSWEET12* Psat7g038200) using five individual plant replicates ( $n = 5$ ) (Figure S1). Retrotranscription and qPCR were performed as described in Morin et al. (2022a) and target gene expression was normalized to the reference gene *PsTFIIA* (Psat5g118600; Die et al., 2010; Knopkiewicz and Wojtaszek, 2019).

## 2.6 | GO term analysis

Gene ontology (GO) enrichment analysis was performed on both up- and down-regulated gene sets. GO terms were assigned based on annotated gene ontology available at the pea genome project (<https://urgi.versailles.inra.fr/Species/Pisum/Pea-Genome-project>). GO term enrichments for biological processes have been performed with the Bioconductor topGO R package (topGO, Alexa and Rahnenfuhrer, 2022) with Fisher exact tests ( $pval < 0.05$ ) using the list of annotated GO in the pea genome as a background. Synonymous GO terms were filtered using REVIGO (Supek et al., 2011).

## 2.7 | Transcription factor and cis-element analyses

The protein sequences of the 44,756 representative gene models in the pea genome (Kreplak et al., 2019) were used as the input in the prediction server available at the PlantTFDB v5.0 (<http://planttfdb.gao-lab.org/prediction.php>; Jin et al., 2016) to identify a total of 1807 TF in the pea genome (<https://zenodo.org/record/7968044>; Kodjovi et al., 2023). We then computed the intersections between this genome-wide TF dataset and the lists of genes regulated by WD in respective plant organs. Significant overrepresentation of TF genes and TF families in the gene lists differentially expressed by WD was determined by a Fisher test using the total number of TF in the genome and the number of genes in each TF family as a background (Kodjovi et al., 2023).

Promoter sequences (−1 kb upstream of the coding sequence) of the sugar transport and starch metabolism genes regulated by WD (Figure S2) were retrieved using the getfasta command available from the bedtools suite v2.30.0 (Quinlan and Hall, 2010). The promoter sequences were scanned for the presence of cis-acting regulatory DNA elements using the PLACE database (Higo et al., 1999) and motifs involved in the transcriptional response to WD (Slawinski et al., 2021) were retrieved.

# 3 | RESULTS & DISCUSSION

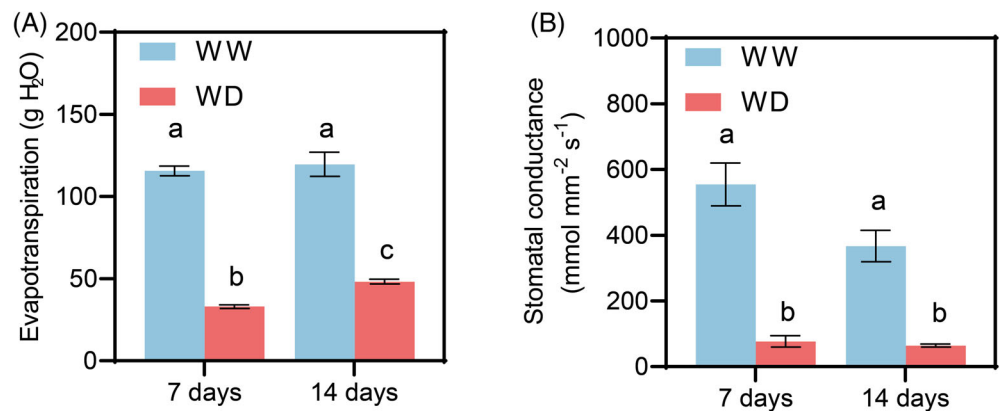
## 3.1 | Water deficit affects phenotypic and physiological traits

### 3.1.1 | Water deficit reduces pod length, the number of flowering nodes and internode length

We applied a water deficit (WD) at the beginning of the reproductive phase, a stress that often occurs in field conditions with major impacts



**FIGURE 2** Physiological measurements of plants subjected to water deficit. (A) Evapotranspiration and (B) stomatal conductance of pea plants subjected to a WD of 14 days following its first flower pollination compared to WW control (n = 5). Letters represent significant differences determined by a Two/K sample permutation test ( $p < 0.05$ ).



on phenotypic traits (Figure 1A). First, pod length was measured to assess the impact of WD on fruit development. WD plants formed significantly shorter pods (Figure 1B). Most likely, this reduction in pod length occurred before 7 days after pollination (dap), since pod length did not vary between 7 dap and 14 dap in well-watered (WW) and WD conditions. Such a reduction in size may be interpreted as a reduction of biomass allocation towards pea pericarps during WD, as previously demonstrated in *Phaseolus vulgaris* during water stress (Morales-Elias et al., 2022) and nutrient starvation (Coello and Martínez-Barajas, 2016). Interestingly, Coello and Martínez-Barajas (2016) showed that pod biomass reduction in common beans during nutrient starvation was linked to the remobilization of storage products from the pods to nourish the seeds. Plant production was negatively affected by WD as shown by a significant reduction in the number of flowering nodes formed in WD conditions (Figure 1C). This apical growth arrest was combined with a shortening of internode length, significantly reduced by 33% after 7 and 14 days of WD (Figure 1D). Such an apical growth arrest is commonly observed in response to WD in pea (Ney et al., 1993; Baigorri et al., 1999; Henriët et al., 2019). This cessation of new phytomer production could be linked to the reallocation of carbon towards developing fruits in order to limit their abscission (Guilioni, 1997; Guilioni et al., 2003). Thus, both pod length reduction and apical growth arrest (Figure 1B, C) illustrate the impact of WD on carbon allocation at the plant level.

### 3.1.2 | Effects of water deficit on evapotranspiration and stomatal conductance

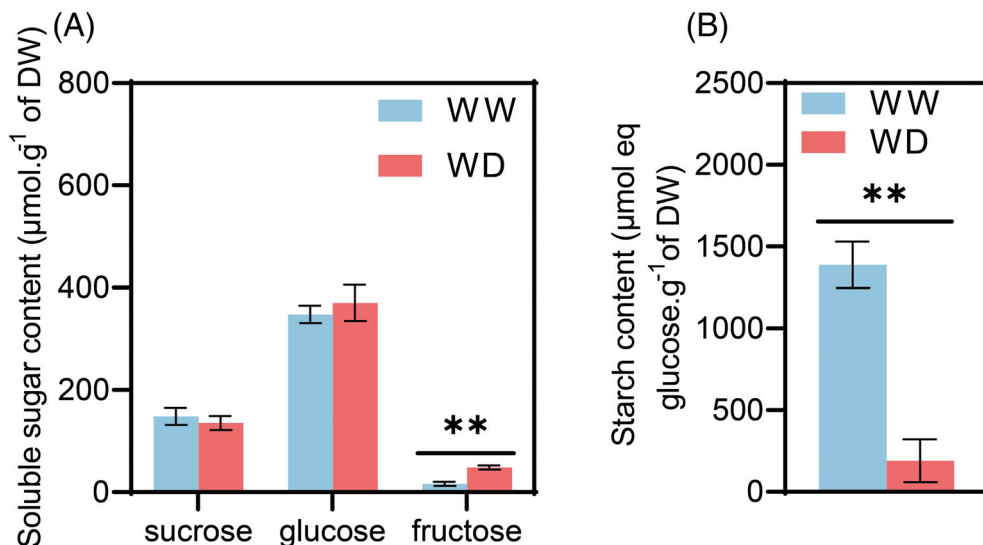
The WD applied at the beginning of the reproductive phase also had major impacts on physiological traits, such as plant evapotranspiration and stomatal conductance (Figure 2). Since less water is available in the soil for the plant, evapotranspiration was reduced in WD conditions, especially after 7 days of WD (a reduction of 71%; Figure 2A). Such a reduction in the use of water by the plant is the result of a decreased fraction of transpirable soil water available for the plant (Lecoeur et al., 1996). Interestingly, the increase in evapotranspiration after 14 days of WD could be linked to an increased water demand related to seed development. Indeed, in soybean, water use increases

during seed development (Karam et al., 2005). Higher water demand could, therefore, explain the high sensitivity to drought of pea crops during early reproductive stages (Andersen and Aremu, 1991; Ney et al., 1993; Henriët et al., 2019). Moreover, a reduction in the soil water content can also limit the net photosynthesis, as previously described in pea leaves (Guilioni et al., 2003), thus reducing carbon production at the plant level.

Since evapotranspiration is tightly linked to leaf transpiration, we also measured a strong reduction in stomatal conductance in WD conditions (86% and 82% reductions at 7 dap and 14 dap, respectively; Figure 2B). Such a reduction in stomatal conductance is often described in pea canopy and limits the CO<sub>2</sub> supply to the chloroplast (Lecoeur et al., 1996; Guilioni et al., 2003). As stomatal conductance is often described as proportional to the photosynthesis rate (Miner et al., 2017), this observed decrease could therefore reduce photoassimilate availability. Thus, an edaphic WD could influence both water use and carbon metabolism at the plant level.

### 3.1.3 | Water deficit impacts fructose and starch contents

In order to explore the effect of WD on carbon metabolism in source organs, soluble sugar and starch contents were assessed in the last leaf developed prior to reproductive development after 14 days of WD. Interestingly, leaf sucrose and glucose contents showed no variation between WW and WD conditions (Figure 3A). However, leaf fructose levels were significantly higher in WD conditions (a 2.9-fold higher content). Moreover, leaf starch contents were strongly reduced in response to WD (86% decrease compared to the WW condition; Figure 3B). Such a reduction in starch contents could be linked to a diversion of sugars from starch metabolism to prevent sugar deficit in this source leaf (Dong and Beckles, 2019). Indeed, in soybean, a higher level of soluble sugars was observed in source leaf despite reduced photosynthesis (Du et al., 2020), which was explained by an increased starch degradation into glucose. Nevertheless, soluble sugar accumulation may be caused by a decrease in starch synthesis in leaves, as shown in barley (Villadsen et al., 2005) and spinach (Geigenberger et al., 1997). In spinach leaves, a decrease in AGPase activity was



**FIGURE 3** Sugar and starch contents in a source leaf subjected to water deficit. (A,B) Measurements of glucose, fructose and starch contents in a leaf of pea plants subjected to a WD of 14 days following its first flower pollination compared to WW control ( $n = 5$ ). The leaf sampled is the last leaf developed prior to the beginning of reproductive development. Asterisks represent significant differences determined by a Two/K sample permutation test (\*\*  $p < 0.01$ ).

associated with decreased starch synthesis and increased leaf sucrose levels (Zrenner and Stitt, 1991; Geigenberger et al., 1997). Therefore, a decrease in starch synthesis could help maintain soluble sugar levels (especially sucrose and glucose) close to WW conditions (Dong and Beckles, 2019). At the molecular level, all the differentially expressed genes involved in the starch biosynthesis pathway were repressed by WD (Figure 6). These results indicate a down-regulation of starch biosynthesis in response to WD. Overall, this regulation in starch metabolism could be part of an acclimation mechanism to help maintain soluble sugar contents and prevent the plant from sugar starvation after two weeks of WD.

### 3.2 | Genome-wide transcriptional responses to water deficit in source and sink organs

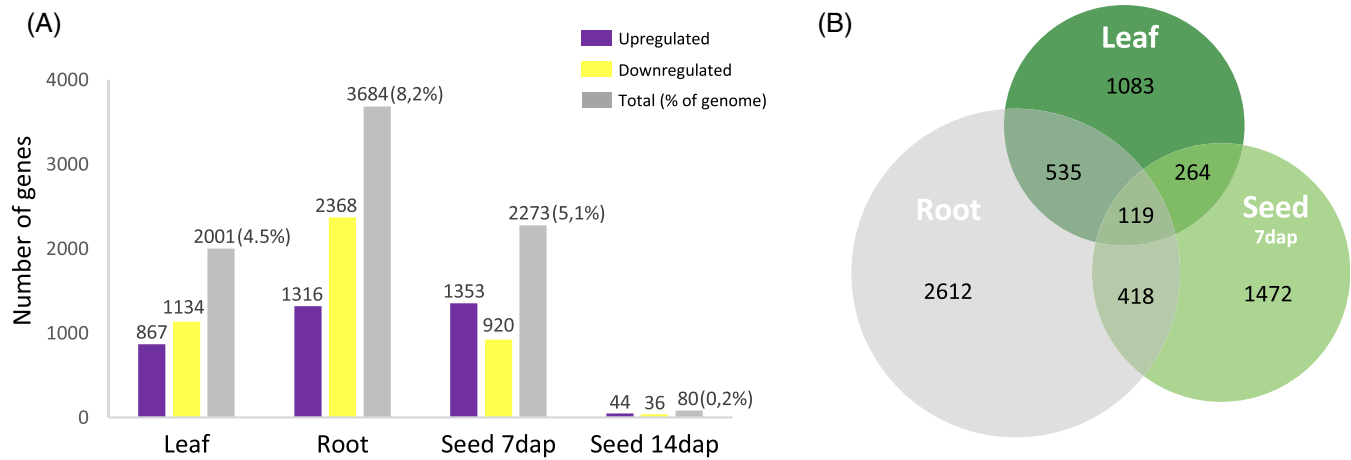
We performed an RNA-seq analysis to identify genes significantly up- and down-regulated by WD ( $p\text{val} < 0.05$  and absolute fold change  $> 2$ ; Tables S2-S6). The largest response to WD (in terms of number of genes) was monitored in root, with a total of 3684 genes (1316 induced and 2368 repressed). This represents approximately 8.2% of the annotated genes in the genome responding to WD in root (Figure 4A). In the leaf, 2001 genes were regulated by WD (867 induced and 1134 repressed by WD), representing 4.5% of the annotated genes in the genome (Figure 4A). Interestingly, the number of genes repressed by WD was greater than the number of genes induced in both organs (Figure 4A), suggesting that several biological pathways get down-regulated in response to water stress in both leaf and root systems. In seed at the embryonic stage (7 dap), we identified a total of 2273 genes differentially expressed (1353 induced and 920 repressed by WD), representing 5.1% of the annotated genes in the genome. In contrast to leaf and root, this higher number of genes induced in embryonic seeds (Figure 4A) suggests that many pathways are being up-regulated in response to water stress. At a later stage of seed development (14 dap), only 80 genes (genome-wide) were regulated in WD vs WW conditions,

suggesting that genome-wide transcriptional changes to water stress no longer occur in seed at the filling stage.

We performed a dataset comparison between different organs (Figure 4B). The gene set from seed at 14 dap was not included because the low number of genes in this dataset (80 genes) prevented any intersection with other gene sets. The resulting Venn diagram showed an intersection of 119 genes constitutively regulated by WD in all organs (leaf, root, and seed at 7dap; Figure 4B). Among these genes consistently regulated by WD, we identified *PsSWEET7* (Psat7g023840) and *GBSS1a* (Psat0s4284g0040) (Figure 6). We also observed an intersection of 535 genes regulated by WD in both shoot and root, 264 genes commonly regulated in both shoot and embryonic seed and 418 genes commonly regulated in both root and embryonic seed (Figure 4B).

#### 3.2.1 | Genome-wide reprogramming to water deficit in seed occurs during embryogenesis

In seeds, genome-wide-reprogramming to WD takes place at the embryonic stage but no longer occurs at the filling stage once the seed has passed the final stage of seed abortion (FSSA). Indeed, large transcriptomics changes occur with 2273 genes regulated by WD at the early stage of embryonic development (Figure 4A). Later, when the seed entered the nutrient-filling stage, only 80 genes were significantly regulated by WD, suggesting that once the seed had passed the FSSA, transcriptomics reprogramming to WD no longer occurs. In maize, a high number of differentially expressed genes was also observed in 5 dap kernels under drought stress (Wang et al., 2019a). Moreover, in 9 dap pea seeds, three days before the beginning of seed-filling, 11 genes were found differentially regulated by a moderate WD (Henriet et al., 2019). Here, we hypothesize that intense reprogramming to water stress is occurring at early stage of seed development to ensure embryonic survival prior to the FSSA. Once seeds have passed this critical checkpoint, a



**FIGURE 4** Genome-wide responses to water deficit in leaf, root and seed organs. (A) Number of genes differentially expressed between the WW and WD conditions, either up or down-regulated (in purple and yellow respectively) in each organ ( $n = 3$ ). The total number of genes regulated by WD (in gray) is indicated with the percentage of genes in relation to the genome (44,756 genes annotated in the pea genome; Kreplak et al. 2019). (B) Venn diagram comparing the datasets regulated by WD in leaf (14 dap), root (14 dap) and seed organs (7 dap).

developmental-driven regulation of storage-associated gene patterns might occur despite water scarcity.

### 3.2.2 | Biological processes affected by water deficit

To categorize the differentially expressed genes based on their biological functions, GO-term enrichment was tested for all gene sets regulated by WD (Figures S3-S6). Several biological processes were highly impacted by WD in the source leaf. Among the 53 biological processes repressed in the leaf, post-translational protein modification, oxidation-reduction process and ion transport were identified (Figure S3, Table S8). On the other hand, among the 63 biological processes identified as being induced in leaf, metabolic processes, biological regulation, and regulation of cellular or metabolic processes were significantly enriched. The root system also displayed numerous biological processes significantly regulated by WD, with 46 and 71 biological processes respectively up- and down-regulated in roots (Figure S4, Table S8). To name a few, post-translational protein modification and phosphorylation were repressed in WD roots, whereas ion transport and carbohydrate derivative biosynthesis were induced. Interestingly, the oxidative reduction process and response to stress were identified both in up- and down-regulated gene sets, suggesting that specific metabolic pathways related to ROS regulation are involved in drought stress response in the root organ (Figure S4). Moreover, few biological processes were found in 14 dap seeds compared to 7 dap (Table S8). In seeds at the filling stage, 21 and 17 biological processes were respectively identified as up- and down-regulated, while 62 were up-regulated and 44 down-regulated by WD in embryonic seeds (Figure S5, Table S8). In 7 dap seeds, oxidation-reduction processes were both up- and down-regulated, cellular

component biogenesis and carbohydrate metabolic processes were specifically down-regulated, and metabolic processes were up-regulated. In 14 dap seeds, oxidation-reduction processes were up-regulated (Figure S6). Noteworthy, oxidation-reduction processes were regulated in both source and sink organs, which indicates a broad response to oxidative stress at the plant level (Fracasso et al., 2016).

### 3.2.3 | Genome-wide reprogramming of transcription factors in response to water deficit

We chose to focus our analysis on transcription factors (TF) as biological processes associated with transcriptional regulation were highly represented in genes differentially expressed between the WW and WD conditions (Figure S3-S6). To do so, we compared the dataset of 1807 transcription factors (TF) identified in the pea genome (Kodjovi et al., 2023) with respective gene lists responding to WD. Thus, we respectively identified 158, 248 and 169 TF responding to WD in leaf, root and embryonic seed (Figure 5), including 11 TF constitutively regulated by WD in all organs (Figure S8). The occurrence of TF was overrepresented in all organs in response to WD, confirming the significance of the transcriptional regulation processes highlighted by the GO term analysis. These results confirm the biological importance of genome-wide transcriptional responses to water deficit (Joshi et al., 2016).

At the TF family level, ERF genes were highly overrepresented among genes regulated by WD in both leaf and root organs (Figure 5, Table S9). The AP2/ERF families are well known for their response to drought stress; for instance, they include the DREB (drought-responsive response binding) genes. The AP2/ERF families were recently investigated in *P. sativum* with several DREB candidates



induced in leaves under drought stress (Jarambasa et al., 2023). We also found an overrepresentation of NAC and WRKY genes regulated by WD in leaf (Figure 5). Previous reports showed that the NAC and WRKY families are involved in plant abiotic stress response, including drought resistance (Li et al., 2021b; Jiang et al., 2016). In root AP2, ERF and GRAS families were found over-represented in the list of genes regulated by WD. In embryonic seed, we found an overrepresentation of GRF, HD-ZIP and ZF-HD families regulated by WD. The ZF-HD family has recently been identified in the pea genome and its role in drought resistance has been reported in multiple plant species (Shi et al., 2023).

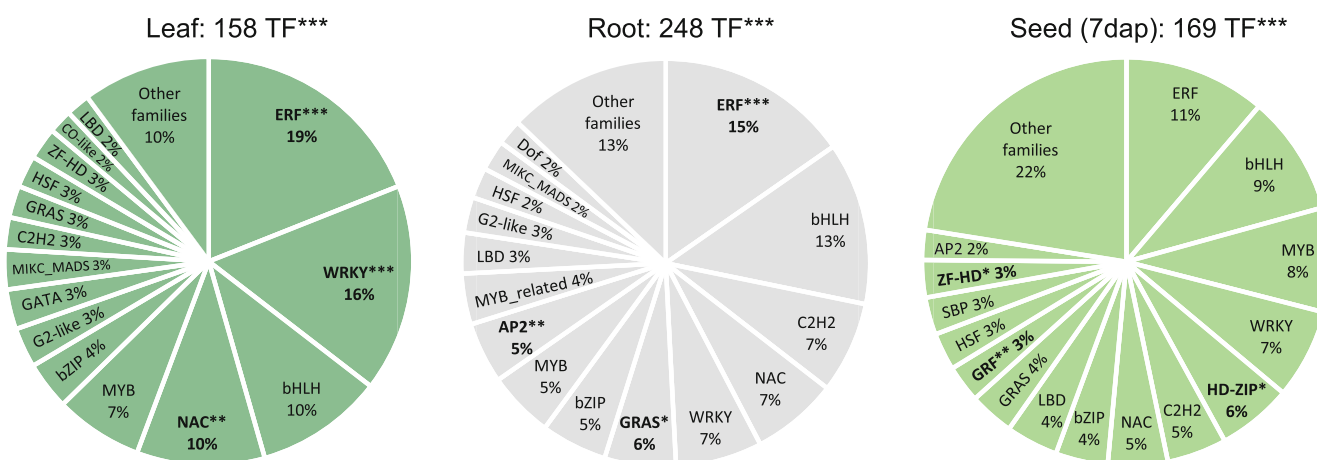
In conclusion, our results suggest that multiple TF belonging to different families (AP2/ERF, GRAS, GRF, HD-ZIP, NAC, WRKY, ZF-HD) orchestrate the genome-wide transcriptional regulation of water deficit in leaf, root and seed organs. The TF dataset identified in this study represents a valuable genetic resource to exploit through breeding programs in legume crops as it provides potential targets for improving crop resilience to environmental stresses.

### 3.2.4 | Sugar transport and starch metabolism genes are transcriptionally regulated by water deficit in leaf, root and seed

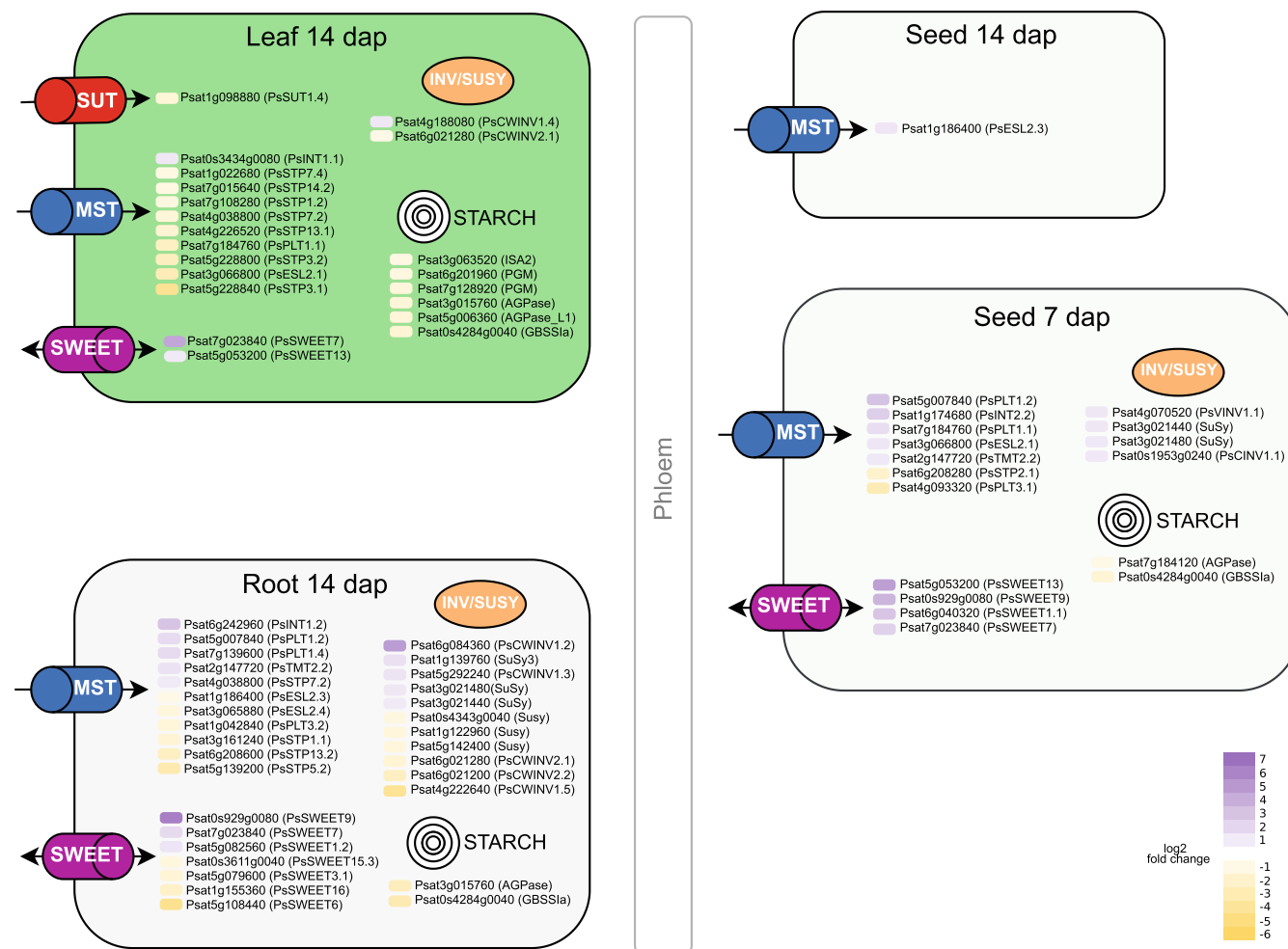
We also focused our analysis on biological processes associated with carbon transport and metabolism as these processes were over-represented in our GO analyses (Figure S3-S6) and are highly relevant to adaptive drought responses (Fracasso et al., 2016; Bray 1997). In addition, our biochemical analysis revealed a higher fructose level and

a strong decrease in starch content in source leaf (Figure 3). More precisely, we focused on genes encoding sugar transporters (SUT, MST and SWEET families), sucrose catabolism enzymes (INV and SUSY families) and enzymes of the starch synthesis pathway (UGPase, PGM, AGPase, GBSS, SS, SBE, ISA and PUL) (Doody et al., 2019; Yu et al., 2021; Morin et al., 2022a). Among the genes differentially expressed between the WW and WD conditions, we identified 54 genes related to sugar transport and starch synthesis, including 33 sugar transporters, 14 sucrose catabolic enzymes and 7 starch metabolism genes in leaf, root and seed organs (Figure 6). These genes presented a signature of “cis” element related to abiotic stress response in their promoter region (Figure S2). For instance, we could map multiple copies of cis-regulatory motifs related to drought response, such as CBFHV, WBOX, MYB, MYC, DRE and ABRE elements (Figure S2). This result suggests that drought-responsive TFs (Figure 5) orchestrate the transcriptional regulation of sugar transport and starch metabolism genes in response to WD.

In source leaf, most genes involved in sugar transport and starch metabolism were repressed after 14 days of WD (Figure 6). For instance, the only SUT genes significantly impacted by WD among all organs (*PsSUT1.4*) was repressed in leaf. Also, all MST regulated by WD were repressed in leaf, except for an induced inositol transporter (*PsINT1.1*). Moreover, all genes involved in the starch synthesis pathway were repressed by WD in leaf (Figure 6). These results indicate that the transport of sucrose, monosaccharides and subsequent starch synthesis seem reduced in leaf cells subjected to WD. This down-regulation of starch biosynthesis in response to WD may explain the significant reduction observed in leaf starch content (Figure 3B). In contrast, all SWEET genes regulated by WD (*PsSWEET7* and *PsSWEET13*) presented



**FIGURE 5** Transcription factors regulated by water deficit in leaf, root and seed. The total number of TF differentially expressed between the WW and WD conditions in each organ is indicated at the top of the pie chart. The top 15 TF families (in terms of number of differentially expressed genes) is proportionally plotted as a pie chart. Asterisks indicate significant overrepresentation determined by a Fisher test (\*  $p < 0.05$ ; \*\*  $p < 0.01$ ; \*\*\*  $p < 0.001$ ). TF families are abbreviated as follows: AP2 (APETALA2), bHLH (Basic Helix-Loop-Helix), bZIP (basic leucine zipper), C2H2 (Cys2/His2-type zinc-finger domain), CO-like (CONSTANS-like), Dof (DNA binding with One Finger), ERF (Ethylene Response element binding Factors), GATA (zinc finger proteins binding the consensus sequence WGATAR), GRAS (GAI, RGA and SCR), GRF (Growth-Regulating Factor), HD-ZIP (homeodomain-leucine zipper), HSF (Heat Stress transcription Factors), LBD (Lateral organ Boundaries Domain), MYB (myeloblastosis), G2-like (Golden 2-like), NAC (NAM, ATAF1/2 and CUC2). MIKC\_MADS (MADS box, Intervening, Keratin binding and C domains), SBP (Squamosa promoter Binding Proteins), ZF-HD (Zinc finger-homeodomain). Also see Figure S8 and Table S9 for supplemental information.



**FIGURE 6** Sugar transporter and starch metabolism genes regulated by water deficit in source leaf, sink root and seed. Genes related to sugar transport of the sucrose transporter (SUT), monosaccharide transporter (MST) and sugar will eventually be exported transporter (SWEET) families. Genes related to sucrose catabolism of the sucrose synthase (SUSY) and invertase (INV) families, including cytosolic (CINV), cell wall (CWINV) and vacuolar (VINV) invertases. Genes related to starch synthesis of the phosphoglucomutase (PGM), ADP-Glc pyrophosphorylase (AGPase), granule-bound starch synthase (GBSS) and isoamylase (ISA) families. Genes with a significant ( $p < 0.05$ ) induction (purple gradient,  $\log_2$  fold changes  $> 1$ ) and repression (yellow gradient,  $\log_2$  fold changes  $< -1$ ) in response to water deficit are represented.

a significant induction of their expression (Figure 6). Transporters of the SWEET family facilitate sugar export from plant cells (Chen et al., 2010); thus, this induction may support that sugars get reallocated from source towards sink organs in response to WD.

In roots, drought induced marked changes in the expression of genes related to sugar transport, as well as modifications of carbon fluxes and remodeling of the root architecture to efficiently forage water in the soil (Durand et al., 2016; Hennion et al., 2019). Our RNA-seq analysis in root identified a total of 29 genes related to sugar transport and starch synthesis that were differentially expressed after 14 days of WD in comparison with the WW condition (Figure 6). Only two genes involved in the starch synthesis pathway were identified in this gene set (AGPase and GBSSla) and both genes were repressed in response to WD. In contrast, several genes involved in the sucrose degradation pathway (SUSY and INV) were significantly induced or repressed in response to WD in roots. Regulation of invertase expression in the roots could vary, depending on the root region. In maize,

IVR2 (a vacuolar invertase) expression is specifically induced by WD in the primary root tips (Kim et al., 2000). Numerous genes of the sugar transport systems (MST and SWEET) were also regulated by WD (Figure 6). Among them, polyol (PLT) and inositol (INT) transporter genes were highly represented, suggesting that sugar alcohol molecules could help maintain the osmotic balance in the root cell subjected to WD (Li et al., 2021a). Finally, SWEET exporters of all clades were found regulated by WD (Figure 6), notably the glucose export system of clade I (PsSWEET1.2 and PsSWEET3.1), the sucrose export system of clade III (PsSWEET15.3) and the vacuolar fructose transporter of clade IV (PsSWEET16) (Doody et al., 2019). Overall, the highest number of genes regulated by WD were identified in the root system (Figure 4) with many of them encoding sugar transporter (MST and SWEET) and sucrose catabolism enzymes (SUSY and INV), confirming that cellular regulation of carbon fluxes and metabolism towards roots is a major response to drought.

In seeds at the embryonic stage subjected to 7 days of WD, the expression of genes involved in monosaccharide transport and sucrose catabolism were induced by WD (Figure 6), confirming the high glucose metabolic activity at this stage of embryonic division. However, both genes related to starch metabolism were repressed (AGPase and GBSS1a; Figure 6). Indeed, at this early stage of seed development, starch synthesis only occurs transiently, as glucose is primarily used to feed the embryo (see Result & Discussion 3.2; Borisjuk et al., 2002; Weber et al., 2005). Notably, the expression of several genes involved in monosaccharide transport (MST and SWEET), as well as genes involved in sucrose cleavage (INV and SUSY), was induced by WD. This up-regulation of the hexose import system and sucrose cleaving enzymes suggests that glucose provision is increased in embryonic seed subjected to WD. The crucial role of sucrose catabolism enzymes in seed development and drought response has been highlighted in previous studies (Ruan et al., 2010) and we recently showed that *PsvINV1.1*, *PscINV1.1* and *PscWINV1.2* responded to WD in pea seed to generate glucose energy and signals (Morin et al., 2022a). A higher glucose metabolic activity is critical during embryogenesis and thereby could promote embryo survival in drought conditions. Genes encoding polyol and inositol transporter (PLT and INT; Figure 6) were also found up-regulated by WD. This transcriptional regulation could help maintain the osmotic pressure in seeds subjected to water stress (Li et al., 2021).

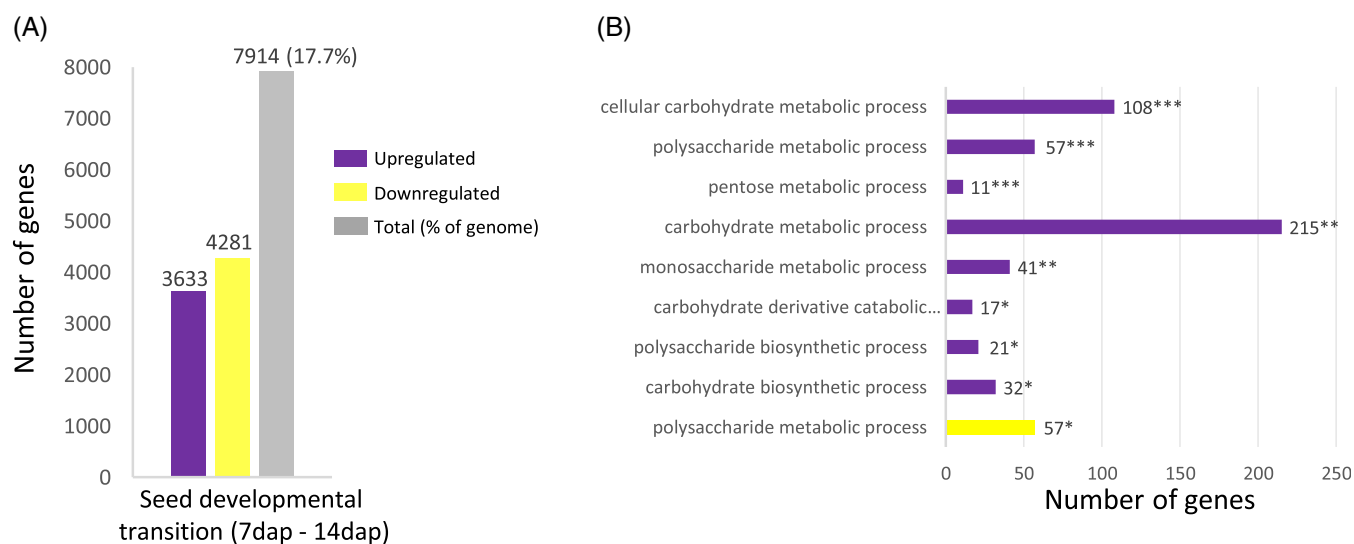
In seed at the filling stage subjected to 14 days of WD, only a small repertoire of genes was found differentially expressed in comparison to the control condition (Figure 4A). Hence, genome-wide transcriptional reprogramming to WD, including genes related to carbon transport and metabolism, seems to occur primarily at earlier stages of seed development (See Results & Discussion 2.1). Only a

single gene related to carbon transport was found induced by WD at the filling stage: *PsESL2.3*, a gene coding for a monosaccharide transporter of the ESL subfamily (Figure 6). ESLs have long been described for their responsiveness to dehydration processes (Kiyosue et al., 1998).

Noteworthy, two genes were consistently regulated by WD in all organs (Figure 6). A sugar transporter (*PsSWEET7*) was found induced by WD in leaf, root, and embryonic seed, and its expression displayed a 20-fold induction by water stress in source organs. The second gene, involved in amylose synthesis (*GBSS1a*; Dry et al., 1992) is consistently repressed in these organs by WD. Moreover, numerous genes of the ESL and STP clades were found regulated in all organs by WD (Figure 6). Both ESL and STP candidates are well characterized for their importance in abiotic stress response, including water deficit (Yamada et al., 2011; Liu et al., 2021; Slawinski et al., 2021). These candidate genes may represent interesting targets for improving the efficiency with which plants take up and use water and nutrients (Schroeder et al., 2013; Varshney et al., 2023).

### 3.3 | Genome-wide responses to seed development

We also looked at genome-wide gene expression changes occurring in response to seed development by comparing the transcriptomes of seeds harvested at the embryonic stage (7 dap) vs seed at the filling stage (14 dap) (Table S7). A total of 7914 genes (3633 induced and 4281 repressed) were differentially expressed, representing approximately 18.4% of the annotated genes in the genome responding to seed developmental stages (Figure 7A). This large number of



**FIGURE 7** Genome-wide responses of seed developmental transition from embryogenesis to filling stages. (A) Number of genes either up- or down-regulated (in purple and yellow respectively) between the two seed developmental stages (7 dap vs 14 dap). The total number of genes differentially expressed from embryogenesis to seed-filling stages (in gray) is indicated with the percentage of genes in relation to the genome (44,756 genes annotated in the pea genome; Kreplak et al. 2019). (B) Significant GO term enrichment related to carbon biological process in the up- or down-regulated (in purple and yellow respectively) dataset. Number of genes present in the dataset corresponding to each GO term is indicated (complete GO term analysis is available in Figure S7 and Table S8).

differentially expressed genes indicates that substantial genome-wide reprogramming occurs during this transition from embryogenesis to seed-filling stages.

### 3.3.1 | Biological processes affected through seed development

Gene ontology analysis of biological processes was performed to assess the main biological pathways affected by seed development. The GO analysis showed 90 and 80 biological processes respectively induced and repressed during seed development (Figure S7 and Table S8). Among the biological processes regulated during seed development, regulation of biological, cellular, and metabolic processes were highly represented with carbohydrate metabolic processes largely induced in 14 dap seeds (Figure 7B). Indeed, the biological processes linked to carbohydrate metabolic processes contained the highest number of significantly up-regulated genes during seed-filling. Therefore, a focus was made on biological processes related to carbohydrates. Among the GO terms identified, 9 were specifically related to carbon metabolism (Figure 7B). Altogether, our results illustrate the important changes in carbon metabolism occurring during the seed-filling stage, which marks the induction of storage-associated gene patterns, including starch biosynthesis (Figure 8B). In maize, such enrichment in carbohydrate synthesis and metabolic processes was also observed during seed-filling (Yin et al., 2020).

### 3.3.2 | Sugar transport and starch metabolism genes regulated through seed development

Among the 7914 genes regulated during seed development (Figure 7A), several genes encoding sugar transporters of the SUT, MST, and SWEET families, sucrose catabolic enzymes like invertases and enzymes of the starch synthesis pathway were differentially expressed from embryogenesis to seed-filling stages (Figure 8).

#### *Feeding embryo with energy-rich monosaccharides during embryogenesis*

During embryogenesis, sucrose is released in the apoplast, separating the seed coat from the embryo, where it is hydrolyzed into glucose and fructose through the enzymatic activity of cell wall invertases (Weber et al., 2005). Consistently, all cell wall invertases (*PsCWINV1.2*, *PsCWINV2.2*, and *PsCWINV1.5*) were highly induced during embryogenesis and then repressed at the filling stage (Figure 8A), including *PsCWINV1.2* the major invertase candidate driving sucrose cleavage from the seed coat and feeding glucose energy to the dividing embryo (Morin et al., 2022a). Moreover, two cytosolic invertases (*PsCINV1.1* and *PsCINV4.1*) and one vacuolar invertase (*PsVINV1.1*) were also significantly down-regulated through seed development. This high expression of invertase genes seems to clearly mark the embryogenesis stage (Morin et al., 2022a). The down-regulation of invertases during early seed-filling (14 dap) could be linked to a preferential sucrose

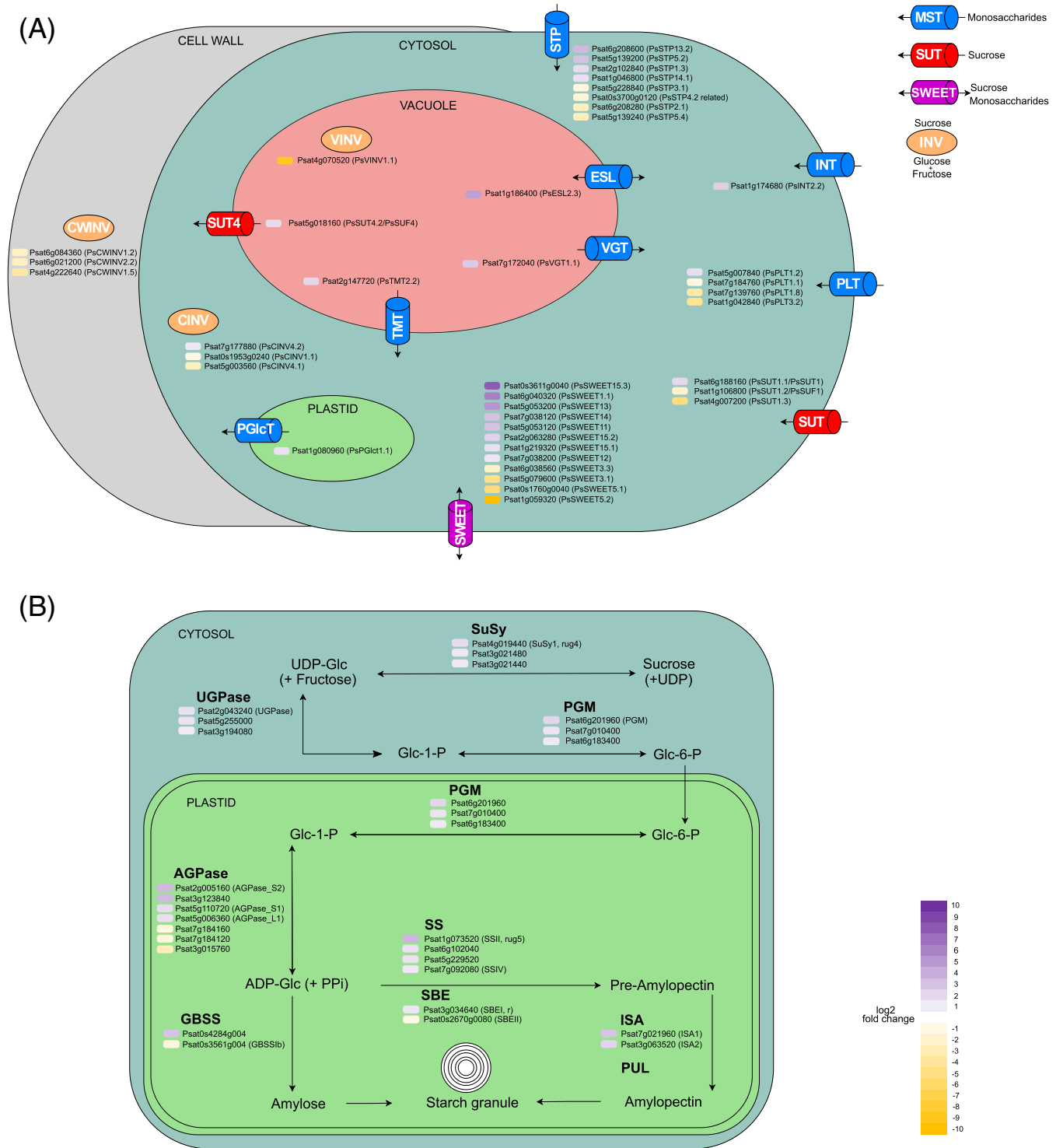
transport toward the embryo during this stage (Weber et al., 2005). When embryogenesis ends, sucrose is no longer cleaved by invertases but rather transported to feed the starch synthesis pathway (Figure 8B). The expression levels of several sugar transporters illustrate this transition from hexose to sucrose transport (Figure 8A).

In embryonic seeds, numerous PsSWEET of clades I and II are strongly expressed (Figure 8A). Clade II PsSWEET, such as *PsSWEET5.1* and *PsSWEET5.2*, have not yet been studied in legume species. However, in *Arabidopsis*, *AtSWEET5* transports preferentially glucose and galactose (Wang et al., 2022) and is involved in pollen development (Gautam et al., 2022). Both genes from the PsSWEET5 subclade could be involved in glucose retrieval after sucrose hydrolysis in the apoplast. *PsSWEET3.2* is also more expressed in embryonic seeds and could be involved in sucrose transport. A close gene homolog in *Lotus japonicus* (*LjSWEET3*) mediates sucrose transport toward nodules (Sugiyama et al., 2017), but its function and localization have not yet been studied in legume seeds. Sucrose uptake by the embryo is catalyzed by both a saturable and a non-saturable component (Patrick and Offler, 1995). In *Vicia faba*, proton-driven import of sugar is localized in the cotyledonary cells' outer layer, and sugar uptake by parenchyma cells is supposed to be passive (McDonald et al., 1996). The up-regulation of several genes of the STP clade also suggests active transport of hexoses towards the embryo (Figure 8A). In *Vicia faba*, *VfSTP1* transports glucose and is expressed in embryonic epidermal cells neighboring young and highly mitotic regions (Weber et al., 1997). Likewise, hexoses originated by invertase activity during embryogenesis could be loaded into the embryo by yet unidentified PsSTP. SUTs could also be involved in sucrose transport towards seeds during embryogenesis, as *PsSUT1.2* is strongly expressed during this stage. In pea, this gene encodes a sucrose facilitator (SUF) and has been found to be predominantly expressed in cotyledonary cells (Zhou et al., 2007). The set of genes identified in this analysis are promising candidates to decipher the pathway of sugar transport toward the developing embryo.

Noteworthy, some genes involved exclusively in starch biosynthesis showed a higher expression during the embryonic stage, namely the putative *AGPase* (*Psat7g184160*, *Psat7g184120* and *Psat3g015760*) and *GBSSIb* (Figure 8B). These genes could contribute to the formation of transitory starch during embryogenesis in pea seed coats as observed in several studies (Rochat et al., 1995; Déjardin et al., 1997).

#### *Loading sucrose to feed starch synthesis at the seed-filling stage*

When embryogenesis ends, the seed enters the seed-filling stage and initiates its nutritional filling. At this stage, sucrose is directly transported from the seed coat by sucrose transporter of the SUT and SWEET families (Hageman and Van Volkenburgh, 2021). The results from our study highlight this switch to sucrose transport and utilization at the filling stage (Figure 8B). Indeed, several clade III SWEETs are strongly up-regulated from the seed-filling stage, namely *PsSWEET11*, *PsSWEET12*, *PsSWEET13*, *PsSWEET14*, *PsSWEET15.1*, *PsSWEET15.1* and *PsSWEET15.3*. Clade III SWEETs are known to be localized at the plasma membrane and mainly transport sucrose (Chen



**FIGURE 8** Sugar transporter (A) and starch metabolism genes (B) regulated during the developmental transition from embryogenesis to seed-filling stages. Genes related to sugar transport of the sucrose transporter (SUT), monosaccharide transporter (MST) and sugar will eventually be exported transporter (SWEET) families. Genes related to sucrose catabolism of the sucrose synthase (SUSY) and invertase (INV) families, including cytosolic (CINV), cell wall (CWINV) and vacuolar (VINV) invertases. Genes related to starch synthesis of the UDP-Glc pyrophosphorylase (UGPase), phosphoglucomutase (PGM), ADPGlc pyrophosphorylase (AGPase), granule bound starch synthase (GBSS), soluble starch synthase (SS), starch branching enzyme (SBE) and starch debranching enzymes of the isoamylase (ISA) and pullulanase (PUL) families. Genes with a significant ( $p < 0.05$ ) induction (purple gradient,  $\log_2$  fold changes  $> 1$ ) and repression (yellow gradient,  $\log_2$  fold changes  $< -1$ ) from embryogenesis to seed-filling stages are represented.



et al., 2012). In pea, clade III SWEETs seem more expressed in the seed coat than in the cotyledons (Grant et al., 2021). Among these SWEETs, three out of four of the *PsSWEET15* gene paralogs (Doidy et al., 2019) are up-regulated in seeds at the filling stage (Figure 8A). As in other crops, SWEET15 could be localized in the plasma membrane of seed coat cells (Chen et al., 2015; Ko et al., 2021), mediating sucrose release towards the apoplast. In soybean, the mutation of both *GmSWEET15a* and *GmSWEET15b* gene copies delayed embryonic development and led to subsequent seed abortion (Wang et al., 2019b). Thus, SWEET15 genes are important candidates in legumes, regulating carbon provision for plant productivity and population fitness. Other clade III SWEETs were also up-regulated during seed-filling (*PsSWEET11*, *PsSWEET12*, and *PsSWEET13*; Figure 8A). These genes are orthologous to *AtSWEET11* and *AtSWEET12*, which are involved in phloem loading in *Arabidopsis* (Chen et al., 2012; Doidy et al., 2019). Up-regulation of clade III SWEETs could be linked to preferential sucrose transport in seeds, as shown previously with the down-regulation of invertases, but also with an increased sink strength in seeds during filling (Jeuffroy and Warembourg, 1991; Munier-Jolain, 2003). Among the seven PsSUTs identified in the pea genome (Doidy et al., 2019), the active transporter encoded by *PsSUT1.1* is induced during the seed-filling stage (Figure 8A). During this stage of development, *PsSUT1.1* is expressed in both the epidermal seed coat and cotyledonary cells (Tegeder et al., 1999; Borisjuk et al., 2002; Zhou et al., 2007) and could be involved in addition to the clade III SWEET identified in this study in sucrose unloading towards seeds. After being loaded in the cotyledonary cells, the content of sucrose which is used to sustain cell metabolism and starch storage will consequently lower, leading to higher SUT expression (Hageman and Van Volkenburgh, 2021). The vacuolar facilitator encoded by *PsSUT4.2* is also expressed at higher levels during seed-filling (Figure 8A). This tonoplast transporter is involved in sucrose export from the vacuole to the cytosol and is mainly expressed in the seed coat (Zhou et al., 2007). Other vacuolar transporters, such as *PsVGT1.1*, *PsTMT2.2*, and *PsESL2.3*, are also up-regulated during the seed-filling stage. They could be involved in sucrose storage in the vacuole to facilitate cellular expansion, as well as sucrose homeostasis necessary for optimal seed growth.

This transition from hexoses to sucrose utilization corresponding to the set of cell differentiation and storage activity is also illustrated by the gene induction of several key enzymes involved in sucrose metabolism and starch synthesis (Figure 8B). Thus, the induction of sucrose synthase (SUSY) and most enzymes of the starch synthesis pathway (UGPase, PGM, AGPase, GBSS, SS, SBE, ISA) seems to clearly mark this transition to the seed-filling stage.

Indeed, among the differentially expressed genes involved in starch synthesis, a wide majority of them are up-regulated from the seed-filling stage (Figure 8B). For instance, SUSY and UGPase encoding genes are up-regulated during this stage, which could be linked to the hydrolysis of sucrose in the cytosol to ensure starch synthesis in the plastid. Such induction of starch biosynthesis was also observed in 20 dap pea seeds (Malovichko et al., 2020) and, therefore, seems to be characteristic of the seed-filling stage. This induction of genes

related to starch biosynthesis could be driven by sugar transporters involved in sucrose loading in seeds. Indeed, in *Vicia faba*, *in vitro* application of sucrose on young explanted cotyledons promotes cotyledonary storage activity (Ambrose et al., 1987; Weber et al., 1996; Buitink et al., 2004) and increased sucrose level up-regulates the expression of ADPG-pyrophosphorylase and sucrose synthase (Heim et al., 1993; Weber et al., 1998). The transition from hexose to sucrose transport in cotyledons, mediated by the transcriptional repression of invertase expression and the induction of clade III SWEETs, could therefore be related to the physiological shift from embryogenesis to seed-filling. In turn, sucrose might enable the beginning of starch accumulation in cotyledonary cells through the induction of genes involved in the starch biosynthetic pathway.

## 4 | CONCLUSION

Our comprehensive study integrating phenotypic, physiological, and transcriptomic approaches uncovered new insights into the regulation of carbon transport and metabolism in response to WD in pea crops. This stress resulted in decreased vegetative and reproductive growth and affected sugar contents in source leaf, suggesting changes in carbon allocation and source-to-sink relationships. Consistently, our genome-wide transcriptome analysis identified a large repertoire of genes regulated by WD in leaf, root and developing seeds, including genes involved in biological processes related to transcriptional regulation, sugar transport and metabolism. The analysis of their transcriptional regulation suggests that sugar is remobilized from leaves by its diversion from starch biosynthesis to be reallocated towards sink organs by SUT and SWEET transporters. The root system displayed the largest genome-wide changes to WD, suggesting intense genetic reprogramming to efficiently forage water in the soil, with broad regulation of transcription factors (TF), sugar transport (MST and SWEET) and metabolism genes (SUSY and INV) in this organ. Noteworthy, different patterns of gene expression were found depending on the seed developmental stage. Seeds in embryogenesis showed induction of numerous genes involved in monosaccharide transport and sucrose catabolism (INV and SUSY), indicating maintenance of glucose provision to the embryo to ensure its survival. In contrast, seeds at the filling stage displayed a rather small gene expression reprogramming in response to WD, which suggests that changes in gene expression take place earlier during seed development. Lastly, we performed a similar analysis to study variations in carbon transport and metabolism during seed development. Our results showed a transition from hexose to sucrose transport during seed-filling. This shift was accompanied by an increase in the expression of genes related to sucrose transport and starch biosynthesis, marking the filling stage.

Altogether, our results illustrate the crucial role of sugar transport and metabolism during water deficit and seed development. The development of climate-resilient crops is a timely challenge and research strategies based on optimized carbon resource allocation are being developed to select crops with a more stable yield under

unfavorable environments (Nadeem et al., 2019; Hageman and Van Volkenburgh, 2021; Morin et al., 2022b). By providing new insights of the genome-wide changes during drought, our integrative picture uncovers new candidate genes that could be targeted in breeding programs to further improve stress resilience and carbon utilization in seed crops.

## AUTHOR CONTRIBUTIONS

The experimental plan was designed by AM, CV, JD, LM, NP and RL. Samples were collected by AM, BP, CV, JD, LM and NP. Phenotypical and physiological analysis were performed by AM, BP, JD and NP. RNA-seq analyses were performed by AM, GCK and JD. Data curation was performed by BM. The manuscript was written by AM and JD and reviewed by all authors.

## ACKNOWLEDGEMENTS

This work was supported by the University of Poitiers, the Centre National de la Recherche Scientifique (CNRS), the Nouvelle-Aquitaine region with the 2015-2020 State-Region Planning Contracts (CPER) and the European Regional Development Fund (FEDER) by a doctoral grant from Grand Poitiers awarded to AM and Ministère de l'Enseignement Supérieur et de la Recherche Scientifique. We thank students of the Master Gphy (Génie Physiologique, Biotechnologique et Informatique, Université de Poitiers) for their contributions to bioinformatics analyses. We thank Mickael Durand for his contribution to RNA-seq analysis; Vincent Lebeurre, Helene Paulhac, Florence Thibault for their technical assistance and all members of the SEVE team (EBI, Université de Poitiers).

## DATA AVAILABILITY STATEMENT

Transcriptome analyses presented in this work can be found in the supplementary data. The raw RNA-seq data have been deposited to NCBI under the Bioproject number PRJNA991334 (<https://www.ncbi.nlm.nih.gov/bioproject/991334>) with the SRA numbers SRR25161569 - SRR25161592.

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## SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

**How to cite this article:** Morin, A., Porcheron, B., Kodjovi, G.C., Moumen, B., Vriet, C., Maurousset, L. et al. (2023) Genome-wide transcriptional responses to water deficit during seed development in *Pisum sativum*, focusing on sugar transport and metabolism. *Physiologia Plantarum*, 175(6), e14062. Available from: <https://doi.org/10.1111/ppl.14062>