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Cloning and Characterization of a cDNA (*TaGB1*) Encoding β Subunit of Heterotrimeric G - protein from Common Wheat cv. S615

Md. Shakhawat HOSSAIN[1], Takato KOBA[1,2]* and Kyuya HARADA[1,2]

[1]*Laboratory of Genetics and Plant Breeding, Graduate School of Science and Technology, Chiba University, 648 Matsudo, Matsudo city, Chiba 271 - 8510, Japan*
[2] *Faculty of Horticulture, Chiba University, 648 Matsudo, Matsudo city, Chiba 271 - 8510, Japan*
**Corresponding author* E–mail address: koba@faculty.chiba - u.jp

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Abstract

We isolated a full - length cDNA clone, *TaGB1*, encoding the β subunit of heterotrimeric G - protein from common wheat, *Triticum aestivum* cv. S615. The predicted amino acid sequence deduced from the cDNA was 70 - 89% and >50% homologous to those of the β subunits of plants and animals, respectively. The *TaGB1* exhibited the highest homology of 89% with the rice G - protein β subunit gene and preserved the most essential seven repeats of the "WD - 40" motif, which are commonly found in all plant and animal G - protein β subunits. Southern hybridization revealed that the wheat genome contains a single - copy gene for the β subunit of heterotrimeric G - protein. The analysis of the expression for Gβ in wheat showed that *TaGB1* mRNA was expressed in all of the organs tested and exhibited high degrees of mRNA accumulation in spikes and internodes as well as dark - grown seedlings. It was also observed by RT - PCR that the transcript of *TaGB1* was expressed throughout the ancestral genomes of wheat.

Accession number: AB090160.

Key words: β subunit, cDNA, common wheat, heterotrimeric G - protein, "WD - 40" motif.

Abbreviations

α, β and γ subunits, Alpha, beta and gamma subunits; Gβ subunit, G - protein beta subunit; GPCR, G - protein coupled receptor; kDa, kilodalton; ORF, Open reading frame; *TaGB1*, *Triticum aestivum* G - protein beta subunit 1.

Heterotrimeic G - proteins, composed of α, β and γ subunits, are involved in a variety of cellular responses through various signaling systems. In animals and simple eukaryotes, GTP - dependent heterotrimeric G - proteins function as mediators that transmit the numerous external signals through G - protein coupled receptors (GPCRs) to the effector molecules on cell surfaces (Kaziro *et al.*, 1991; Simon *et al.*, 1991; Neer, 1995). By binding a ligand, GPCR changes its conformation and activates the G - protein by promoting the Gα subunit to convert GDP to GTP. Then, the heterotrimer is separated into a GTP - bound Gα subunit and a $\beta\gamma$ - dimer. In this active state, the GTP - bound Gα and $\beta\gamma$ - dimer interact with a number of downstream effectors that will ultimately elicit the cellular response. In hydrolysis of the GTP molecule, the intrinsic GTPase activity of the α subunit reconstitutes the inactive heterotrimer and accepts the next signal for continuing cellular processes.

It is now evident that the Gα subunit complex is involved in various signaling pathways. Through the suppression of the Gα subunit gene and the analysis of gibberellin insensitive dwarf mutant (*d1*) in rice, it was suggested that the rice Gα subunit gene plays an important role in plant growth and development, particularly, in the formation of internodes and seeds (Ashikari *et al.*, 1999; Fujisawa *et al.*, 1999). Jones *et al.* (1998) reported that roles of G - proteins are implicated in gibberellin induction of α - amylase gene in oat aleurone layer. Moreover, the Gα subunit gene also plays a role in a variety of signaling processes (Fujisawa *et al.*, 2001). Conversely, little is known about the role of the $\beta\gamma$ complex in signal transduction processes. Recent studies indicate that the $\beta\gamma$ complex is able to

interact directly with effectors such as adenylyl cyclase, K^+ channels, and phospholipase A_2 (Clapham *et al.*, 1993; Iniguez-Lluhi *et al.*, 1993). Furthermore, in some cases, the $\beta\gamma$ complex is involved in receptor recognition (Kleuss *et al.*, 1992, 1993).

The cDNA or genes encoding G-protein β subunit homologs have been isolated from a few plant species including *Arabidopsis* and maize (Weiss *et al.*, 1994), rice (Ishikawa *et al.*, 1996), oat (Jones *et al.*, 1998), potato (Kang *et al.*, 2002), and tobacco (Kusnetsov and Oelmuller, 1996; Ando *et al.*, 2000; Kaydamov *et al.*, 2000). Recently, the first plant G-protein γ subunit has been reported in *Arabidopsis* (Mason and Botella, 2000).

The reports on the G-protein involvement in a number of different cellular functions raise the possibility that G-protein β subunits are also directly involved in signaling in plants, as G-protein α subunits. We have attempted to elucidate the structure and function of heterotrimeric G-protein in common wheat, *Triticum aestivum* (genome formula = AABBDD; $2n = 6x = 42$). In our previous work, we reported the molecular characterization of two cDNAs, *TaGA1* and *TaGA2* (DDBJ/EMBL/GenBank accession nos. AB090158 and AB090159) that showed genome-specific and differential expression patterns for the α subunits of heterotrimeric G-protein in common wheat (Hossain *et al.*, 2003). In this report, we describe the cloning and characterization of a cDNA encoding the G-protein β subunit, *TaGB1*, in hexaploid wheat.

The two oligonucleotides shown below were designed based on the sequence of maize G-protein β subunit (*ZGB1*, GenBank accession no. U12233) and used as primers for RT-PCR: (1) 5' GACAAGCCAGAAAACACATGCC 3', corresponding to the sequence ranging from position 483 bp to position 504 bp for the sense primer and (2) 5' GTCCCACACATAACAGTCACCA 3', corresponding to the sequence ranging from position 1173 bp to position 1152 bp for the anti-sense primer of *ZGB1* (Weiss *et al.*, 1994). The mRNA of one-week-old seedlings purified by Nucleo-Trap mRNA Kit (MARCHEREYNAGEL, GMBH & CO.) from total RNA (treated with DNaseI) of wheat was used as the template for PCR. PCR was performed under the conditions recommended by the manufacturer (QIAGEN). After PCR, a single reproducible partial cDNA fragment of 691 bp was obtained and subsequently cloned into the vector pCR 2.1 by TA cloning Kit (Invitrogen) and sequenced. Alignment of the amino acid sequence deduced from the partial cDNA sequence revealed a high degree of sequence similarity to those of the plant and animal G-protein β subunits (70-85%). To obtain a full-length cDNA encoding the G-protein β subunit of wheat, we further designed a sense primer (5' GTTGCTTGTGGCGGTCTTGATAGTG 3') for the amplification of the 3' RACE-PCR fragment and two anti-sense primers (a. 5' AGCGACAGATGTAACAATAGGGAG 3' and b. 5' CCGAACTGCCCGACTTGTAATCCTG 3') for the amplification of the 5' RACE-PCR fragment from the isolated partial G β cDNA. In RACE-PCR, first strand cDNA was synthesized from poly $(A)^+$ RNA by using SMART^{TM} RACE-PCR method (CLONTECH) following the manufacturer's instructions. To perform the second strand synthesis of the cDNA fragment, PCR was carried out using an anti-sense primer for 5' RACE-PCR and a sense primer for 3' RACE-PCR (indicated above) by using Advantage2 PCR Kit (CLONTECH) following the manufacturer's instructions. After agarose gel electrophoresis, a single fragment from the products of 3' RACE-PCR and a single band with smear from the 5' RACE-PCR was obtained. Then, we performed nested PCR for obtaining a specific single fragment of the 5' RACE-PCR product using the second anti-sense primer indicated above. Both 5' and 3' RACE-PCR fragments were cloned using TA cloning Kit (Invitogen) and sequenced. After sequencing, a full-length cDNA encoding the G-protein β subunit was obtained which was designated as *TaGB1* (*Triticum aestivum* G protein β subunit 1). The sequence of *TaGB1* (DDBJ, EMBL and GenBank accession no. AB090160) was 1, 688 bp in length with 171 bp of 5' UTR and 374 bp of 3' UTR with a putative polyadenylation signal sequence, and contained an open reading frame of 380 amino acid residues with a molecular mass of 48.5 kDa. To date, *TaGB1* has the longest nucleotide sequence among the Gβ subunit genes identified in plants and animals. However, the length of the ORF (380 aa) of *TaGB1* was similar to those of Gβ subunits in cereals and monocotyledons, while the total molecular mass of *TaGB1* (48.5 kDa) was greater than those observed in other plants and animals.

The deduced amino acid sequence encoded by *TaGB1* showed significant similarities to the plant and animal G-protein β subunits (**Fig. 1**). *TaGB1* shared 71-89% and >50% amino acid sequences with plant and animal G-protein β subunits, respectively. The highest similarity was 89% against rice Gβ subunit, *RGB1* (Ishikawa *et al.*, 1996) and 51% against that of *Rattus norvegicus* (Sharma and Das, unpublished data). To our knowledge, *TaGB1* is the first plant G-protein β subunit that shows >50% amino acid similarity with the animal G-protein β

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TaGB1   1 MASVAELKEKHAAATASVNSLRERLRQRRQTLLDTDVEKYSKAQGRTAVSFNPTDLVCCR    60
 RGB1   1 MASVAELKEKHAAATASVNSLRERLRQRRQMLLDTDVERYSRTQGRTPVSFNPTDLVCCR    60
 ZGB1   1 MASVAELKEKHAAATASVNSLRERLRQRRETLLDTDVARYSKSQGRVPVSFNPTDLVCCR    60
  oat   1 MASVAELKERHAAATASVNSLRERLRQRRQTLLDTDVEKYSKAQGRTAVSFNQTDLVCCR    60
 AGB1   1 MS-VSELKERHAVATETVNNLRDQLRQRRLQLLDTDVARYSAAQGRTRVSFGATDLVCCR    59
          *..*.****.**.**..**.**..*****..******..**..***. ***..*******

                      Repeat 1                          Repeat 2
       61 TLQGHSGKVYSLDWTPEKNWIVSASQDGRLIVWNA LTSQKTHAIKLHCPWVMECAFAPNG   120
       61 TLQGHSGKVYSLDWTPEKNWIVSASQDGRLIVWNA LTSQKTHAIKLHCPWVMTCAFAPNG   120
       61 TLQGHSGKVYSLDWTPEKNWIVSASQDGRLIVWNA LTSQKTHAIKLHCPWVMACAFAPNG   120
       61 TLQGHSGKVYSLDWTPEKNWIVSASQDGRLIVWNA LTSQKTHAIKLHCPWVITCAFAPNG   120
       60 TLQGHTGKVYSLDWTPERNRIVSASQDGRLIVWNA LTSQKTHAIKLPCAWVMTCAFSPNG   119
          *****.**********.*.**************** ***********.*.**..***.***

                                           Repeat 3
      121 QSVACGGLDSACSIFNL SSQADRDGNMPVSRVLTGHKGYASSCQYVPDQETRLITGSGDQ   180
      121 QSVACGGLDSACSIFNL NSQADRDGNIPVSRILTGHKGYVSSCQYVPDQETRLITSSGDQ   180
      121 QSVACGGLDSACSIFNL NSQADRDGNMPVSRILTGHKGYVSSCQYVPDQETRLITSSGDQ   180
      121 QSVACGGLNSACSIFNL NSQVDRNGNMPVSKLLTGPKGYVLSCQYVPDQETRMITGSGDP   180
      120 QSVACGGLDSVCSIFSL SSTADKDGTVPVSRMLTGHRGYVSCCQYVPNEDAHLITSSGDQ   179
          ********.*.****.* .*..*..*..***. ***..**...*****......**.***.

                                   Repeat 4
      181 TCVLWDV TTGQRISIFGSEFPSGHTADVLSLSINSLNANMFISGSCDTTVRLWDL RITSR   240
      181 TCVLWDV TTGQRISIFGGEFPSGHTADVLSLSINSSNSNMFVSGSCDATVRLWDI RIASR   240
      181 TCVLWDV TTGQRISIFGGEFPSGHTADVQSVSINSSNTNMFVSGSCDTTVRLWDI RIASR   240
      181 TCVLWDV TTGQRISIFGGEFPSGHTADVLSLSINSLNTNMFVSGSCDTTVRLWDL RIASR   240
      180 TCILWDV TTGLKTSVFGGEFQSGHTADVLSVSISGSNPNWFISGSCDSTARLWDT RAASR   239
          **.**** ***...*.**.**.*******.*.**...* *.*.*****.*.**** *..**

                   Repeat 5
      241 AVRTYHGHEGDINSVKFFPDGQRFGTGSDDGTCRLFDM RTGHQLQVYNREPDRNDNELPI   300
      241 AVRTYHGHEGDINSVKFFPDGQRFGTGSDDGTCRLFDV RTGHQLQVYSREPDRNDNELPT   300
      241 AVRTYHGHEDDVNSVKFFPDGHRFGTGSDDGTCRLFDM RTGHQLQVYSREPDRNSNELPT   300
      241 AVRTYHGHEGDINSVKFFPDGHRFGTGSDDGTCRLFDM RIRHQLQVYSREPDRNDNELPS   300
      240 AVRTFHGHEGDVNTVKFFPDGYRFGTGSDDGTCRLYDI RTGHQLQVYQPHGDGENG--PV   297
          ****.****.*.*.******* *************.*. *..******....*......*.

                  Repeat 6                          Repeat 7
      301 VTSVAFSISGRLLFAGYSNGD-CYVWDT LLAEMVLNLGTLQNSHEGRISCLGLSSDGSAL   359
      301 VTSIAFSISGRLLFAGYSNGD-CYVWDT LLAEVVLNLGNLQNSHEGRISCLGLSSDGSAL   359
      301 VTSIAFSISGRLLFAGYSNGD-CYVWDT LLAEVVLNLGNLQNSHDGRISCLGMSSDGSAL   359
      301 VTSIAFSISGRLLFAGYSNGD-CYAWDT LLAEVVLNLGTLQNSHEGRISCLGLSSDGSAL   359
      298 T-SIAFSVSGRLLFAGYASNNTCYVWDT LLGEVVLDLGLQQDSHRNRISCLGLSADGSAL   356
          ..*.***.*********.... **.*** **.*.**.** .*.**..******.*.*****

      360 CTGSWDKNLKIWAFSGHRKIV   380
      360 CTGSWDKNLKIWAFSGHRKIV   380
      360 CTGSWDKNLKIWAFSGHRKIV   380
      360 CTGSWDKNLKIWAFSGHRKIV   380
      357 CTGSWDSNLKIWAFGGHRRVI   377
          ******.*******.***...
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Fig. 1 Alignment of the predicted amino acid sequence of *TaGB1* with those of *RGB1* of *O. sativa* (accession no. Q40687, Ishikawa *et al.*, 1996), *ZGB1* of *Z. maize* (accession no. P49178, Weiss *et al.*, 1994), G-protein β subunit of wild oat (accession no. T08036, Jones *et al.*, 1998), and *AGB1* of *A. thaliana* (accession no. P49177, Weiss *et al.*, 1994). Amino acid sequences were aligned using the Genetyx-Mac ver. 10.1 software and then adjusted manually. Boldfaced codes are assigned to residues showing the repetitive 'WD-40' motif which generally starts with an arginine (R) and/or Gly-His (GH) dipeptide and ends with the Try-Asp (WD) dipeptide (modified from Kaydamov *et al.*, 2000). Open boxes represent seven repeats (1-7) of the WD-40 motif which are conserved among plant, animal and yeast β subunits (modified from Ishikawa *et al.*, 1996). The consensus amino acid residues are indicated as asterisks below the sequences.

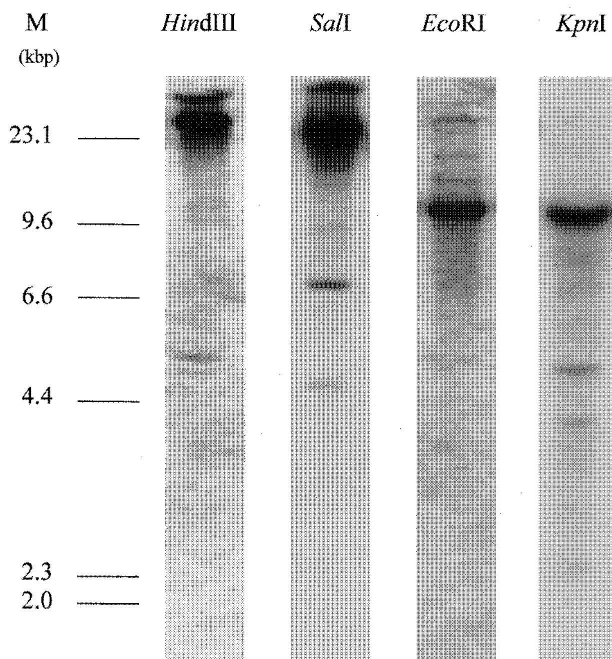

Fig. 2 Southern blot hybridization of the genomic DNA from *Triticum aestivum* cv. S615 (AABBDD). Genomic DNA (15 μg in each lane) was digested with *Hin*dIII, *Eco*RI, *Kpn*I and *Sal*I and fractionated on a 0.8% agarose gel, followed by blotting onto a nylon membrane (positively charged, Amersham). The membrane was hybridized with the ECL-labelled 3' RACE PCR product of *TaGB1* according to the instructions of ECL detection and hybridization system (Amersham Pharmacia). M represents the size marker, λ-*Hin*dIII.

subunit. With respect to the number of amino acid residues compared among higher organisms, Gβ sequences differ not only between plants and animals, but also among higher plants. Gβ of the dicots such as *Arabidopsis* (Weiss *et al.*, 1994), potato (Kang *et al.*, 2002; Provart *et al.*, unpublished data), tobacco (Ando *et al.*, 2000; Lein and Saalbach, unpublished data), *Nicotiana plumbaginifolia* (Kaydamov *et al.*, 2000) and *Pisum sativum* (Lapik *et al.*, unpublished data; Wu and Tuteja, unpublished data) contained 377 aa, whereas, Gβ of the monocots such as rice (Ishikawa *et al.*, 1996), maize (Weiss *et al.*, 1994), oat (Jones *et al.*, 1998) and *TaGB1* (present work) contained 380 aa.

To determine the copy number of the gene encoding the β subunit in wheat genome, genomic Southern blot analysis was performed (**Fig. 2**). Genomic DNA from leaves of *T. aestivum* cv. S615 was purified as described by Murray and Thompson (1980). For blotting, samples of DNA (15 μg per lane) were digested with *Hin*dIII, *Eco*RI, *Kpn*I and *Sal*I restriction endonucleases, fractionated on 0.8% agarose gel and subsequently blotted onto nylon membranes (Hybond N^+, Amersham). The hybridization was carried out at 42 ℃ for overnight using the ECL-labelled 3' RACE PCR product of *TaGB1* as a probe, and the stringency of the subsequent washes was as follows: 2x10 min at 55 ℃ in buffer containing 0.1% SSC (pH 7.0) and 0.4% SDS, and 2x15 min at room temperature in 2x SSC. Signal detection was carried out following the instruction of ECL™ Direct Nucleic Acid Labelling and Detection Systems (Amersham). A major single band with a few weak bands for Gβ was detected following the enzymatic digestion of the genomic DNA. Thus, the result indicates that the wheat genome contains only a single copy of β subunit gene, as previously reported in *Arabidopsis* and maize (Weiss *et al.*, 1994). Conversely, multiple copies of β subunit genes were reported in mammalian genomes (Kaziro *et al.*, 1991; Simon *et al.*, 1991; Neer *et al.*, 1995).

Two primers (1) 5' GCCTCACAAGATGGAAGACTAA 3', corresponding to the sequence ranging from position 421 bp to position 442 bp for the sense primer and (2) 5' GTCCCACACATAACAGTCACCA 3', corresponding to the sequence ranging from position 1149 bp to position 1128 bp for the anti-sense primer were designed based on the sequence of *TaGB1* to analyze the *TaGB1* expression in wheat. Total RNAs were isolated from one-week-old seedlings, seeds harvested one week after anthesis, young spikes and first internodes as described by Fukazawa *et al.* (1985) and treated with DNaseI for eliminating any genomic contamination before being subjected to RT-PCR to amplify the PCR fragment of *TaGB1* (728 bp) using the RT-PCR Kit (QIAGEN) following the manufacturer's instructions. In RT-PCR, a half microgram of the total RNA was used as the template, and initial reverse transcription was performed at 50 ℃ for 30 min and subsequent inactivation at 95 ℃ for 15 min. The second strand synthesis was continued in a total of 50 μl volume using hot lid PCR under the following conditions: 30 cycles of 94 ℃ for 15 s, 58 ℃ for 30 s, 72 ℃ for 2 min with an elongation of 5 min at 72 ℃. As a positive control, we used two oligonucleotides designed based on the sequence of maize actin gene (Moniz *et al.*, 1996) and performed RT-PCR as described above. **Fig. 3A** shows the RT-PCR-amplified DNA fragments corresponding to *TaGB1* (728 bp) and the actin control (809 bp) obtained using the total RNAs isolated from the tissues. The transcript of *TaGB1* was expressed in all of the organs tested and showed high degrees of mRNA accumulation in spikes and internodes. The analysis of the expressions of the β subunit genes in different organs have also been

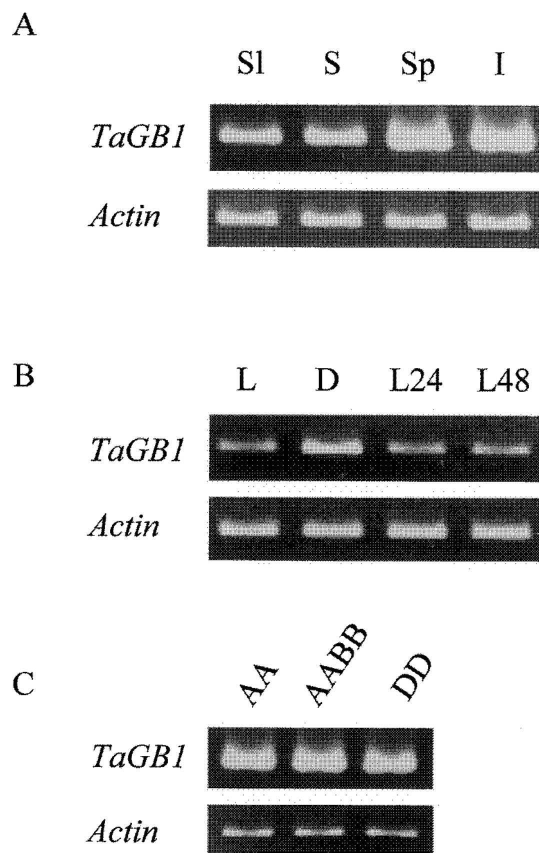

Fig. 3 RT-PCR analysis of *TaGB1* mRNA expression.
(A) A half microgram each of total RNAs from one-week-old seedlings (Sl), seeds harvested one week after anthesis (S), young spikes (Sp), and first internodes (I) was used to amplify DNA fragments corresponding to *TaGB1* (728 bp).
(B) A half microgram each of total RNAs from seedlings grown in 16 h white fluorescent light / 8 h darkness for 7 days (L), under continuous darkness for 7 days (D), and under continuous darkness for 6 days and white fluorescent light for 24 h (L 24) or 48 h (L 48) was used for RT-PCR for the amplification of DNA fragments corresponding to *TaGB1* (728 bp).
(C) A half microgram each of total RNAs from seedlings of *T. monococcum* (AA), *T. turgidum* ssp. *durum* (AABB), and *Ae. squarrosa* (DD) was used to amplify DNA fragments corresponding to *TaGB1* (728 bp). Actin (809 bp) was used as the control. The products were visualized by ethidium bromide staining.

reported in *Arabidopsis*, maize, rice and potato (Weiss *et al.*, 1994; Ishikawa *et al.*, 1996; Kang *et al.*, 2002). Since G-protein plays a role in the light signaling process in plants, the *TaGB1* expression patterns in dark and light-grown seedlings were observed. Total RNA was isolated from seedlings grown at 20 ℃ in an incubator under the following conditions: 16 h white fluorescent light and 8 h darkness for 7 days (L), continuous darkness for 7 days (D), and darkness for 6 days and exposure to white fluorescent light for 24 h (L24) or 48 h (L48). RT-PCR was performed as described above. **Fig. 3B** represents the RT-PCR-amplified DNA fragments of *TaGB1* (728 bp) and the actin control (809 bp) obtained from the total RNAs of the tissues. The transcript of *TaGB1* showed high degrees of mRNA accumulation in dark-grown seedlings compared with light-grown seedlings. The result indicates that the *TaGB1* expression differed between the light and dark treated tissues. To examine the distribution of the *TaGB1* gene expression in the progenitors of wheat, total RNAs were isolated from *T. monococcum* (genome formula = AA), *T. turgidum* ssp. *durum* (AABB), and *Aegilops squarrosa* (DD), and RT-PCR was performed to amplify the DNA fragments corresponding to *TaGB1* and actin as the control. The result shown in **Fig. 3C** represents the expression pattern for the *TaGB1* in the ancestral wheat genomes and suggests that *TaGB1* is expressed throughout the ancestral genomes of wheat. We observed similar patterns of *TaGB1* expression in each experiment when the quantity of template RNA was changed (data not shown).

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