Transcriptome sequencing reveals hotspot mutation regions and dwarfing mechanisms in wheat mutants induced by γ-ray irradiation and EMS

Hongchun Xiong*, Chunyun Zhou*,†, Huijun Guo Yongdun Xie, Linshu Zhao, Jiayu Gu, Shirong Zhao, Yuping Ding and Luxiang Liu*

Institute of Crop Sciences, Chinese Academy of Agricultural Sciences/National Engineering Laboratory for Crop Molecular Breeding, National Center of Space Mutagenesis for Crop Improvement, Beijing 100081, China

*Corresponding author. Institute of Crop Sciences, No. 12 Zhongguancun South Street, Haidian District, Beijing, 100081. Tel: 86 010 62122719; Fax: 86-10-82108543; Email: liuluxiang@caas.cn)

†These authors contributed equally to this work.

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ABSTRACT

Induced mutation is an important approach for creating novel plant germplasms. The introduction of dwarf or semi-dwarf genes into wheat has led to great advancements in yield improvement. In this study, four elite dwarf wheat mutants, named dm1–dm4, induced from γ-ray irradiation or ethyl methane sulfonate (EMS) mutagenesis, were used to identify transcriptome variations and dwarfing mechanisms. The results showed that the hotspot regions of mutations distributed on the chromosomes were consistent among the four mutant lines and these regions were mainly located around the 50, 360 and 400 Mb positions of chromosome 1A and the distal regions of chromosomes 2A and 2BL. Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses suggested that ‘protein processing in endoplasmic reticulum’ was the most common significantly enriched pathway based on the differentially expressed genes (DEGs) between wildtype (WT) and the mutants. Notably, 18 out of 20 genes involved in this process encode heat shock proteins (HSPs). The results implied that HSPs might participate in wheat dwarfism response and function in the dwarfism process through protein folding and/or degradation. Moreover, seven genes in dm4 involved in modulating auxin levels were down-regulated and dm4 was more sensitive to auxin treatment compared with WT, indicating the important roles of auxin in regulation of dwarf phenotype in dm4. This study not only identified transcriptome sequence variation induced by physical and chemical mutagenesis but also revealed potential dwarfing mechanisms in the wheat mutant lines.

Keywords: induced mutation; wheat; transcriptome variation; dwarfism; differentially expressed genes; hormone

INTRODUCTION

The introduction of randomly induced mutations in crop plants is a powerful tool for crop improvement because it accelerates genetic variations and produces enhanced phenotypes [1, 2]. Recently, the development of high-throughput sequencing techniques has resulted in several studies focused on the molecular nature of chemically and physically induced mutations [3–6]. Whole genome sequence analyses have suggested that the mutations generated through fast-neutron irradiation in rice [3] or chemical mutagenesis in sorghum [6] are evenly distributed across their genomes. However, the distribution of sequence variations induced by physical or chemical mutagens in wheat plants has rarely been reported. Due to its large size and the highly repetitive sequences in the hexaploid wheat genome [7], transcriptome sequencing is more economical and feasible in wheat.

Plant height is a key agronomic trait that is tightly connected with crop yield improvement [8]. In the 1960s, breeding for dwarf wheat and rice resulted in dramatic increases in crop yields due to enhancements in lodging resistance and harvest index [8]. The wheat varieties containing ‘Green Revolution’ Rht-B1b or Rht-D1b genes had reduced plant height but increased grain yield. Both gene sequences possess a premature stop codon in a gibberellin (GA) responsive region where DELLA proteins that are negative regulators of GA signal transduction are blocked [9]. Additionally, dwarfism has been observed in wheat varieties with other nucleotide mutations in DELLA binding regions,
such as Rht-B1d, Rht-B1c [10, 11], and Rht-B1p [12]. In addition to these GA-insensitive dwarfing genes, several GA-sensitive dwarfing genes have also been found in wheat, such as Rht4, Rht5, Rht8, Rht9, Rht12, Rht13 and Rht18 [13–18]. Until recently, the most commonly utilized dwarfing genes were Rht-D1b, Rht-B1b and Rht8 [19, 20]. Some limitations have also been observed when incorporating dwarfing genes into agricultural cultivars. Many Rht genes have been shown to decrease thousand-grain weight [15, 21, 22]. Although >20 dwarfing genes have been identified in wheat, the genes utilized in agricultural practice are restricted and the dwarfing mechanism requires further investigation.

Dwarfism has been shown to be tightly connected with plant hormone signaling. In addition to GA, brassinosteroids (BR) are another group of hormones that affect plant height. In wheat, reduced sensitivity to BR resulted in the semi-dwarf phenotype of Rht8 [16]. Loss of function or knockdown of the BR receptor in rice [23], maize [24] and Brachypodium distachyon [25] also resulted in dwarf stature. The plant hormone auxin is also an integral factor in dwarfism phenotypes. The height reduction in maize br2 and sorghum dw3 mutants was a result of the loss of P-glycoproteins known to modulate polar auxin transport [26]. In dwarf apple trees, the levels of the auxin indoleacetic acid (IAA) and the expression abundance of auxin-related genes were significantly different than in the non-dwarfed trees [27, 28]. Additionally, strigolactones [29] and cytokinin [30] also affect plant height. These hormones have been shown to interact with each other for dwarfism modulation [28].

In recent years, the high-throughput RNA-Seq technique has been used to study dwarfism mechanisms in plants. Transcriptome expression analysis of a dwarf soybean mutant revealed that genes involved in hormone biosynthetic pathways were significantly affected in the mutant [31]. Three specific pathways were shown to have differentially expressed genes (DEGs) between WT and dwarf cotton. These pathways were carotenoid biosynthesis, plant–pathogen interaction and plant hormone signal transduction [32]. Agapanthus praecox treated with the plant growth retardant paclobutrazol showed expression changes in genes associated with stimulus response, hormonal signaling, carbohydrate metabolism and cell-related biological processes [33]. In Kentucky bluegrass, DEGs between WT and dwarf mutants were mainly involved in terpenoid biosynthesis and plant hormone metabolism [34].

In this study, four identified semi-dwarf wheat mutants that did not show significant negative effects on thousand-grain weight were created by induced mutation via γ-ray irradiation and ethyl methanesulfonate (EMS). We provide new insights into the mutations in hotspot regions at the transcriptome level induced by γ-rays and EMS in wheat plants. Moreover, the dwarfism mechanisms in these mutants were revealed by transcriptional expression profile analysis and IAA treatment. This transcriptome dataset offers a valuable public information platform for studies seeking to characterize transcriptome sequence variation by induced mutagenesis as well as studies of dwarfism mechanisms in wheat plants.

**Materials and Methods**

**Plant materials and growth conditions**

The four dwarf wheat mutants, dm1, dm2, dm3 and dm4 at the jointing stage were sampled and quickly put into liquid nitrogen and kept at −80 °C for RNA extraction. Total RNA was extracted using TRIzol® Reagent (Tiangen Biotech). RNA purification and library construction was based on a previously described procedure [36]. DNA contamination was removed using DNase I (Takara) and the total RNA was purified using an RNA purification kit (Tiangen Biotech). A NanoPhotometer® spectrophotometer (IMPLEN), a Qubit® RNA Assay Kit with Qubit® 2.0 Fluorometer (Life Technologies) and an RNA Nano 6000 Assay Kit with the Bioanalyzer 2100 system (Agilent Technologies) were used for assessment of RNA purity, concentration and integrity, respectively. According to the manufacturer’s recommendations, sequencing libraries were constructed using NEBNext Ultra™ RNA Library Prep Kit for Illumina® (NEB) and library quality was assessed with the Agilent Bioanalyzer 2100 system. This process was repeated three times for each genotype group independently, successfully creating 15 cDNA libraries.

**Transcriptome sequencing and single-nucleotide polymorphism analysis**

The constructed libraries were deep sequenced on the Illumina HiSeq platform. After removing reads containing adapters, poly-N homopolymers, and other low quality reads from the raw data, clean reads were obtained for further analysis. The Q20, Q30 and GC content in the clean reads were calculated for quality assessment. Using TopHat2 (http://tophat.cbcb.umd.edu/), paired-end clean reads were aligned to the reference genome Chinese Spring wheat v1.0 in the public databases (http://www.wheatgenome.org). Picard-tools v1.96, SAMtools (http://samtools.sourceforge.net) and GATK2 software (https://software.broadinstitute.org/gatk) were used for single-nucleotide polymorphism (SNP) analysis. All sequencing datasets are deposited in the National Centre for Biotechnology Information (NCBI) under the BioProject ID PRJNA417210 with the Sequence Read Achieve (SRA) submission ID SUB3198378.
Functional annotation and DEGs analysis
NCBI non-redundant protein sequences (NR), UniProt/Swiss-Prot, Gene Ontology (GO), Clusters of Orthologous Groups of proteins (KOG/COG), Protein family (Pfam), and Kyoto Encyclopedia of Genes and Genomes (KEGG) databases were used for gene function annotation. Unigene expression levels were estimated as fragments per kilobase of transcript per million fragments mapped (FPKM). The DESeq R package that provides statistical routines for determining DEGs using a model based on a negative binomial distribution [37] was used for identification of DEGs between WT and the mutants. The Benjamini and Hochberg approach for controlling the false discovery rate (FDR) was used for adjustment of P-values. Unigenes with an adjusted P-value determined to be < 0.05 (FDR < 0.05) and a fold change value ≥2 ([log2 fold changes] ≥ 1) between WT and mutants were designated as differentially expressed. DEGs were annotated with KEGG assignments (http://www.genome.jp/kegg/) and the statistical significance of the enrichment of DEGs in KEGG pathways was assessed using KOBASE software (http://kobas.cbi.pku.edu.cn).

Quantitative real-time PCR validation
For qPCR confirmation, the same stage and tissue of wheat samples as those used in the RNAseq analysis were sampled. Total RNA was isolated using TRNzol-A+ Reagent (Tiangen Biotech) and DNA contamination was removed using DNase I (Takara); RNA was purified using an RNA purification kit (Tiangen). An iScript cDNA synthesis kit (Bio-Rad) was used to synthesize first-strand cDNA. Quantitative real-time PCR was conducted on a CFX 96 Real-Time System (Bio-Rad) using iScript EvaGreen Supermix Kit (Bio-Rad). Primers used for quantitative real-time PCR are listed in Table S6 (see online supplementary material); the actin gene was used as an internal control.

IAA treatment
The full seeds of WT, dm1 and dm4 were selected for sterilization. After germination for 2–3 days, the WT, dm1 and dm4 were grown in Hoagland nutrient solution without indole-3-acetic acid (3-IAA) or with 500, 1000 and 5000 µg/L 3-IAA under 200–300 µmol m−2 s−1 light at 21 °C in a growth chamber. The nutrient solution and 3-IAA were changed every 4 days. After 8 days of treatment, the seedling lengths from eight replicates for each treatment were measured.

RESULTS
Dynamic variation of plant height in four dwarf wheat lines derived from γ-ray and EMS mutagenesis
To compare the effects of γ-rays and EMS on phenotypic and transcriptomic variations, four dwarf wheat mutant lines derived from γ-rays (dm1 and dm2) and EMS (dm3 and dm4), were used in this study. These four dwarf mutant lines showed similar thousand-grain weight to the WT in both years (Fig. S1, see online supplementary material). Three weeks after the turning-green stage, the plant height of these four mutants was similar to the WT. At the fifth week after the turning-green stage, the EMS-induced dm3 and dm4 plants were shorter than the γ-ray-induced dm1 and dm2 plants (Fig. 1A and B). The second to fifth internode lengths in dm1 were significantly shorter than in WT, while the first to fourth internode lengths of dm2 were significantly decreased compared with the WT. Moreover, all of the internode lengths in dm3 and dm4 were significantly lower than in WT (Fig. 1C).

Comparison of SNPs and Indels in the transcriptome of the wheat mutant lines
RNAseq was conducted to compare the transcriptome sequence variation and stem transcript levels between WT and the dwarf mutants. After removing poor-quality reads, adapter contamination and low-quality regions from the 15 libraries, 42–66 million clean reads were obtained and 75.60–84.45% of reads were mapped to the wheat reference genome, including 69.47–77.76% that were unique (Table S1, see online supplementary material).

The numbers of homozygous SNPs and Indels between WT and mutants showed that the number of SNPs in all four mutants were obviously higher than the number of Indels. The number of SNPs in dm1 and dm2 were almost the half that in dm3 and dm4 while the numbers of Indels were similar in the four mutants (Fig. 2A). The different types of SNPs in the homozygous variations between WT and mutants suggested that there were more transitions (C/G to T/A and T/A to C/G) than transversions (C/G to G/C, C/G to A/T, T/A to G/C and T/A to A/T) in the four mutants (Fig. 2B). The Ti:Tv (transitions:transversions) ratios of dm1, dm2, dm3 and dm4 were 1.86, 1.98, 2.27 and 4.45, respectively. The numbers of C/G to T/A transitions were significantly higher than other types of SNPs in dm3 and dm4 (Fig. 2B). Among the four types of transversions, the numbers of C/G to G/C transversions were generally higher in the four mutants (Fig. 2B). We further analyzed the mutation types of the SNPs and found that the numbers of synonymous and nonsynonymous mutations were the highest. The numbers of mutations in 3′-UTR were higher than that in 5′-UTR and the numbers of stop-gain mutations were the lowest (Fig. 2C).

The distribution and hotspot regions of mutations across wheat chromosomes
The number of homozygous SNPs between WT and mutants in 21 chromosomes showed that chromosome 1A contained the highest number of SNPs. Chromosomes 2A and 2B also showed higher numbers of SNPs than the other 18 chromosomes in three of the four mutants (Fig. 3A). To analyze the hotspot regions for mutations in the transcriptome of wheat, the SNP frequencies in the chromosomes of the four mutant lines were analyzed. The distribution of SNPs in these three chromosomes showed that the mutation region in the chromosomes was very similar in the four mutants (Fig. 3B–D). The results suggested that the mutated SNPs were mainly observed around the 50, 360 and 400 Mb positions of chromosome 1A (Fig. 3B), the ends of both short and long arms of chromosome 2A (Fig. 3C) and the distal regions of the long arm of chromosome 2B (Fig. 3D).

Differentially expressed genes and functional categorization
DEGs were characterized as having an FDR < 0.05 and a fold change ≥2 and were identified in comparisons between WT and all mutant
Transcriptome analyses of dwarf wheat mutants

Fig. 1. The phenotypes, plant height and internode length of WT, dm1, dm2, dm3 and dm4. (A) Phenotypes of WT and the four dwarf mutants. Bars = 10 cm. (B) Dynamic changes of plant height. The x-axis 1 W to 7 W denotes 1–7 weeks after the turning-green stage. (C) The internode lengths of WT, dm1, dm2, dm3 and dm4 at mature stages. The x-axis 1st to 5th indicates the first to fifth internodes measured starting from the internodes below the ear. Data are means ± SD of five replicates and different letters represent significant differences between WT and the four mutants at \( P < 0.05 \).

The KEGG pathway is a collection of manually drawn pathway maps reflecting the molecular interaction, reaction and relation networks (http://www.genome.jp/kegg) and the enriched KEGG pathways based on the DEGs were analyzed. Rich factor reflects the proportion of DEGs in a given pathway and \( q \) value indicates FDR-adjusted \( P \) value. A higher rich factor and lower \( q \) value suggested a greater degree of pathway enrichment. For dm1 vs WT, ‘cyanoamino acid metabolism’ and ‘protein processing in endoplasmic reticulum’ were significantly enriched (Fig. 5A). In contrast, more genes were enriched in ‘ribosome’ and ‘starch and sucrose metabolism’ in the dm2 vs WT group (Fig. 5B). In the dm3 vs WT comparison, the DEGs were significantly enriched in ‘starch and sucrose metabolism’ and ‘protein processing in endoplasmic reticulum’ (Fig. 5C). The DEGs involved in ‘protein processing in endoplasmic reticulum’ were significantly enriched in the dm4 vs WT group (Fig. 5D). In total, DEGs in dm1 vs WT, dm3 vs WT and dm4 vs WT comparisons were commonly and significantly enriched in ‘protein processing in endoplasmic reticulum’.

Expression patterns of DEGs involved in ‘protein processing in endoplasmic reticulum’

To identify genes involved in plant height, the DEGs participating in the commonly enriched ‘protein processing in endoplasmic reticulum’ were further analyzed. Eighteen out of 20 genes were annotated as ‘heat shock protein’ and the last two genes (Fig. 6A) were annotated as ‘derlin-2’ and ‘cell division control protein’. The expression patterns of the DEGs showed that 17 genes encoding HSPs were up-regulated in the three mutants compared with the WT (Fig. 6A). The qPCR confirmation of eight genes showed similar expression patterns with the RNAseq results (Fig. 6B-I).
Several DEGs related to auxin were down-regulated in the dwarf mutant lines

Due to the important roles of plant hormones in the regulation of plant height, the expression levels of DEGs related to plant hormones were specifically analyzed (Table 1, Fig. 7). Among the four mutants, DEGs involved in plant hormones were not detected in dm2. In dm1 vs WT, one gene encoding coleoptile phototropism protein 1, known to play an important role as a signal transduction component in lateral translocation of auxin [38], and another gene encoding the two-component response regulator ARR12, which directly regulates cytokinin-responsive genes, showed decreased expression levels in dm1 compared with the WT. In dm3 vs WT, two genes encoding auxin transporter-like protein 2 and one gene for coleoptile phototropism protein 1 were down-regulated. Additionally, one gene encoding auxin-induced protein and abscisic acid receptor had increased expression levels in dm3. In dm4 vs WT, three genes encoding auxin transporter-like proteins, three genes putatively for indole-3-acetic acid-amido synthetase and one gene encoding indole-3-acetic acid-induced protein, had decreased expression abundance in dm4. One gene encoding 12-oxophytodienoate reductase and two genes for protein TIFY 10B, involved in jasmonate biosynthesis or signaling processes [39], showed up-regulated expression in the dm4 vs WT comparison. Moreover, genes encoding shaggy-related protein kinase and DELLA protein had obviously decreased transcript levels in dm4 compared with the WT. The expression levels of thirteen DEGs involved in hormones were confirmed by qPCR analysis and showed similar expression patterns between RNAseq and qPCR data (Fig. 7).

The dm4 mutant was more sensitive to IAA treatments compared with WT and dm1

Seven genes related to auxin had changed expression levels in the dm4 mutant, so the sensitivities of WT and mutants with IAA treatment were investigated (Fig. 8). Under normal conditions, the seedling length of dm1, dm4 and WT showed no difference. In contrast, when the plants were treated with 500, 1000 and 5000 μg/L IAA for 8 days, the seedling length of dm4 was significantly shorter than that of WT and dm1. Generally, the seedling length of dm4 was significantly reduced when the concentrations of IAA were increased, while the seedling lengths in WT and dm1 were slightly increased with 500 μg/L IAA treatment (Fig. 8).

DISCUSSION

Induced mutation is one of the most important and useful techniques for the creation of new crop materials [1]. Development of dwarf or semi-dwarf wheat advances agricultural productivity due to its lodging resistance [40] but many dwarfing genes have some negative effects on yield components, in particular decreased thousand-grain weight [15, 21, 22, 41]. The four dwarf wheat mutant lines derived from induced mutation without negative effects on thousand-grain weight in this study are promising materials for dwarf wheat breeding. This study further characterized transcriptomic variations and dwarf mechanisms in the four mutant lines.
New insights into the transcriptomic sequence variations induced by γ-rays and EMS

Mutant plants are usually generated by chemical or physical mutagenesis. Limited information regarding the hotspot region of mutations across the genome induced by chemical or physical mutagenesis has been reported [5, 42]. Sequencing the rice genome showed fast-neutron-induced mutations were distributed evenly across the genome [5]. However, in EMS-induced wheat, mutated genes were mainly detected in the distal region of the chromosomes [42]. Additionally, the highest frequency of segmental duplications was found on chromosome ends compared with other regions of the chromosome in fast neutron-irradiated soybean [43]. Consistently, this study showed that the transcriptome-wide distribution of mutated regions induced by EMS and γ-rays were mainly enriched on the ends of chromosomes 1A, 2A and 2B (Fig. 3). It is possible that the unique components such as repetitive DNA in centromeres stabilize their structure [44] and thus lead to low mutations near pericentromeric chromosomal regions. The similar mutation regions between γ-rays- and EMS-induced mutants indicate that wheat chromosomes contain hotspot regions where mutations easily occur. The high similarity of mutation regions among the four mutants may due to the DNA structure and/or chromosome position of those regions which were often damaged by mutagens. Similarly, a previous study on distribution of breakpoints within chromosomes of human lymphocytes induced by atomic bomb showed that the breakpoints were not randomly distributed and a large proportion of breakpoints in the telomeric region were observed [45]. Cytological studies on X-irradiation effects suggested that the number of chromosome breaks produced by X-irradiation were relatively higher in distal regions of all the chromosomes than in proximal regions [46]. The regions with a high frequency of SNPs distributed across the chromosomes were consistent among the four mutants (Fig. 3B–D), suggesting that these hotspot regions of mutations were not affected by the different mutagens. Interestingly, the DEGs between WT and mutants were also mainly distributed on the distal regions of chromosomes 1A, 2A and 2B (Fig. S2, see online supplementary material), indicating the genomic mutations may affect gene expression levels to some extent.

EMS is a widely used chemical mutagen, most often producing C/G to T/A transitions [47, 48]. This is consistent with our finding that the highest numbers of C/G to T/A transitions were detected in the EMS-induced dm3 and dm4 (Fig. 2B). In contrast, γ-ray-induced dm1 and dm2 showed equal amounts of C/G to T/A and T/A to C/G transitions (Fig. 2B). Irradiation with γ-rays produces equal numbers of transitions and transversions in the genome of tomato mutants [47]. In our study, both EMS-induced dm3 and dm4 and γ-ray-induced dm1 and dm2, had more transitions than transversions at the transcript level.
Fig. 4. Volcano plots of DEGs between WT and each mutant. (A) $dm_1$ vs WT. (B) $dm_2$ vs WT. (C) $dm_3$ vs WT. (D) $dm_4$ vs WT. FC denotes fold change and FDR in the vertical coordinates indicate the adjusted $P$-values. The red dots represent up-regulated genes and green dots represent down-regulated genes. The black dots indicate genes without significant differences.
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Fig. 5. KEGG enrichment by the DEGs. (A) dm1 vs WT. (B) dm2 vs WT. (C) dm3 vs WT. (D) dm4 vs WT. The rich factor reflects the proportion of DEGs in a given pathway and the circle size indicates the number of DEGs in the pathway. The circle color represents the range of q values (corrected P-values).

(Fig. 2B), suggesting that in wheat plants γ-ray irradiation also easily induces transitions at the transcript level. These results also imply that the variations at the transcriptome level are different from those at the whole genome level. Our recent study indicated that among the four types of transversions, the number of C/G and G/C transversions were the highest in the space-flight-induced wheat mutant [36]. Interestingly, the EMS- and γ-ray-induced wheat mutants also showed the highest number of C/G and G/C transversions (Fig. 2B). One limitation of this analysis is that only SNPs and small indels could be detected and the frequently occurring larger fragment mutations from γ-rays need further study using whole genome sequencing. Transcriptome sequencing focusing on gene regions lowers the cost and is therefore a good alternative to genome sequencing. Actually, transcriptome sequencing reflects the mutations in the gene regions and thus provides important information on induced mutagenesis in a functional gene. Since M₆ mutant lines were used for sequencing, we could not exclude variations occurring during the growth of many generations. But this possibility may be very low and therefore most of the SNPs are really induced by mutagen treatment.

Several studies have reported genome-wide variations induced by chemical or physical mutagens [5,47,48]. However, research regarding genomic mutation differences among similar phenotypes is limited. This study also focused on phenotypic variation comparisons among the four mutants and showed decreased plant height (Fig. 1A and B) among the mutants. Consistent with the more dramatic phenotype variations found in the dm3 and dm4 dwarf wheat mutants (Fig. 1), the number of SNP mutations (Fig. 2A) and DEGs (Fig. 4) between WT and dm3/dm4 were greater than those found in the WT and dm1/dm2. These results suggested that the mutation efficiency in dm3 and dm4 was higher than in dm1 and dm2 at the transcript level, possibly leading to the larger variations observed between dm3/dm4 and the WT phenotypes.
Fig. 6. The expression patterns of DEGs enriched in 'protein processing in endoplasmic reticulum'. (A) Expression heat map of the DEGs in 'protein processing in endoplasmic reticulum'. Each column represents one of the three replicates for each wheat genotype. (B–I) qPCR confirmation of the top eight genes from the heat map. Values are means ± SE from three independent replicates.
Fig. 7. Expression comparisons of RNA-seq and qPCR in the selected genes from Table 1. The values for RNA-seq represent the average ratio of the expression level in the dwarf mutants to WT from three replicates. For qPCR, the data are means ± SE from five biological replicates, and the significant differences between WT and mutants of the qPCR data were assessed using a t-test, *P < 0.05, **P < 0.01.
Table 1. DEGs between WT and the mutants involved in hormone processes

| Gene ID       | FDR     | log2 FC | Gene annotation                                    |
|---------------|---------|---------|---------------------------------------------------|
| dm1 vs WT     |         |         |                                                   |
| TraeCSU01G114000 | 1.15E-03 | -1.25  | Coleoptile phototropism protein 1                 |
| TraeCS7D01G148200 | 1.28E-07 | -1.98  | Two-component response regulator ARR12            |
| dm3 vs WT     |         |         |                                                   |
| TraeCS1A01G278400 | 1.21E-02 | -1.16  | Auxin transporter-like protein 2                  |
| TraeCS1B01G287300 | 2.90E-03 | -1.22  | Auxin transporter-like protein 2                  |
| TraeCS7A01G315300 | 3.15E-02 | 1.28   | Auxin-induced protein                              |
| dm4 vs WT     |         |         |                                                   |
| TraeCS1B01G095900 | 9.76E-07 | -1.36  | Auxin transporter-like protein 3                  |
| TraeCS4A01G070500 | 1.20E-05 | -1.82  | Auxin transporter-like protein 2                  |
| TraeCS1B01G287300 | 4.77E-03 | -1.34  | Auxin transporter-like protein 2                  |
| FDR indicates the adjusted P-values and FC denotes fold change.

Fig. 8. The effects of IAA treatment on the seedling length of WT, dm1 and dm4. The plants were grown in nutrient solution with 0, 500, 1000 and 5000 μg/L 3-IAA for 8 days. Values are means ± SD from eight replicates and different letters indicate the significant difference among WT, dm1 and dm4 at P < 0.05.

Key pathways and genes involved in wheat dwarfism response

Studies on transcriptome profiling have suggested that the genes involved in carotenoid biosynthesis, plant–pathogen interaction, terpenoid biosynthesis and plant hormone pathways have altered expression in dwarf plants [31, 32, 34]. In contrast, our study showed that ‘protein processing in endoplasmic reticulum’, ‘cyanoamino acid metabolism’, and ‘starch and sucrose metabolism’ pathways were significantly enriched by DEGs between the dwarf mutants and WT (Fig. 5). These results show the different pathways enriched by DEGs pertaining to the dwarfism phenotype and establish novel information concerning gene pathways in the dwarfism response. In addition, DEGs in the dm1 vs WT, dm3 vs WT, and dm4 vs WT were significantly enriched in ‘protein processing in endoplasmic reticulum’, indicating the important role of this process in regulation of dwarfism in wheat. Eighteen out of 20 genes involved in this process encode HSPs (Fig. 6A). The HSP family is an important factor ensuring correct protein folding that plays a significant role in degradation pathways, such as endoplasmic reticulum-associated degradation [49]. In addition, most of the HSPs showed higher expression in dm1, dm3 and dm4 compared with the WT (Fig. 6). These results suggest that HSPs might participate in wheat dwarfism response and function in the dwarfism process through protein folding and/or degradation. Some studies have reported that HSPs are involved in plant tolerance to heat [50, 51] and drought stresses [52]. HSPs function as molecular chaperones, aiding in the refolding of proteins denatured by heat as well as protecting cellular organelles from hyperthermia damage [50]. The highly expressed HSPs in the dwarfing mutants compared with the WT calls for further research regarding the different abilities of these dwarf mutants to resist heat and drought compared with the WT wheat plants. However, the direct mechanisms involved in the dwarf phenotype in the four mutants need further study. The comparisons
of DEGs between the lines derived from the repeated back-cross of the original mutants to the parent genotype is an optimum approach for identification of the dwarf mechanism in the mutants.

The possible roles of auxin in dwarf phenotypes of the mutant lines

Plant hormones play crucial roles in plant height regulation [53]. Several transcriptome analyses have suggested that hormone biosynthesis or signal transduction genes are significantly affected by dwarfism [31–34]. It is well known that GA biosynthesis or signaling is tightly connected with plant height. The ‘Green Revolution’ Rht-B1b or Rht-D1b alleles carrying mutations in DELLAs resulted in dwarfing [54]. The Rht-B1b and Rht-D1b dwarfing genes were highly expressed in both node and internode but the GA biosynthesis genes such as TaGA20ox1 and TaGA3ox1 involved in dwarfism were mainly expressed in the node [11]. We therefore sampled the node and part of internode for DEGs analysis. Previous studies have reported that mutation and expression changes of auxin-related genes are responsible for dwarfism [26–27]. In our study, several genes crucial for hormones had changed expression abundance in dm1, dm3 and dm4 (Table 1, Fig. 7). The expression levels of seven genes involved in modulating the levels of active auxin in dm4 had decreased expression compared with WT (Table 1, Fig. 7F–I). Due to the limited reports about wheat seedlings in responsive to different concentrations of IAA treatment, we selected various IAA concentrations for investigation and found that dm4 had a different response to IAA treatment compared with WT, which is consistent with the gene expression patterns (Fig. 8). These results indicate that the homeostasis of IAA levels may be changed in dm4 and thus lead to the reduced plant height. Further studies are required to fully explore the roles and mechanisms of IAA in reduction of plant height in dm4. In contrast, only one gene related to auxin was identified as differentially expressed in dm1 vs WT (Table 1, Fig. 7A) and dm1 showed the same trends of IAA response as the WT (Fig. 8), suggesting the low possibility of IAA contributing to decreased plant height in dm1. In dm3, three genes related to auxin transporter and signal transduction had decreased expression (Table 1, Fig. 7C–D), indicating that auxin may be responsible for the reduction of plant height in dm3. It has been reported that cross-talk can occur between plant hormones and this interaction can affect plant height [28, 54, 55]. In this study, DELLAs (Table 1) involved in GA signaling [9] and shaggy-related protein kinase (Table 1, Fig. 7M) regulating BR signaling [56] showed lower expression in dm4. Thus, it is also possible that auxin interacts with GA and/or BR to affect plant height in dm4. Although indirect mechanisms of dwarfism in the mutant lines were identified in this study, the causal mutations affecting the dwarf phenotype would be different in each line and need to be further investigated using gene mapping.

CONCLUSION

Our study identified the hotspot regions of mutations across the chromosomes induced by γ-rays and EMS at the transcriptome level. In addition, the associated pathways and genes for dwarfing mechanisms were investigated in the four dwarf wheat mutant lines. Several genes encoding HSPs might be involved in the dwarfism responses in the dwarf mutants and the change of active IAA levels may be responsible for the reduced plant height in the dm4 mutant. This sequence data will be a valuable resource for dwarfing-associated gene mapping in the mutant lines.

SUPPLEMENTARY DATA

Supplementary data is available at RADRES Journal online.

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CONFICT OF INTEREST

The authors declare that there are no conflicts of interest.

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