Wheat gibberellin oxidase genes and their functions in regulating tillering

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Abstract

Multiple genetic factors control tillering, a key agronomy trait for wheat (*Triticum aestivum* L.) yield. Previously, we reported a *dwarf-monoculm* mutant (*dmc*) derived from wheat cultivar Guomai 301, and found that the contents of gibberellin acid 3 (GA_3) in the tiller primordia of *dmc* were significantly higher. Transcriptome analysis indicated that some wheat gibberellin oxidase (*TaGAox*) genes *TaGA20ox-A2, TaGA20ox-B2, TaGA3ox-A2, TaGA20ox-A4, TaGA2ox-A10* and *TaGA2ox-B10* were differentially expressed in *dmc*. Therefore, this study systematically analyzed the roles of gibberellin oxidase genes during wheat tillering. A total of 63 *TaGAox* genes were identified by whole genome analysis. The TaGAoxs were clustered to 4 subfamilies, GA20oxs, GA2oxs, GA3oxs and GA7oxs, including 7 subgroups based on their protein structures. The promoter regions of *TaGAox* genes contain a large number of *cis*-acting elements closely related to hormone, plant growth and development, light, and abiotic stress responses. Segmental duplication events played a major role in *TaGAoxs* expansion. Compared to *Arabidopsis*, the gene collinearity degrees of the *GAoxs* were significantly higher among wheat, rice and maize. *TaGAox* genes showed tissue-specific expression patterns. The expressions of TaGAox genes (*TaGA20ox-B2, TaGA7ox-A1, TaGA2ox10* and *TaGA3ox-A2*) were significantly affected by exogenous GA_3 applications, which also significantly promoted tillering of Guomai 301, but didn't promote *dmc*. *TaGA7ox-A1* overexpression transgenic wheat lines were obtained by *Agrobacterium* mediated transformation. Genomic PCR and first-generation sequencing demonstrated that the gene was integrated into the wheat genome. Association analysis of *TaGA7ox-A1* expression level and tiller number per plant demonstrated that the tillering capacities of some *TaGA7ox-A1* transgenic lines were increased. These data demonstrated that some *TaGAoxs* as well as GA signaling were involved in regulating wheat tillering, but the GA signaling pathway was disturbed in *dmc*. This study provided valuable clues for functional characterization of *GAox* genes in wheat.

Keywords Wheat (*Triticum aestivum* L.), Gibberellin oxidase (GAox), Tillering, Expression profiles, Gibberellin (GA), Gene transformation

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Introduction

Gibberellins (GAs) comprise a large hormone family that modifies many aspects of plant growth and development (*Spielmeyer et al., 2004*), including seed germination (*Yamaguchi et al., 2001*;

68 *Urbanova & Leubner-Metzger, 2016*), stem elongation (*Tian et al., 2022*), leaf expansion (*Xu et*
69 *al., 2016*), flowering and fruit development (*Yu et al., 2004*). Currently, more than 136 GAs
70 (*Macmillan & Takahashi, 1968*; *Hedden, 2020*) have been identified, and only GA_1, GA_3, GA_4
71 and GA_7 are recognized as major bioactive GAs in plants. Therefore, many non-bioactive GAs
72 exist in plants as precursors for the bioactive forms or deactivated metabolites (*Yamaguchi,*
73 *2008*; *Hedden & Phillips, 2000*).
74 For more than a century, the biosynthesis and metabolic pathways of GAs have been studied in
75 detail. GAs is biosynthesized from geranylgeranyl diphosphate (GGDP). Three different classes
76 of enzymes are required for the biosynthesis of bioactive GAs from GGDP in plants: terpene
77 synthases, cytochrome P450 monooxygenases, and 2-oxoglutarate-dependent dioxygenases
78 (2ODDs) (*Hedden & Thomas, 2012*). Among them, three types of GA oxidases (*GAoxs*) in
79 2ODD gene superfamily are the key enzymes in the synthesis and degradation of GAs (*Hu et al.,*
80 *2021*). They are mainly responsible for the mutual transformation of different GAs, and are
81 particularly important in regulating the level of bioactive GAs. GA20-oxidases (GA20oxs) and
82 GA3 Beta-hydroxylases (GA3oxs) are GA biosynthetic enzymes, they convert inactive GAs of
83 GA_{12} and GA_{53} into bioactive GAs of GA_1, GA_3, GA_4 and GA_7 (*Yamaguchi, 2008*). In addition,
84 it has been reported that GA7-oxidases (GA7oxs) are also involved in the biosynthesis of GAs.
85 In pumpkin (*Cucurbita maxima* L.) and cucumber (*Cucumis sativus* L.), GA7-oxidases converted
86 GA_{12}-aldehyde to GA_{12} efficiently (*Frisse et al., 2003; Lange et al., 2013*). GA2-oxidases
87 (GA2oxs) are recognized as GA deactivation enzymes, they convert GA precursors or bioactive
88 GAs into inactive GAs by 2β-hydroxylation (*Thomas et al., 1999*; *Kawai et al., 2014*). GA1-
89 oxidases (GA1oxs) are GA deactivation enzymes. In cucumber, GA1oxs convert GA_9 to GA_{61}
90 and GA_4 to GA_{88}, respectively (*Lange et al., 2020a)*. In wheat, GA1oxs convert GA_9 to GA_{61}
91 (*Pearce et al., 2015*).
92 So far, *GAox* genes have been identified in many plant species, including Arabidopsis
93 (*Arabidopsis thaliana* L.) (*Lange et al., 2020b*), maize (*Zea mays* L.) (*Ci et al., 2021*), rice
94 (*Oryza sativa* L.) (*He et al., 2022*), soybean (*Glycine max* L.) (*Han & Zhu., 2011*), jute
95 (*Corchorus capsularis* L.) (*Honi et al., 2020*), tea plant (*Camellia sinensis* L.) (*Pan et al., 2017*),
96 grape (*Vitis vinifera* L.) (*He et al., 2019*), and peach (*Prunus davidiana* L.) (*Cheng et al., 2021*).
97 The functions of many *GAox* genes have also been elucidated. *AtGA2ox9* contributes to freezing
98 tolerance and *AtGA2ox10* regulates seed production in Arabidopsis (*Lange et al., 2020b*). Studies
99 of *GA3ox* mutants suggested that bioactive GAs synthesized in the stamens and/or flower
100 receptacles were transported to petals to promote their growth in Arabidopsis (*Hu et al., 2008*).
101 The *semi-dwarf* gene, *sd1* of rice loses the function of *GA20ox-2*, leading to the *sd1* mutant

102 contained less GA levels than wild-type plants (*Ashikari et al., 2002*). *OsHox4* gene was

103 involved in GA metabolism, and controlled the expression of *OsGA2ox* and *OsGA3ox* family

104 genes. Overexpression of *OsHox4* gene caused bushy tillers (*Zhou et al., 2015*).

105 Tillering is an important trait of cereal crops that determines spike number, plant type structure,

106 thereby affected the final crop yield (*Wang et al., 2019*). Tillering is a very complex trait, except

107 the genetic factors and environmental factors, it can also be affected by plant hormones. Some

108 hormones such as IAA (*Cai et al., 2018*), ABA (*Liu & Hou, 2018*; *Lin et al., 2020*), SLs

109 (*Nakamura et al., 2013*) and CTK (*Yang et al., 2020*) are directly involved in regulating the tiller

110 bud growth. GAs play promotable and/or inhibitory roles during shoot branching and tillering in

111 different plant species. Several studies showed that GA treatment promoted axillary bud

112 development and shoot branching in woody plants, such as Jatropha curcas, papaya

113 (*Chaenomeles sinensis*), hybrid aspen and sweet cherry (*Prunus avium*) (*Elfving et al., 2011*; *Ni*

114 *et al., 2015a*; *Rinne et al., 2016*). Some studies showed that GA affected tiller bud growth in

115 annual herbaceous plants. In rice, the application of high concentration GAs could promote the

116 degradation of rice OsSHR1 or MOC1, leading to fewer tillers (*Lin et al., 2015*; *Lin et al., 2020*).

117 The inhibitory effects of GAs on tiller production were dose-dependent in tall fescue (*Festuca*

118 *arundinacea* L.) (*Zhuang et al., 2019*). In bahiagrass (*Paspalum notatum* Flugge),

119 overexpression of *AtGA2ox1* resulted in a significant reduction of the endogenous bioactive GA_1

120 contents, and increased the number of vegetative tillers (*Agharkar et al., 2007*). In tomato

121 (*Solanum lycopersicum* L.), C_{19} GA2oxs silencing led to higher contents of active GA_4 in

122 axillary buds and few branches (*Martínez-Bello et al., 2015*). Overexpressing *OsGA2oxs*

123 increased rice tiller number (*Lo et al., 2008*). In wheat, *Rht24* encodes TaGA2ox-A9, which

124 confers higher expression of *TaGA2ox-A9* in stems, leading to a reduction of bioactive GAs in

125 stems (*Tian et al., 2022*).

126 Previously, we have reported a *dwarf-monoculm* wheat mutant (*dmc*) derived from Guomai 301,

127 and found the content of GA_3 in the tiller primordia of *dmc* was significantly higher than that in

128 Guomai 301. Transcriptome analysis revealed that GA biosynthetic genes (*TaGA20ox-A2*,

129 *TaGA20ox-B2* and *TaGA3ox-B2*) were lowly expressed, one GAs biosynthetic genes

130 (*TaGA20ox-A4*) and two GAs catabolic genes (*TaGA2ox-A10* and *TaGA2ox-B10*) were highly

131 expressed in the tiller primordia of *dmc* (*He et al., 2018*; *An et al., 2019*). It indicates the

132 existence of a negative feedback mechanism that regulates *TaGAox* genes expression. This study

133 was to systematically analyze the roles of gibberellin oxidase genes played during wheat

134 tillering. We identified 63 *TaGAox* genes in wheat genome, and comprehensive analyzed their

135 gene and protein structures, and evolution. The expression profiles of *TaGAox* genes in tiller

136 primordia of Guomai 301 and *dmc* after exogenous GA₃ application were explored, and the

137 effect of exogenous GA₃ on wheat tillering was also evaluated. These results were reported here.

Materials & methods

Plant materials and growth conditions

Guomai 301(Wild type, WT) is a representative semi-winter wheat (Its vernalization requirement

is between winter wheat and spring wheat; Vernalization at 0~12℃ for 15~35 days) cultivar in

Henan, China. Mutant *dmc* was obtained from ethyl methyl sulfonate (EMS) treated Guomai 301

(*Ni et al., 2015b*). For GA₃ treatment, seeds with full grains and the same size were selected,

disinfected with 70% ethanol on the surface for 5 minutes, rinsed with distilled water, and placed

in a petri dish for germination at 27℃. After 48 h, the germinated seedlings were transplanted in

experimental field, Henan Agricultural University, Zhengzhou, Henan Province, China (34 51 N,

113 35 E, 95 m asl). Each treatment had 8 seedlings of Guomai 301 and 8 seedlings of *dmc*, they

were sown in the same plot, and each treatment had three replicates. For the hydroponics test, the

germinated WT and *dmc* seeds were transplanted in tanks with 1.5 L of 1/2 Hoagland nutrient

solution, and cultivated in an incubator. The seed germination conditions for genetic

transformation were the same as above.

Identification of TaGAoxs

Wheat genome data and protein data were downloaded from the Ensembl Plants database

(IWGSC refseqv1.1, http://plants.ensembl.org/index.html), and were used to identify all

members of wheat GAox family. The Hidden Markov Model (HMM) profile of 2OG-FeII_Oxy

(PF03171) and DIOX_N (PF14226) were downloaded from the Pfam database

(http://pfam.xfam.org). HMMER software (http://www.ebi.ac.uk/Tools/hmmer) was used to

search the *TaGAox* genes from wheat genome database. Meanwhile, the protein sequences of

Arabidopsis GAox family members, maize GAox family members and rice GAox family

members were downloaded from The Arabidopsis Information Resource (TAIR,

http://www.Arabidopsis.org), Maize Genetics and Genomics Database (Maize GDB,

http://www.maizegdb.org/gene_center/gene) and Rice Genome Annotation Project Database

(RGAP http://plantbiology.msu.edu), and these sequences were used as input sequences to

BLASP in the wheat protein database. The output putative GAox protein sequences were

confirmed by SMART (http://smart.embl-heidelberg.de) and Pfam searching for the presence of

the 2OG-FeII_Oxy and DIOX_N domains. All output protein sequences with e-value ≤ 1e-10

were collected, removing the redundant sequences. The longest transcript sequence

corresponding to each candidate gene was selected as the final sequence. Finally, obtained

TaGAoxs were named mainly referred in Pearce et al (*2015*).

170   The Expasy Prot Param tool (http://web.expasy.org/protparam) was used to predict the physical

171   and chemical properties of wheat GAox proteins, including amino acid length, molecular weight,

172   and theoretical isoelectric point and so on.

173   Construction of the phylogenetic tree

174   The multiple sequence alignment of wheat, Arabidopsis, maize and rice GAox protein amino

175   acid sequences were carried out using MEGA software (http://www.megasoftware.net). Based

176   on the sequence alignment results, the phylogenetic tree was constructed using Neighbor-joining

177   method in MEGA software, setting the bootstrap parameter to 1000 and using the default values

178   for other parameters.

179   Analyses of conserved motif distributions and gene structures

180   The online software MEME (http://meme-suite.org) was used to analyze conserved motifs for

181   each TaGAox protein sequences and the maximum number of motif finding was 10. The wheat

182   Generic Feature Format 3 (GFF3) file was downloaded from the wheat genome database and

183   used to elucidate the structure information of the *TaGAox* genes. Illustration depicting of protein

184   motifs, conserved domains and gene structures of TaGAox genes was constructed using the

185   TBtools software (*Chen et al., 2020*).

186   Analysis of the *cis*-acting elements

187   The 2,000 bp upstream sequences before transcription start positions of *TaGAox* genes were

188   extracted from the wheat genome sequence, and the *cis*-acting elements were predicted and

189   analyzed using the PlantCARE (http://bioinformatics.psb.ugent.be/webtools/plantcare/html/).

190   Gene duplication and synteny analysis of *TaGAoxs*

191   Multiple Collinearity Scan toolkit (MCScanX) was adopted to analyze the *TaGAox* gene

192   duplication events, with the default parameters (*Wang et al., 2012*). Tandem duplication events

193   were defined as chromosomal regions containing two or more genes within 200 kb (*Holub,*

194   *2001*). The synteny relationship of the orthologous *GAox* genes obtained from wheat,

195   Arabidopsis, maize and rice was analyzed, and the syntenic maps were visualized by TBtools

196   (http://github.com/CJ-Chen/TBtools). TBtools was used to calculate the synonymous rate (Ks)

197   and nonsynonymous rate (Ka) substitutions of each duplicated gene pairs and their ratios

198   (Ka/Ks) (*Hurst, 2002*).

199   Tissue specific expression analysis of *TaGAoxs*

200   The public wheat RNA-Seq datasets were downloaded from the Wheat Expression Browser

201   (http://www.wheat-expression.com). It was used to analyze the expressions of *TaGAox* family

202   genes in different tissues or organs (roots, stems, leaves, spikes and seeds) of Chinese Spring

203   (Table S1). The gene expression level was represented by transcripts per million (TPM). The

204   gene expression values present as $\log_2$-transformed normalized TPM values and visualized with

205   TBtools.

GA_3 treatment and RNA extraction

207   The seedlings at the three-leaf stage were treated with 2×10^{-4} mol/L GA_3 to analyze the

208   expression patterns of *TaGAox* genes. The tiller primordia of Guomai 301 and *dmc* at 0 h

209   (untreated control), 1 h and 2 h after GA_3 application were sampled respectively. The RNAs of

210   the samples were immediately extracted for gene expression analysis (*Zhang et al., 2021*).

qRT-PCR

212   qRT-PCR was performed as described previously (*Zhang et al., 2021*). The primers of *TaGAoxs*

213   were designed using primer-blast of NCBI (www.ncbi.nlm.nih.gov/tools/primer-blast). All the

214   primers were listed in Table S2. The *Actin* gene was used as an internal control. Each sample had

215   three biological replicates. The relative expressions of *TaGAoxs* were calculated by $2^{-\Delta\Delta Ct}$

216   methods (*Livak & Schmittgen, 2001*). All data were statistically analyzed. The values shown in

217   the form of means ± SD were obtained from three independent experiments (*Li et al., 2021*).

Evaluation of the effects of GA_3 on tillering

219   The wheat seedlings at the two-leaf stage were sprayed with distilled water and 2×10^{-4} mol/L

220   GA_3 solution respectively on the leaves until all the leaves were wet. Each seedling was sprayed

221   with 5 mL solution, and treated once every three days for a total of 10 times. The tiller numbers

222   were observed and counted once every seven days from the sixth application, when the tiller

223   number was obviously different among different treatments (*Li et al., 2021*).

Wheat transformation and expression analysis of *TaGA7ox-A1*

225   The CDS of *TaGA7ox-A1* was isolated from Chinese Spring. *TaGA7ox-A1* and plant expression

226   vector of pCAMBIA1304 were digested with *Nco* I and *Spe* I . An expression vector

227   pCAMBIA1304-CaMV35S:TaGA7ox-A1 was constructed by inserting *TaGA7ox-A1* under of

228   pCAMBIA1304-CaMV35S promoter, and transformed into *Agrobacterium* GV3101.

229   pCAMBIA1304-CaMV35S:TaGA7ox-A1 was introduced into Guomai 301 by *Agrobacterium*

230   mediated genetic transformation. When the seedlings of Guomai 301 grew to 0.5-1cm, they were

231   infected by *Agrobacterium* containing the expression vector. Agrobacterium infection solution

232   was diluted to OD_{600}=0.6 with 100 μmol/L of acetyl syringone. The transformed plants were

233   transferred to a sterilized petri dish and cultured in a dark environment for 48 hours. After 30

234   days of culture at 4°C for vernalization, they were transplanted into an artificial climate

235   incubator (*Dong et al., 2018*; *Yang et al., 2021*; *Cao et al., 2021*).

236   Specific primers F2/R2 were designed according to the sequences of the vector and the target

237   gene (*TaGA7ox-A1*), and the positive TaGA7ox-A1-OE transgenic lines were detected by

238 genomic PCR. The leaf DNAs of Guomai 301 and the transgenic plants were extracted at two-
239 leaf stage and tillering stage. The amplified products were sequenced to demonstrate whether
240 *TaGA7ox-A1* gene was successfully transferred into Guomai 301. Under natural tillering
241 conditions, tiller numbers of the transgenic and wild-type lines were observed and recorded
242 every seven days. The tiller primordia RNAs of Guomai 301 and the transgenic plants were
243 extracted at tillering stage for transgene expression analysis. The primers used in gene isolation,
244 transgenic plant verification and qRT-PCR were listed in Table S2.

245 **Results**

246 **Identification of TaGAoxs**

247 A total of 64 candidate wheat gibberellin oxidase (TaGAox) proteins were identified by
248 HMMER and BLASTP in wheat Genome. The conserved motif search showed that one of the 64
249 proteins lacked the DIOX_N domain (TraesCS7D02G046300.1), the other 63 proteins were
250 typical TaGAoxs family members (Table S3). The 63 TaGAox proteins included 13 TaGA20oxs,
251 3 TaGA7oxs, 6 TaGA3oxs, 40 TaGA2oxs and 1 TaGA1ox. The genes in the three *TaGAox*
252 families were unevenly distributed on wheat chromosomes (Table S3).

253 The lengths of the amino acid sequences of TaGAoxs ranged from 305 aa (TaGA2ox-A5) to 442
254 aa (TaGA20ox-A2), with an average of 361.35 aa. The molecular weights of the TaGAoxs
255 ranged from 33.30 KDa to 48.13 KDa, with an average of 39.23 KDa. The TaGA20ox-A2 was
256 the heaviest and TaGA2ox-A5 was the lightest. The predicted values of the isoelectric points of
257 TaGAoxs ranged from 4.92 to 9.19. Among them, 14 TaGAoxs with theoretical pI greater than 7
258 were slightly alkaline, while the remaining TaGAoxs with theoretical pI less than 7 were slightly
259 acidic. It was speculated that most of them were acidic proteins. Their aliphatic index ranged
260 from 72.13 to 91.27, with an average of 80.97. For grand average of hydropathicity (GRAVY),
261 TaGA2ox-A16 and TaGA2ox-D16 were 2 positive negative hydrophobic proteins, the other 61
262 were hydrophilic proteins (Table S3).

263 **Phylogenetic tree of wheat GAox proteins**

264 To investigate the phylogenetic relationships of TaGAox proteins, the phylogenetic tree was
265 constructed using 127 GA oxidases of wheat, Arabidopsis, maize and rice (Fig. 1). Similar to
266 previous report (*Ci et al., 2021*), the GAox proteins were clustered to 7 distinct subgroups: C_{19}-
267 GA2ox (Ⅰ, Ⅱ), C_{20}-GA2ox (Ⅲ, Ⅳ), GA7ox (V), GA20ox (VI), and GA3ox (VII). They
268 contain 19, 6, 12, 3, 3, 13 and 6 TaGAox proteins respectively. According to previous reports,
269 TaGA1ox-B1 did not fit well into GA3ox subfamily, and TaGA1ox-B1 was shown to encode a
270 GA1-oxidase (*Pearce et al., 2015*). The evolutionary relationship showed that the distribution of
271 GA20oxs, GA3oxs and GA2oxs in each species was similar. There was no Arabidopsis GA7ox

272   protein in GA7ox (V) subgroup. Most of TaGAox family members were clustered with maize

273   and rice GAox family members. In summary, wheat GAox proteins were more similar to those

274   of maize and rice than those of Arabidopsis.

Structures and conserved motifs of TaGAox genes

276   A phylogenetic tree of TaGAoxs was constructed using the 63 TaGAox protein sequences (Fig.

277   2A). The clusters of TaGAoxs were basically consistent with those in Fig. 1. To characterize the

278   structures of TaGAox proteins, 10 motifs were identified using the MEME motif search tool

279   (Table S4). The motif compositions and arrangements of the TaGAoxs in the 7 subgroups were

280   similar. Among them, motifs 1, 2, 3 and 7 were discovered in all of the TaGAox proteins. Motifs

281   3, 5 and 8 belonged to the DIOX_N domain, and motifs 1, 2, 4 and 6 belonged to the 2OG_FeII

282   oxygenase domain (Fig. 2B). Motif 5 was unique to TaGA2ox subfamily. Motif 8 was found in

283   GA3ox (VII) and GA20ox (VI) subgroups, and TaGA2ox-D11. Except for TaGA2ox-A5, motif

284   10 was found in five subgroups of C_{19}-GA2ox (I , II), C_{20}-GA2ox (III , IV) and GA20ox (VI).

285   A more interesting thing was that motif 10 was located in N-terminal of C_{19}-GA2ox (I , II)

286   subgroups and in the C-terminal of C_{20}-GA2ox (III , IV) subgroups. The exon-intron structure

287   diagram of *TaGAoxs* showed that their genomic DNA sequence lengths were significantly

288   different (Fig. 2C). The longest was *TaGA2ox-B2*, its length was about 7,500 bp, and the shortest

289   was about 1,500 bp. The exon number of *TaGAoxs* was 1-5, most genes contained 2 or 3 exons.

290   *TaGA2ox5* only had one exon, *TaGA3ox-D1* had 5 exons. As shown in the Fig. 2C, the

291   homoeologous genes had more similar structures, such as *TaGA3ox2* had 3 exons, which had

292   little difference in structure, length and distribution and might have the same functions.

***Cis*-acting elements in the promoters of TaGAoxs**

294   To gain a deeper understanding of the potential functions of the TaGAox family genes, we

295   analyzed the *cis*-acting elements in the promoter regions of 51 *TaGAox* genes (the promoter

296   sequences of the other 12 *TaGAox* genes contained a large number of 'N', so they hadn't been

297   analyzed) (Fig. 3). The results showed that there were a large number of *cis*-acting elements

298   related to growth and development, abiotic stress and hormones in the promoter regions of the

299   *TaGAox* genes. Among them, many *cis*-acting elements were involved in auxin response (TGA-

300   element, AuxRR-core); abscisic acid response (ABRE), gibberellic response (P-box, GARE-

301   motif, TATC-box), methyl jasmonate response (CGTCA-motif, TGACG-motif), salicylic acid

302   response (TCA-element, SARE), light response (G-Box, Box 4, Sp1), and those growth-related

303   *cis*-elements (CAT-box, GCN4_motif, O2-site, circadian, RY-element, CCAAT-box) and abiotic

304   stress related elements (ARE, GC-motif, LTR, MBS, TC-rich repeats). The large numbers of

305   elements were those of light-responsive elements, jasmonic acid-responsive elements, and ABA-

306 responsive elements. Although all *TaGAox* genes encoding gibberellin oxidase, some of them did
307 not contain gibberellin response elements, such as, *TaGAox-D2*, *TaGA2ox-A1* and *TaGA3ox-D1*.
308 The existence of various *cis*-acting elements in the gene promoter regions suggested that
309 *TaGAoxs* played important roles in regulating wheat growth and development, stress response
310 and hormone response.

311 **Synteny relationships of *TaGAoxs***

312 During plant evolution, whole-genome duplications, transpositions, tandem gene duplications
313 and segmental duplications play important roles in gene expansion and generation (*Hu et al.,*
314 *2021*). In order to discover the gene duplication events of *TaGAoxs*, the 63 *TaGAox*s were
315 investigated. A total of 74 duplicated gene pairs were discovered, including 72 segmentally
316 duplicated gene pairs, they distributed on different chromosomes (Fig. 4, Table S5). According
317 to the methodology of Holub (*Holub, 2001*), there were 2 pairs of tandem duplication genes
318 (Table S5). These results indicated that the expansions of *TaGAox* genes were mainly segmental
319 duplications or tandem duplications, and the segmental duplication events played major roles in
320 *TaGAox* genes evolution. To better determine the selective evolutionary pressure on *TaGAox*
321 gene divergence, we calculated the ka/ks ratios of all the syntenic gene pairs. Except for the
322 ka/ks ratios of *TaGA2ox4-1A* and *TaGA2ox4-1B-1* were 1.037, indicating that they had
323 undergone neutral evolution, the ka/ks ratios of the other 73 pairs of replicated genes were less
324 than 1 (Table S6), indicating that the *TaGAox* genes mainly underwent purifying selection.
325 In order to further infer the evolutionary relationships of the *GAox* genes, the comparative
326 syntenic maps associated with wheat genome were constructed with other species, including
327 Arabidopsis, rice, and maize. No syntenic gene of wheat *GAox* genes was found in Arabidopsis
328 (Fig. 5A). A total of 30 *TaGAox* genes had syntenic genes in rice (Fig. 5B) and 34 *TaGAox* genes
329 had syntenic genes in maize (Fig. 5C). The number of the orthologous gene pairs between wheat
330 and rice, wheat and maize were 43 and 47, respectively. The ka/ks ratios of these gene pairs
331 (Table S7) were calculated. All *GAox* gene pairs had ka/ks < 1, suggesting that *TaGAox* genes
332 had undergone a strong purification selection pressure.

333 **The expression patterns of *TaGAoxs* in different tissues**

334 To gain insight into the putative functions of *TaGAox* genes, the expression profiles of *TaGAoxs*
335 were analyzed using public RNA-Seq data from different organs/tissues of wheat (Fig. 6A, Table
336 S1). Because it expressed at relative high levels in all tested tissues, *TaGAox1* might play an
337 important role during wheat development. *TaGA2ox4*, *TaGA2ox3* and *TaGA3ox2* were highly
338 expressed in stem. *TaGA20ox2* and *TaGA2ox4* were highly expressed in spikes. Most of *TaGAox*
339 genes were lowly expressed or not detected in the 5 tissues, implying functional redundancy of

340 the *TaGAox* genes. The above results indicated that different *TaGAox* genes may be involved in

341 different growth and development processes of wheat. The expressions of some genes were

342 tissue-specific, which would contribute to different morphogenesis in plant development.

343 Based on our published transcriptome data (*He et al., 2018*), the expression profiles of all the

344 *TaGAox* genes in Guomai 301 and *dmc* were further analyzed (Fig. 6B, Table S8). The

345 expression profiles of *TaGAoxs* in tiller primordia showed that most genes expressed very lowly

346 in all detected samples, these genes probably were not necessary for wheat tiller development.

347 *TaGA3ox-B2* and *TaGA3ox-D2* were highly expressed in Guomai 301 and *dmc*. High expression

348 levels suggested they played important roles during tiller development. Compared to WT,

349 *TaGA20ox-A4*, *TaGA2ox-A10* and *TaGA2ox-B10* were highly expressed, and *TaGA20ox-A2*,

350 *TaGA20ox-B2* and *TaGA3ox-A2* were lowly expressed in *dmc*. Differential expression of these

351 genes might be one of the main causes containing tillering of *dmc*.

352 **Expression patterns of *TaGAoxs* in response to GA$_3$ application**

353 In order to explore whether the expressions of *TaGAox* genes in wheat tiller primordia could be

354 activated by GA$_3$. Some *TaGAox* genes were selected to test according to their expression levels

355 in *dmc* (Fig. 6B). It was found that GA$_3$ significantly affected the expressions of *TaGAoxs* (Fig.

356 7). In *dmc*, the expressions of *TaGA2ox* genes were highest at 1 h after GA$_3$ treatment. The

357 expressions of *TaGA3ox-B2* and *TaGA3ox-D2* continuously decreased and those of *TaGA7ox-A1*

358 and *TaGA7ox-D*1 continuously increased. The expressions of *TaGA20ox-B2* and *TaGA3ox-A2*

359 had no significant changes. The expressions of *TaGA20ox-A4* and *TaGA20ox-A1* were down-

360 regulated at 2 h after GA$_3$ treatment. The expression profiles of *TaGAoxs* in WT were different

361 from those of *dmc*. The expressions of 6 *TaGAoxs* had no significant changes after GA$_3$

362 treatment. The expressions of *TaGA2ox-A1* and *TaGA20ox-B2* decreased at 1 h after GA$_3$

363 treatment, and recovered after 2 h. In both WT and *dmc*, *TaGA7ox-D1*, *TaGA2ox10*, and

364 T*aGA2ox-A16* were very sensitive to GA$_3$ stimulation, the reason was considered as there were

365 *cis*-acting elements of gibberellin response in their promoter regions. Although the gibberellin

366 *cis*-acting elements of the *TaGAoxs* maybe the same, most expression profiles of *TaGAoxs* in

367 WT and *dmc* were significantly different.

368 In summary, after GA$_3$ application, the expressions of GAs catabolic genes and *GA7ox* genes

369 were up-regulated, the expressions of GA biosynthetic genes were down-regulated.

370 **Effects of GA$_3$ on tiller formation of wheat**

371 After the sixth time of GA$_3$ application, the tiller number of Guomai 301 began to appear

372 differences. Compared to the control, continuous GA$_3$ treatment significantly increased the tiller

373 number of Guomai 301, but didn't affect tillering of *dmc* (Fig. 8). The results indicated that the

374   exogenous GA_3 could significantly promote tiller development of Guomai 301, but the *dmc*

375   lacked response to GA_3.

376   **Function of *TaGA7ox-A1* in regulating wheat tillering**

377   Overexpression transgenic plants of *TaGA7ox-A1* were obtained (Table S9). The positive

378   transgenic plants were confirmed by PCR at early tillering stage and the amplified target

379   fragments were sequenced at the late tillering stage (Fig. S1). These results indicated that the

380   *TaGA7ox-A1* was transferred into the positive transgenic plants (Fig. S2). Statistical analysis

381   indicated that the average tiller numbers of *TaGA7ox-A1*-OE transgenic plants were significantly

382   higher than those of WT from the TN4 stage. At the TN6 stage, the tiller number of wild-type

383   plants was 6-7, while the tiller number of transgenic plants was 9-10 (Fig. 9A, Table S9).

384   Compared to WT, the *TaGA7ox-A1* gene relative expression levels and the tiller numbers of the

385   transgenic plants increased significantly (Fig. 9A, 9B, Table S9). The wheat tiller number was

386   significantly correlated with *TaGA7ox-A1* gene expression level ($r = 0.613$, $p < 0.05$) (Table S9).

387   This result indicated that *TaGA7ox-A1* was involved in regulating wheat tillering.

388   **Discussion**

389   **Characteristics and evolution of wheat *GAox* gene family members**

390   Gibberellins are important hormones in plants, and act during the whole life cycle of plants

391   (*Hernandez-Garcia et al., 2021*). In model plant Arabidopsis, the signaling pathways related to

392   GAs have been well elucidated, and gibberellin oxidases are involved in the last step of GA

393   biosynthesis pathway (*Yamaguchi, 2006*). However, the specific functions of the most *GAox*

394   genes are largely remained unknown. Genome-wide predictions of *GAox* genes have become

395   possible when many plants genomic sequences have been reported. In this study, we analyzed

396   the structure, phylogenetic relationships, chromosomal locations, gene duplication events, *cis*-

397   elements, and expression patterns of *GAox* genes in wheat.

398   A total of 63 TaGAox proteins with typical conserved domains, 2OG-FeII_Oxy and DIOX_N,

399   had been identified in this study. Similar to other plant GAox proteins in Arabidopsis, rice, and

400   soybean (*Han & Zhu, 2011*), the TaGAox proteins belonged to the 2OG-Fe (II) oxygenase

401   superfamily. In this study, 13 TaGA20oxs, 40 TaGA2oxs, 6 TaGA3oxs, 3 TaGA7oxs and 1

402   TaGA1ox were identified, respectively. The preliminarily identified *TaGAox* genes included 10

403   *TaGA20oxs*, 29 *TaGA2oxs*, 6 *TaGA3oxs* and 1 *TaGA1oxs*, and they were proved to have the

404   corresponding oxidase activities (*Pearce et al., 2015*). Kumagai et al (*2022*) identified 13

405   *TaGA20oxs*, this result was consistent with that of this study. Previous research identified the

406   full-length genes of *TaGA20ox2* and *TaGA20ox3* in B and D genomes, but only partial

407   sequences in A genome. In this study, the newly identified *TaGA20ox-A2* and *TaGA20ox-A3*

408   were their homologous genes in A genome. The sequences of *TaGA20ox-D3-2* and *TaGA20ox-*

409   *D3-1* were highly similar, and they were homologous genes. A study indicates OsGA20ox5 and

410   OsGA20ox8 were clustered into GA7ox subfamily with CsGA7ox1 and CsGA7ox2 (*Huang et*

411   *al. 2015*; *Kawai et al., 2014*; *Sun et al., 2018*). In this study, 3 TaGA7oxs and OsGA20ox5 and

412   OsGA20ox8 are divided into subgroup V. So far, GA7ox activity was reported in pumpkin and

413   cucumber, but has not been found in other species (*Frisse et al., 2003*; *Lange et al., 2013*).

414   Therefore, whether the 3 *TaGA7ox*s have GA7ox activity needs to be further verified.

415   Compared to previous studies (*Pearce et al., 2015*), 11 *TaGA2ox* genes were newly identified in

416   this study. Among them, *TaGA2ox-A2*, *TaGA2ox-A7*, *TaGA2ox-A8* and *TaGA2ox-D8* were

417   homologous genes of *TaGA2ox2*, *TaGA2ox7* and *TaGA2ox8*. Their homologous genes were used

418   to prove that they have reduced substrate specificity for C_{19}-GAs, as it effectively converted

419   GA_{12} to GA_{110} as well as GA_9 to GA_{51} (*Pearce et al., 2015*). It is speculated that these four

420   newly identified *TaGA2ox* genes also have similar activities. In addition, TaGA2ox5 and

421   AtGA2ox9-10 were divided into subgroup IV (C_{20}-GA2ox) (*Lange et al., 2020b*). *TaGA2ox15*

422   and *TaGA2ox16* and *TaGA2ox8* were paralogous genes. Whether these newly identified *TaGAox*

423   genes have the activity of GA2-oxidases oxidase needs further study.

424   Some reports indicated that the enzyme specificities of a few GAoxs were different from that of

425   predicted by amino acid sequences, however, the enzyme activities were yet gibberellin oxidases

426   (*Frisse et al., 2003*; *Sun et al., 2018*; *Lange et al., 2020b*; *Lange & Lange, 2020*). One of the

427   reasons may be that some key nucleotide variations of a gene lead to amino acid sequence

428   variations, subsequently the three-dimensional protein structure variations, which results in the

429   changes of substrate specificity. Although it's not completely accurate to functional predict the

430   substrate specificities of gibberellin oxidases only by amino acid sequences, it's the major

431   methodology for studies of plant gene families, and are widely used in most studies (*He et al.,*

432   *2019*; *Ci et al., 2021*; *Du et al., 2022*). Therefore, the classification of the *TaGAox* genes is

433   reliable, though their substrate specificities need further research.

434   The 63 *TaGAox* genes were unevenly distributed on 21 wheat chromosomes. There were 2 pair

435   tandem repeat genes and 72 pairs segmental duplications, which indicated that the gene

436   duplications played an important role in the amplification of *TaGAox* genes (*Xu et al., 2020*), and

437   there were functional redundancy among these genes (*Panchy et al., 2016*). Phylogenetic tree

438   analysis divided the TaGAox proteins into 7 distinct subgroups (Fig. 1), which suggested the

439   similarities of the gene structures and functions. Most of the TaGAox proteins clustered together

440   with or close to OsGAoxs and ZmGAoxs and far away from AtGAoxs. This means that wheat,

441   rice and maize are close relatives. There were no Arabidopsis GAox proteins in GA7ox (V)

442 subgroup (Fig. 1). Apparently, some *TaGAox* genes evolved independently after differentiation

443 of monocots and dicots. Gene structure analysis discovered that most *TaGAox* genes in the same

444 subfamily had similar exon/intron structures, most of the genes contained 2 or 3 exons, and the

445 result was similar to GAox genes in other species, such as *Setaria italica*, *Sorghum bicolor*,

446 *Hordeum vulgare*, *Brachypodium distachyon*, maize, and rice (*Zhang et al., 2022*; *Ci et al.,*

447 *2021*). Obviously, the structures of *GAox* genes in the same subgroup are conserved, which

448 imply their similar biological functions.

449 The last step of bioactive GAs synthesis is catalyzed by GA20oxs and GA3oxs to convert GA_{12}

450 and GA_{53} into active GAs (*Yamaguchi, 2008*), degradation of active C_{19}-GAs and C_{20}-GAs by

451 GA2oxs through 2β-hydroxylation yields inactive GA products (*Schomburg et al., 2003; Otani*

452 *et al., 2010*). Most common motifs of TaGAox proteins were shared by the 7 GAox subgroups.

453 However, some motifs of TaGAox proteins had been lost or added in the domains of 2OG-FeII

454 oxygenase and DIOX_N, which might lead to gene functional changes (Fig. 2). For example,

455 motif 4 were absent in TaGA7ox-D2, motif 5 was found in GA2ox. The motif 10 can distinguish

456 the C_{19} GA2ox and C_{20} GA2ox subgroups. It was found that the conserved sequence LPWKET

457 of GA20ox was located in motif 8 (Table S4) (*Huang et al., 2015*; *Pan et al., 2017*). Compared

458 with TaGA20ox (VI), TaGA7ox (V) lacks motif 8, suggesting that it may have different oxidase

459 activities. In summary, some unique motifs existed only in specific families. To better

460 understand the functions of TaGAoxs, the biological functions of these special motifs need to be

461 characterized further.

462 **Signal transduction pathway of GAs is disturbed in *dmc***

463 GA signal transduction is a series of responses induced by cells after GA stimulation. Among

464 them, GA-GID1-DELLA tricomplex plays an important role in the induction of plant growth and

465 germination (*Jiang & Fu, 2007*). GA signal is perceived by GA receptor GIBBERELLIN

466 INSENSITIVE DWARF1 (GID1), and regulate gene expression by promoting degradation of the

467 transcriptional regulator DELLA proteins (*Murase et al., 2008*). In plants, when GA levels

468 decreased, GA did not bind to GID1, so that DELLA gene inhibited the expression of GA

469 responsive genes, thereby limited plant growth. When the GA level increased, GA and GID1

470 were combined to further GA-GID1-DELLA tricomplex. DELLA protein was hydrolyzed by

471 ubiquitin-proteasome. The degradation of DELLA relieved the inhibition of GA response genes,

472 and the plants showed normal GA response. DELLA can also induce the expression of upstream

473 GA biosynthesis genes and GA receptors by feedback regulation (*Wang & Deng, 2014*). The

474 *Rht1* gene is a gain-of-function allele caused by an N-terminal truncation near the DELLA

475 domain and *Rht1* plants produce much more productive tillers (*Kertesz et al., 1991*; *Velu et al.,*

476   *2017*). Our studies had shown that DELLA and *PIF3* were involved in GA signaling pathway

477   and were highly expressed (Fig. S3). E3 ubiquitin-protein ligase were also lowly expressed in

478   *dmc* (*He et al., 2018*; *Li et al., 2016*). It is speculated that the decrease of sensitivity to GAs is

479   one of the factors constraining tillering in *dmc*.

480   **Exogenous GA$_3$ affects the expressions of *TaGAox* genes**

481   Studies have shown that GA is one of the main hormones regulating elongating stems (*Peng et*

482   *al., 1999*; *Schomburg et al., 2003*; *Yamaguchi, 2008*). The *semidwarf* rice *sd1* is caused by the

483   loss of function of the *OsGA20ox2* gene. The precursor GA$_{53}$ accumulates in the stems of *sd1*,

484   the content of GA$_1$ in *sd1* is lower than that in tall lines (*Spielmyer et al., 2002*). This study found

485   that the expression levels of *TaGA20ox-A2* and *TaGA20ox-B2* in *dmc* were lower than that in

486   Guomai 301 (Fig. 6, 7). Therefore, we speculated that homologous genes of *TaGA20ox2* might

487   be related to dwarfing of *dmc*. Plants can regulate GA balance by regulating expressions of *GAox*

488   genes. In some crops, exogenous GA reduces the expressions of *GA20oxs* and *GA3oxs*, and

489   increases the expressions of *GA2oxs* (*He et al., 2019*; *Ci et al., 2021*). The transcriptional

490   analysis showed that the low expression levels of *TaGA20ox-A2, TaGA20ox-B2*, *TaGA2ox-D7*

491   and *TaGA3ox-A2*, and the high expression levels of *TaGA20ox-A4 TaGA2ox-10A* and *TaGA2ox-*

492   *10B* might be the feedback regulation of GA$_3$ (Fig. 6B). qRT-PCR showed that GA$_3$ treatment

493   affected the expression of *TaGAoxs*. In *dmc*, *TaGA2oxs* were up-regulated after GA treatment,

494   and GA2ox played a role in maintaining GA balance in GA synthesis and metabolism in plants

495   (*Thomas et al., 1999*). *TaGA3oxs* were basically down-regulated by exogenous GA$_3$, while

496   *TaGA20oxs* showed different trends. Among them, *TaGA7ox-D1, TaGA2ox10, TaGA2ox-A16*

497   were most significantly regulated by GA$_3$ (Fig. 7). *TaGA7ox-D1* was highly expressed after GA$_3$

498   treatment, but no GA responsive element was detected within its promoter. It may be that there

499   are GA responsive elements outside its 2000 bp promoter region, or there are other regulatory

500   modes to promote the expression of *TaGA7ox-D1*. In summary, *TaGA20ox-A2*, *TaGA20ox-B2*,

501   *TaGA7ox1, TaGA2ox10, TaGA2ox-A16* and *TaGA3ox-A2* may play a major role in regulating the

502   level of bioactive GAs. But how do they regulate wheat tillering needs further research.

503   **GA$_3$ can promote wheat tillering**

504   Studies indicate that GAs has a certain effect on plant tillering or branching. GAs negatively

505   regulates tiller-related genes *OsH1* and *TB1* in rice, thus regulating the occurrence of tiller (*Lo et*

506   *al., 2008*). In the tall fescue, GAs may inhibit tiller development by expressing *FaTB1* in axillary

507   buds (*Zhuang et al., 2019*). It is demonstrated that gibberellin synthesis inhibitor paclobutrazol

508   (PBZ) can promote wheat tiller formation (*Assuero et al., 2012*). These results suggest that high

509   levels of active GAs in plants inhibit branching or tillering. GA$_3$ can inhibit the growth of tiller

510 buds by controlling the content of IAA or cytokinin (CTK) in plants (*Liu et al., 2011*). Studies

511 have shown that GA$_3$ application can inhibit the growth of wheat tiller when wheat tiller buds

512 begin to elongate (*Cai et al., 2013*). However, some researches have demonstrated that

513 application of different concentrations of GA$_3$ can increase the number of wheat tillers (*Islam &*

514 *Mehraj, 2014*). Similarly, GA$_3$ can stimulate tiller development in palmarosa (*Cymbopogon*

515 *martinii*) (*Khan et al., 2015*). In this study, continuous application of GA$_3$ from the second leaf

516 stage could promote tillering of Guomai 301, but it had no obvious effect on tillering of *dmc*

517 (Fig. 8). It was speculated that wheat at different stages had different responses to GAs. Our

518 previous studies showed that the contents of GAs were significantly higher, and the contents of

519 IAA were significantly lower in the tiller buds of *dmc* (*An et al., 2019*). These data demonstrated

520 that GA signaling was involved in regulating wheat tillering, but the GA signaling pathway was

521 disturbed in *dmc*.

522 ### *TaGA7ox-A1* can promote wheat tillering

523 The plant overexpression vector of pCAMBIA1304-CaMV35S:TaGA7ox-A1 was constructed

524 and transferred into *Arabidopsis*. The primary functional analysis showed that overexpression of

525 *TaGA7ox-A1* could significantly increase the branch numbers of the transgenic Arabidopsis

526 plants (Fig. S4; unpublished data). Another study found that overexpression of *OsGA20ox2*

527 promoted plant height and tiller number (*Qiao et al., 2013*). In switchgrass (*Panicum virgatum*

528 L.), overexpression of *ZmGA20ox* promoted tiller number (*Do et al., 2016*). In this study,

529 overexpression of *TaGA7ox-A1* significantly increased the tiller numbers of the transgenic wheat

530 plants (Fig. 9, S2). The tiller number was positive correlated with the expression level of

531 *TaGA7ox-A1* ($r = 0.613$, $P < 0.05$). This result demonstrated that *TaGA7ox-A1* could promote

532 wheat tillering. However, the accurate molecular mechanism needs further study.

533 ## Conclusions

534 A total of 63 *TaGAox* genes distributed on 21 wheat chromosomes were identified. *TaGAox*

535 genes belong to seven subgroups. The promoter regions of *TaGAoxs* contained a large number of

536 *cis*-acting elements related to plant growth, hormone signaling pathway and stress response.

537 Segmental duplication played a major role in *TaGAoxs* amplification. The *TaGAox* genes are

538 tissue-specifically expressed. Genes of *TaGA7ox-A1, TaGA20ox-A1, TaGA20ox-B1, TaGA2ox4*

539 and *TaGA3ox-2* played basic roles during wheat tillering. The abnormal expressions of

540 *TaGA20ox-A2, TaGA20ox-B2, TaGA3ox-A2, TaGA2ox10* and *TaGA20ox-A4* were involved in

541 the synthesis and metabolism of GAs in *dmc* tiller primordia, thereby affected tiller formation.

542 The expressions of *TaGAoxs* were significantly affected by exogenous GA$_3$. Exogenous GA$_3$

543 significantly promoted tillering of Guomai 301, but the GA pathways were disturbed in *dmc*.

544 Overexpression *TaGA7ox-A1* promoted the transgenic wheat tillering.

545

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Figure 1

The phylogenetic tree of the GAoxs in *T. aestivum* L. (Ta), *A. thaliana* (At), *Z. mays* (Zm) and *O. sativa* (Os).

Figure 2

Phylogenetic relationships, conserved protein motif patterns and gene structures of TaGAoxs.

(A) The phylogenetic tree of TaGAox proteins. Clusters are indicated with different colors. (B) The motif compositions of TaGAoxs. The 1-10 motifs are displayed in different colored boxes, the scale at the bottom indicates the length of the amino acid sequences. The green dotted boxes represent DIOX_N domains and the brown dotted boxes represent 2OG_FeII oxygenase domains. (C) Exon-intron structures of *TaGAoxs*. Gray boxes indicate 5 '- and 3'-untranslated regions; yellow boxes indicate exons; black lines indicate introns.
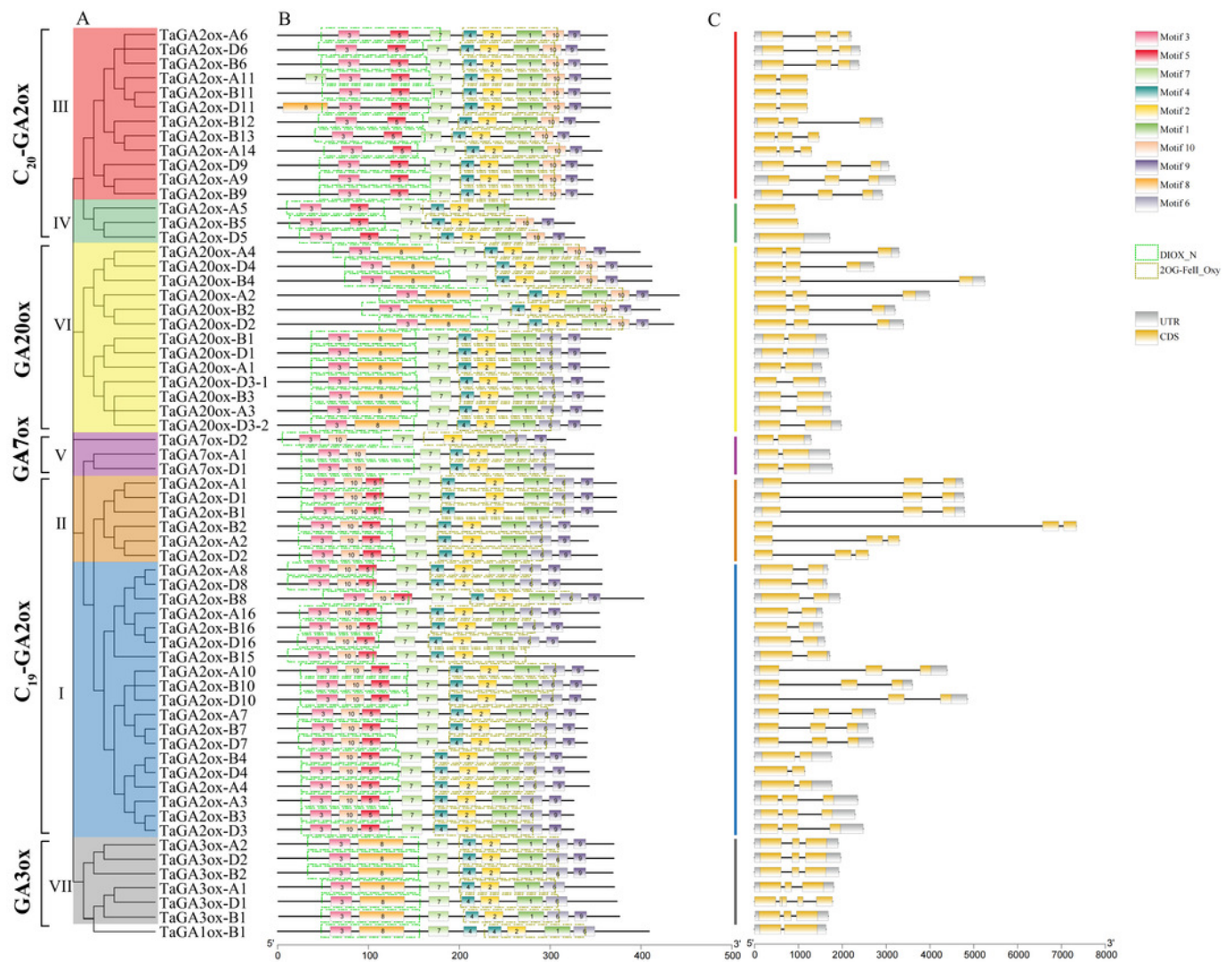
Figure 3

The *cis*-acting elements in the promoters of *TaGAoxs*. The shade of the blue represents the quantity.
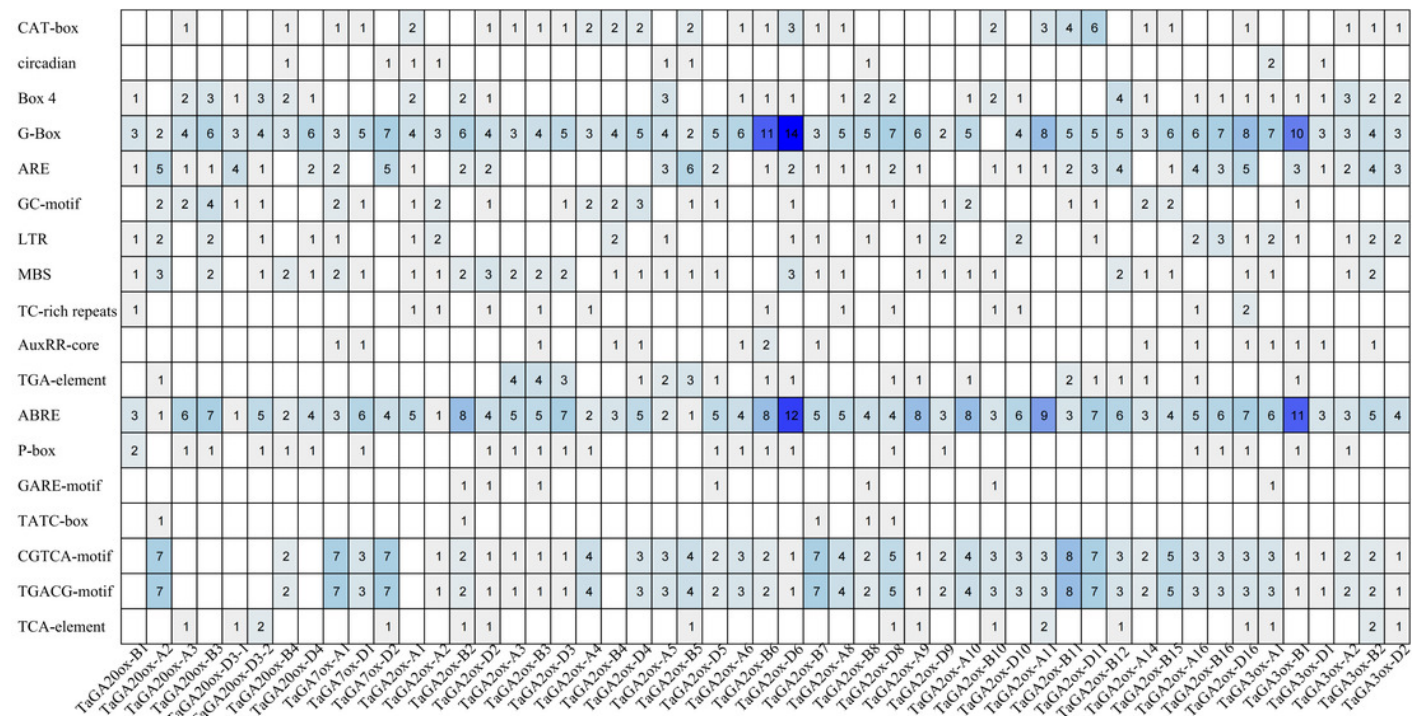
Figure 4

Schematic diagram of the chromosome distribution and inter chromosome relationships of *TaGAoxs*.

The gray lines indicate all duplicated gene pairs in wheat, the highlighted blue lines indicate probably duplicated *TaGAox* gene pairs.
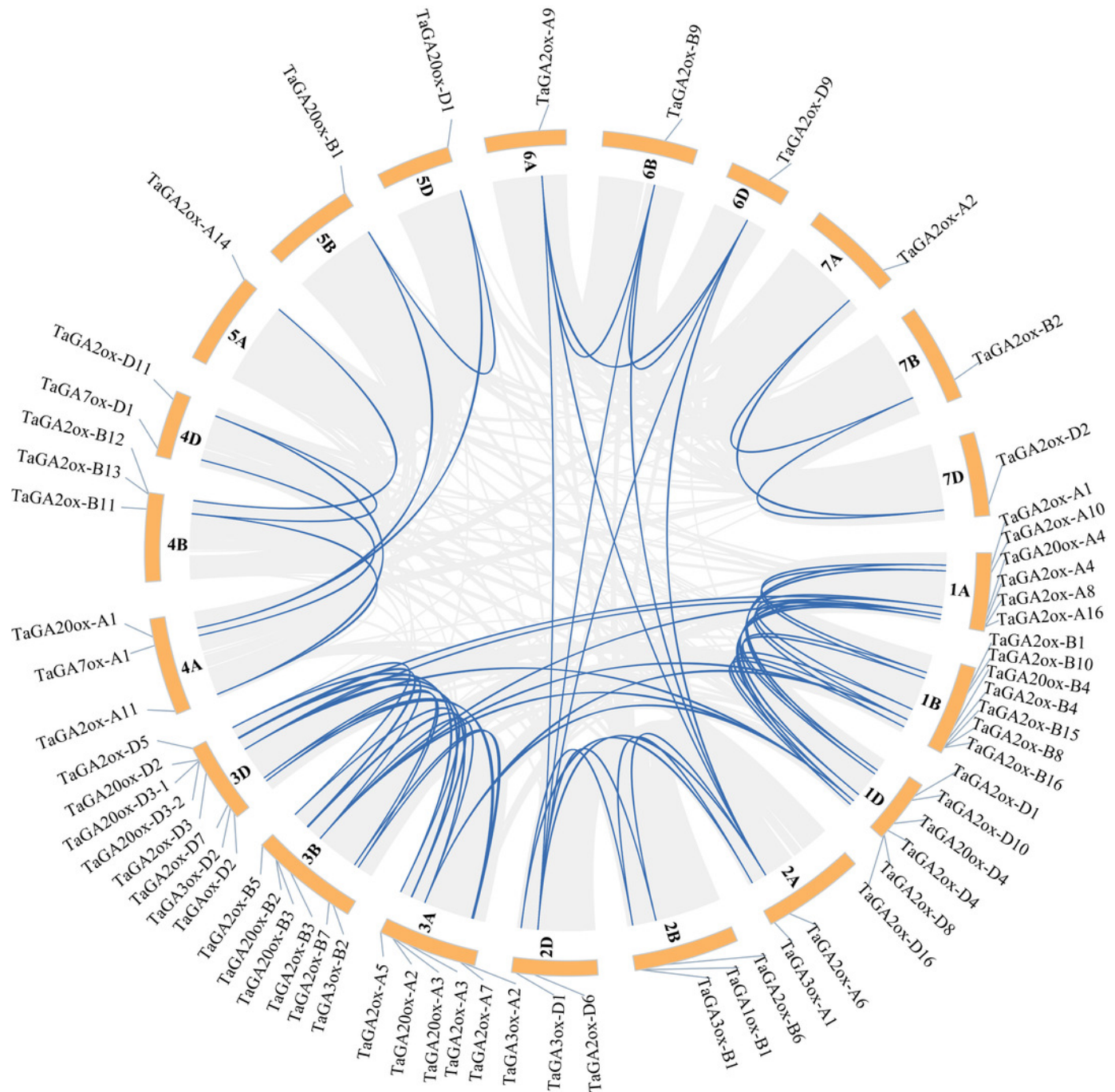
Figure 5

Syntenic relationships of *GAox* genes between wheat and three representative species.

Gray lines in the background indicate the collinear blocks within wheat and other plant genomes, while the blue lines highlight the syntenic *GAox* gene pairs.
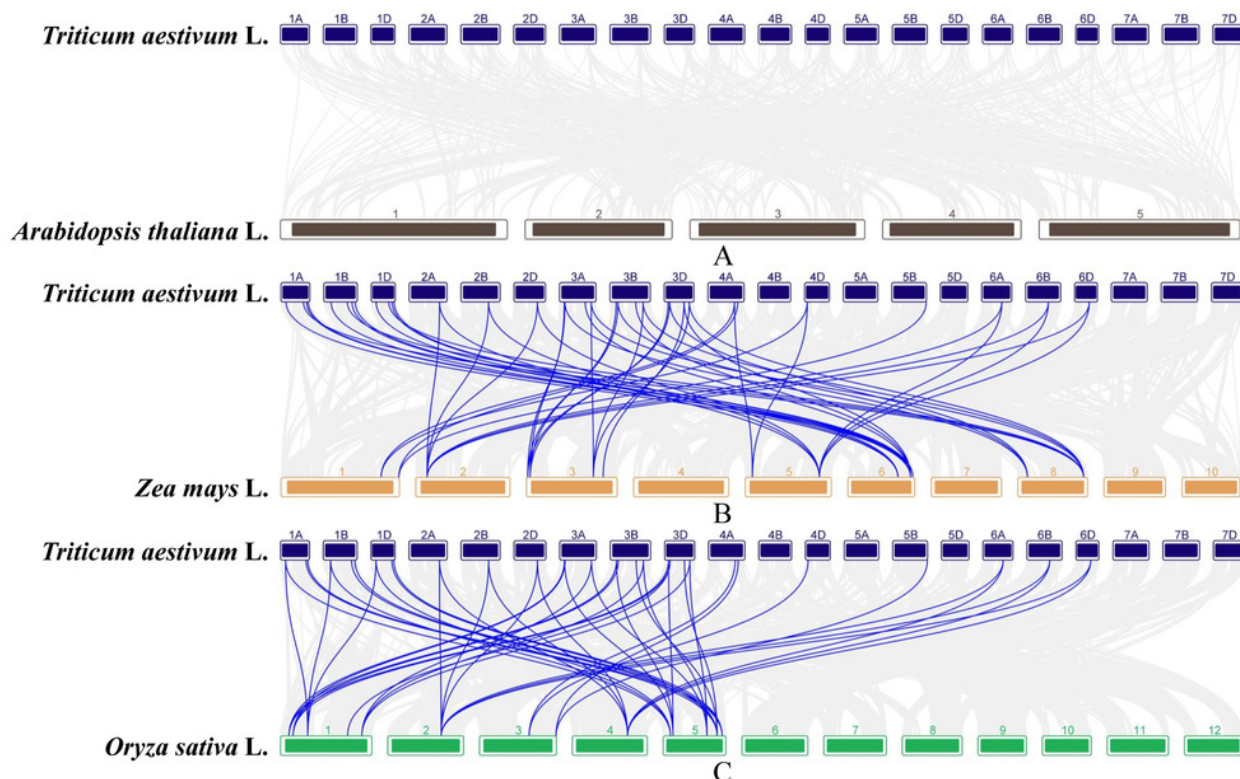
Figure 6

Expression profiles of *TaGAoxs* in various organs or tissues.

(A) Heatmap of expression profiles of *TaGAoxs* in various organs or tissues of Chinese Spring.

(B) Heatmap of expression profiles of *TaGAoxs* in tiller primordia of Guomai 301 and *dmc* based on transcriptome data. Three biological replicates were set up in Guomai301 (T01, T02 and T03) and *dmc* (T04, T05 and T06). The gene expression values present as $\log_2^{(FPKM)}$. Note: Blue: Low expression level; Red: High expression level.
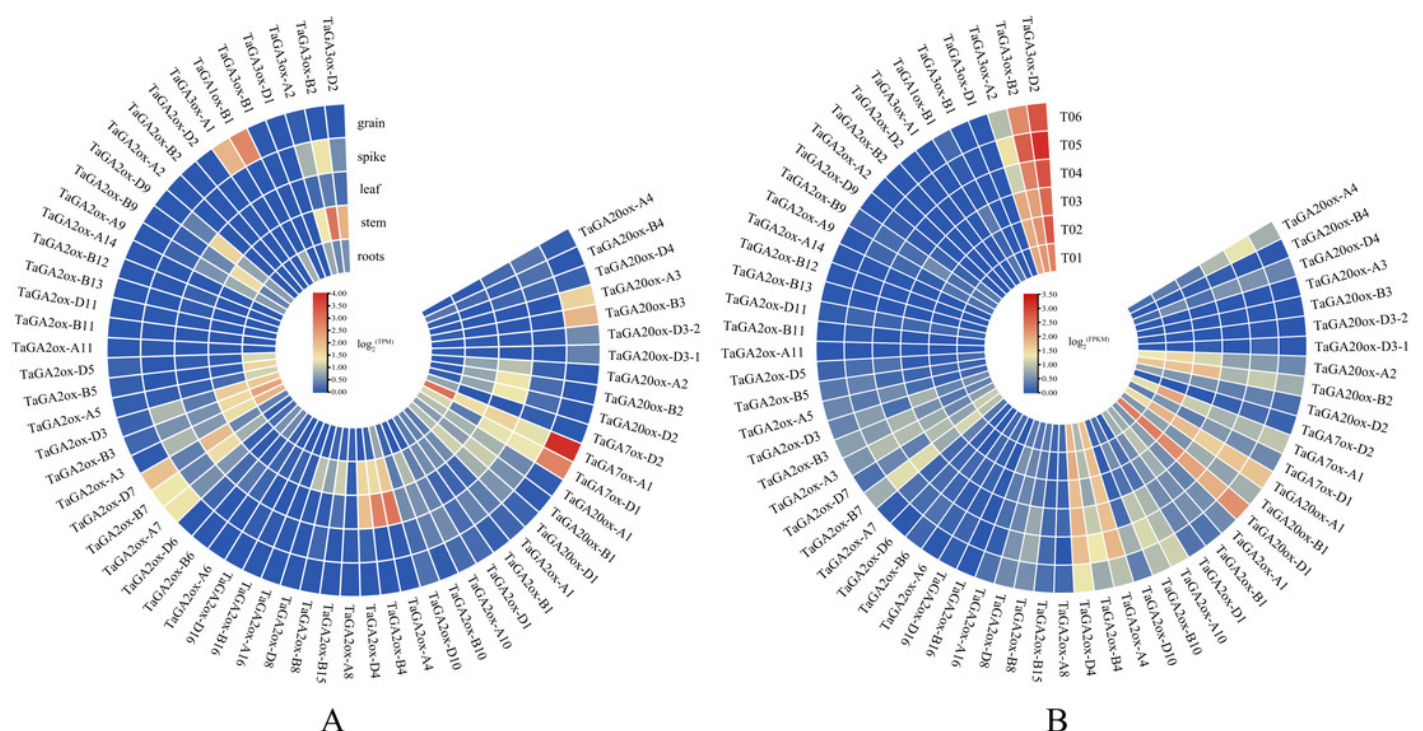
Figure 7

Expression profiles of *TaGAoxs* in response to GA$_3$ stimulation.

WT_0h, *dmc*_0h: untreated controls; WT_1h, *dmc*_1h: 1 h after GA$_3$ treatments; WT_2h,

*dmc*_2h: 2 h after GA$_3$ treatments. Data were normalized to *Actin* gene and vertical bars

indicated standard deviation. Asterisks indicate significant difference or highly significant

difference between WT and *dmc*. An asterisk (*) and two asterisks (**) indicate significant

difference (*P* < 0.05) and highly significant difference (*P* < 0.01) using Student's *t*-test,

respectively
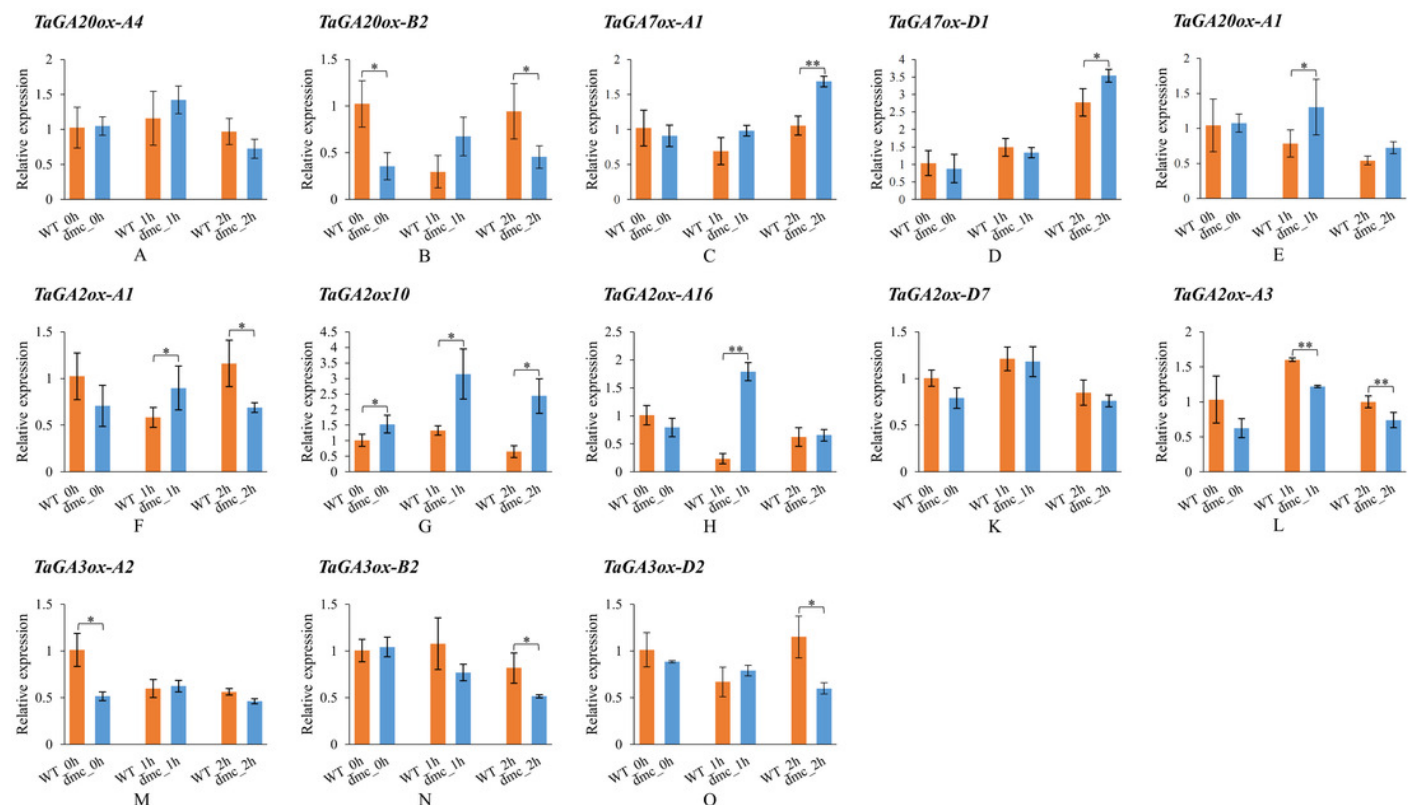
Figure 8

Tiller number changes of Guomai 301 and *dmc* in response to GA$_3$ stimulation.

T1-T6: the GA$_3$ treatment time points, the intervals were 7 days.
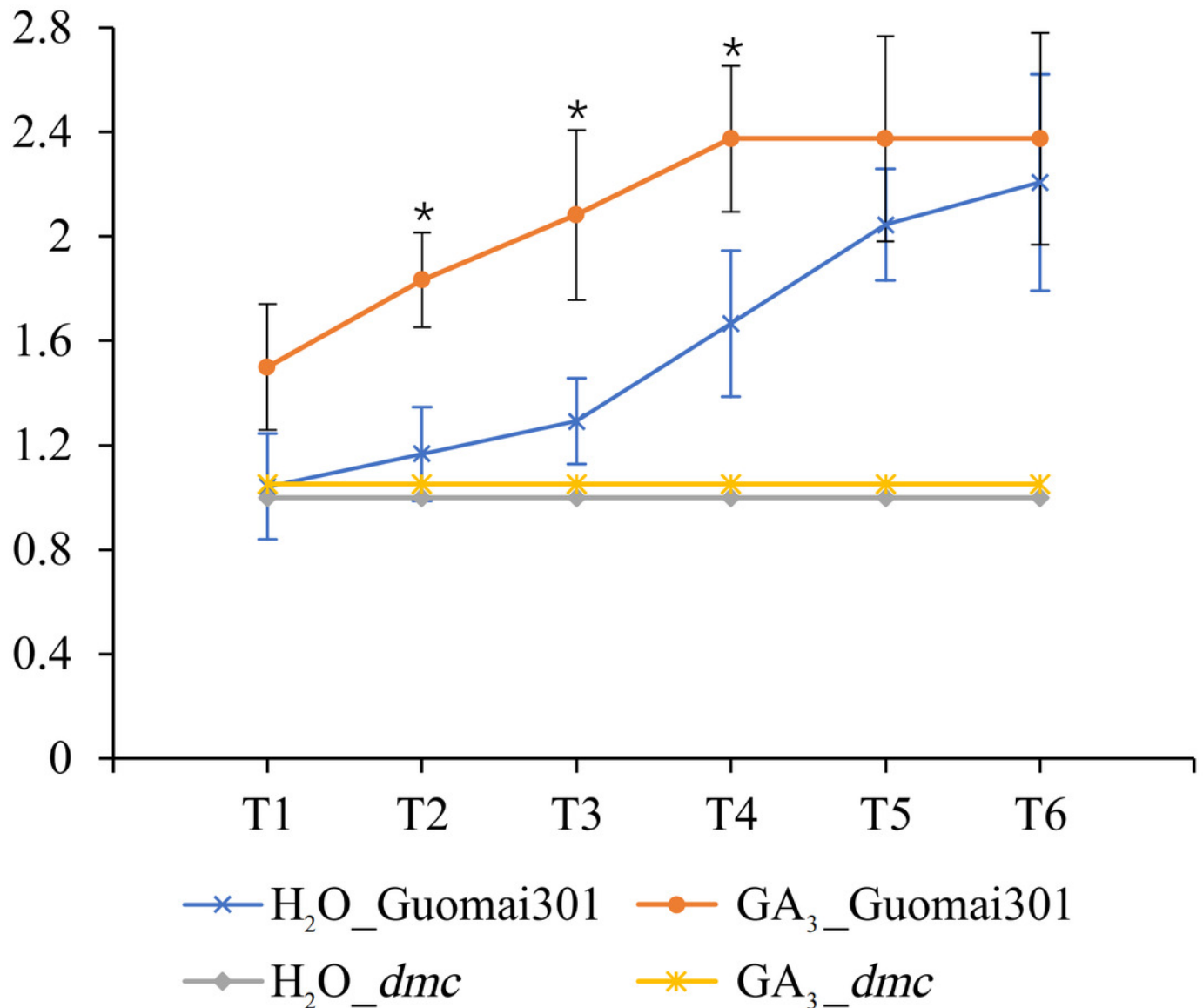
Figure 9

The tiller numbers and qRT-PCR analysis of *TaGA7ox-A1*-OE transgenic lines.

(A) Average tiller numbers of the transgenic plants and controls (WT) at different stages. TN1-TN6: time points of the tiller number record, the intervals were 7 days. (B) *TaGA7ox-A1* expression levels of the transgenic plants and WT. (C) Tiller numbers of the transgenic plants and WT (TN4 stage). An asterisk (*) indicate significant difference ($P < 0.05$) using Student's *t*-test.