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Original Paper

Overexpression of *Q*/*q*-related homoeoalleles of hexaploid wheat reveals distinct recovery of flower transformation in the *apetala2* mutant of *Arabidopsis*

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Abstract The *Q* gene has played substantial roles in wheat domestication. As it pleiotropically governs domestication-related traits, such as free threshing, glume shape and tenacity, rachis fragility, spike length, plant height, and flowering time, wheat is cultivated in widespread adaptation. The *Q* gene located on the A genome encodes the *APETALA2*-like transcription factor *WAP2A*^{Q}. The allelic mutation from *q* (*WAP2A*^{q}) to *Q* took place in the polyploidy wheats, and the B and D genomes of bread (hexaploid) wheat conferred its homoeoalleles (*WAP2B* and *WAP2D*, respectively). Although *WAP2A*^{q} and *WAP2D* revealed allelic phenotypes against *WAP2A*^{Q}, their functions remain to be clarified. We overexpressed full-length cDNAs of *WAP2A*^{Q}, *WAP2A*^{q}, and *WAP2D* in the *ap2* mutant line of *Arabidopsis*. *WAP2A*^{Q} fully recovered their flower organs similar to the wild type, *WAP2D* showed less recovery, and *WAP2A*^{q} rescued the least mutant flower phenotype. Use of a yeast two-hybrid system showed that *WAP2A*^{Q} formed the most homodimers, *WAP2A*^{q} formed the next highest, and *WAP2D* formed the least. The sequence comparisons between the three transcription factors and with *AP2* of *Arabidopsis* revealed that *WAP2A*^{q} confers two single protein substitutions, I_{329}-to-V substitution and K_{108}-to-E in the nuclear translocation signal, *WAP2D* harbors SNPs of I_{329}-to-L similar to *Arabidopsis*, and other 6 substitutions. These data support the idea that a critical point mutation at the functional domain and structure alteration(s) resulting from sequence diversifications caused functional differences in the genes. Mutant lines of *Arabidopsis* can become a powerful tool for analyzing foreign gene functions as in the case of wheat.

Key words: *Arabidopsis* mutant, homoeogene function, overexpression, *Q* gene, wheat domestication.

Wheat has the characteristic allopolyploid nature (Kihara 1944). Bread wheat (*Triticum aestivum* L., $2n=6x=42$, AABBDD as genome formula) was produced by the spontaneous hybridization of cultivated tetraploid wheat, *T. turgidum* L. ($2n=4x=28$, AABB), with wild goatgrass, *Aegilops tauschii* Coss. ($2n=2x=14$, DD). After allopolyploidization, wheat acquired better agronomic traits, such as nonshattering and threshing, as well as wide adaptation (Chalupska et al. 2008). Wheat is a staple food, together with two other major crops, rice and maize, consumed by humans. The *Q* is considered the most important gene for wheat domestication because it governs free-threshing characteristics and other domestication-related traits, such as rachis fragility, glume toughness, spike morphological traits, flowering time, and plant height (Simons et al. 2006). Mutation from *q* to *Q* was found only in cultivated tetraploid and hexaploid wheat, suggesting that the mutation occurred after the domestication of polyploid wheat (Faris et al. 2005). The *Q* gene pleiotropically controls the spike morphological traits of polyploid wheat, and *Q* and *q* show a dosage effect on spike phenotypes (Muramatsu 1963). *Q* and its allele *q*, which are located on the long arm of chromosome 5 of the A genome (5AL), reveal their typical spike morphological traits, that is, square head (*Q*) and speltoid (*q*), respectively (Muramatsu 1963). Their homoeoloci were assigned to genomes 5BL and 5DL, although the homoeogene in the 5BL of Chinese Spring wheat was inactive because of truncation (unpublished; Zhang et al. 2011). Although the *Q* and *q* genes had been cloned as an *APETALA2*-like gene of *Arabidopsis* (Simons et al. 2006), the function(s) of the *Q*/*q* genes and their homoeoalleles remain unclear. The *AP2* gene is known to regulate flower organ identity (Bowman et al. 1989; Jofuku et al. 1994; Kunst et al. 1989), as well as flowering time (Yant et al. 2010), shoot

Abbreviations: Col, Columbia; CS, *Triticum aestivum* cv. Chinese Spring; CTAB, Cetyl trimethyl ammonium bromide; WAP2, wheat APETALA2; SNPs, Single nucleotide polymorphisms.

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apical meristem maintenance (Wuerschum et al. 2006), seed development (Jofuku et al. 1994; Ohto et al. 2005), and fruit development (Ripoll et al. 2011). Structurally, the AP2 contains two repeats of the AP2 domain (AP2-R1 and AP2-R2) for protein-protein interaction and DNA binding, a highly acidic serine-rich region that may provide transcriptional activation, and a 10-amino acid basic region that contains a putative nuclear localization signal (Jofuku et al. 1994; Riechmann and Meyerowitz 1998).

Here, we isolated full-length cDNAs of *Q* (designated as *WAP2A*^{Q}) from Chinese Spring wheat (AABBDD), *q* (*WAP2A*^{q}) from *T. spelta* (AABBDD), and a homoeoallele of the D genome (*WAP2D*) from Chinese Spring. Their functions are implicated by overexpression of those genes in an *APETALA2* mutant of *Arabidopsis thaliana* (*ap2-5*). The *ap2-5* mutant was originally isolated as a homoeotic transformer of flower organs (Kunst et al. 1989). Those overexpressors showed distinct recovery of homoeotic transformations in flower organs based on nucleotide variations in the gene. Furthermore, we examined the homodimer formation ability of three wheat AP2s using a yeast two-hybrid system. The three wheat AP2s revealed different degrees of homodimer formation. Distinct functional differentiations of the three wheat AP2s can be ascribed to structural alterations due to amino acid substitutions. *Arabidopsis* mutant lines can become powerful tools for the study of the functions of foreign genes from other plants such as wheat, from which it is difficult to obtain transformants.

Materials and methods

Plant materials and growth condition

Arabidopsis thaliana ecotype Columbia (Col) conferring the mutated *apetala 2* gene (*ap2-5*) with ethylmethane sulfonate (EMS) was supplied from the *Arabidopsis* Biological Resource Center (ABRC). The plants were grown in a growth chamber under long day conditions of a 16-h light/8-h dark cycle at 22°C. The genomic DNA of *ap2-5* was extracted from the rosette leaves using the cetyl trimethyl ammonium bromide (CTAB) method (Murray and Thompson 1980). The specific forward primer 5′-GGACCAAGATCAAGAAGTTC-3′ and reverse primer 5′-CGTAAGCTCTAGCAGCTTC-3′ were synthesized to amplify the *Arabidopsis APETALA 2* gene. Polymerase chain reaction (PCR) analysis was carried out using a standard procedure. Amplified DNA fragments were determined using the dideoxy sequencing method.

WAP2 genes and generation of transgenic Arabidopsis

Full-length cDNAs of wheat *APETALA 2*-like genes located on the homoeologous chromosome 5 of the A genome from *T. aestivum* cv. Chinese Spring (AABBDD as genome formula; *WAP2A*^{Q}), its allele from *T. spelta* var. duhamelianum (*WAP2A*^{q}), and the homoeoallele from 5D of Chinese Spring wheat (*WAP2D*) were used in the present study (unpublished). A pair of primers, WAP2-F (5′-GGACCAAGATCAAGAAGTTC-3′) and WAP2-R (5′-CGTAAGCTCTAGCAGCTTC-3′) were synthesized to amplify the three *WAP2* genes. The amplified products were inserted into pENTER/D-TOPO to make entry clones (Invitrogen, Carlsbad, CA), and those entry clones were confirmed by DNA sequencing. Three *WAP2* genes in the entry clones were recombined into the binary vector (pBCR79) under the 35S promoter with the LR reaction (Invitrogen). The three constructs, 35S:cWAP2A^{Q}:pBCR79, 35S:cWAP2D:pBCR79, and 35S:cWAP2A^{q}:pBCR79, were then transformed to the *Agrobacterium tumefaciens* strain C581GV3101 (pMP90) using electroporation (Weigel and Glazebrook 2002). After the sequences of these inserts were confirmed, the constructs were transformed into two-month-old *ap2-5* plants using the floral dip method (Weigel and Glazebrook, 2002). The T_1 seeds were screened in a Murashige and Skoog medium plate containing carbenicillin and kanamycin. The genomic DNAs of the T_1 plants were extracted from the rosette leaves to confirm transformation. The primer 35S (5′-CGAGAAAGAGGATCCACCTGAGGATCACAAG-3′) was used as a common the forward primer for all the three genes. The primer WAP2-ADQ-R2 (5′-CATCCCCATTGTAGGAGCTG-3′) was designed as the reverse primer for *WAP2A*^{Q} and *WAP2D*. For *WAP2A*^{q}, the primer WAP2-qR2 (5′-CAGCATCAACAATTGCCTC-3′) was used as the reverse primer. PCR was performed for 3 min at 95°C, followed by 40 cycles of 30 s at 95°C, 30 s at 55°C, and 30 s at 72°C. The T_2 seeds were also screened against antibiotics and genomic PCR analysis.

The T_1 transgenic *Arabidopsis* plants were grown to set flowers. Young flowers were collected once they were fully opened and imaged using an Olympus-SZ-ILLB100 microscope (Olympus, Tokyo, Japan).

Real-time PCR analyses

The young flowers of the *Arabidopsis* plants were collected, immediately frozen in liquid nitrogen, and stored at −80°C until use. Total RNA was extracted using a PureLink Plant RNA Reagent Kit (Invitrogen). One microgram of total RNA was supplied for cDNA synthesis according to the ReverTra Ace (Toyobo, Osaka, Japan) protocol. Real-time (RT)-PCRs were carried out for initial denature at 94°C for 2 min and 30 subsequent cycles under the following conditions: 94°C for 15 s, 62°C for 30 s, and 68°C for 30 s. Primer pairs of WAP2-ADQ-F2 (5′-GTGACGAGGCAGCTCTTCC-3′) and WAP2-ADQ-R2 (5′-CATCCCCATTGTAGGAGCTG-3′) were used for *WAP2A*^{Q} and *WAP2D*, and WAP2-qF2 (5′-GTTCGTCACGAGGCAGCTC-3′) and WAP2-qR2 (5′-CAGCATCAACAATTGCCTC-3′) for the *WAP2A*^{q} gene. The *Arabidopsis ACTIN* gene was used as a control for the RT-PCR.

Quantitative RT-PCR (qRT-PCR) was carried out using the SYBER green dye marker with the ABI Prism 7000 (Applied Biosystems, Tokyo, Japan). The qRT-PCR mixture included

cDNA obtained from 1 μg of total RNA, 0.3 μM of each primer, and 1× SYBR Premix Dimer Eraser in a final volume of 25 μl. Two sets of primers, F-WAP2A-As-F (5′-CCGCCGTACCCG GACCAC-3′)/R-WAP2A-As (5′-CGGCGAGAGACCAGC CAGTA-3′) and F-WAP2BD-As (5′-CCGTCGTACCCG ATCACC-3′)/R-WAP2D-As (5′-ACATGGCCTGGCAAC GTGT-3′), were used for specific amplification of the *WAP2* genes from the A and D genomes, respectively. The *Arabidopsis ACTIN* gene was used as an internal control with the primers F-Actin2-5r (5′-CATTGTGCTCAGTGGTGGAA-3′) and R-Actin2-5r (5′-ATTAAGGTCGTTGCACCACC-3′). The cycling condition for qRT-PCR was as follows: 95°C for 10 min, 40 cycles of 95°C for 5 s, and 60°C for 30 s. Two replications of each reaction were performed.

Yeast two-hybrid analysis

A Matchmaker Yeast Two-Hybrid System (Clontech, CA, USA) was used in the yeast 2-hybrid assay. The full-length cDNAs of the *WAP2A*^{Q}, *WAP2A*^{q}, and *WAP2D* genes cloned in the pENTER/D-TOPO vector were amplified using the M13(–20) F (5′-GTAAAA CGACGGCC-3′) and M13-R (5′-GTCATA GCTGTTTCCTG-3′) primers. One microliter of the purified PCR products was used to recombine the *WAP2* gene into the pGBKT7 and pGADT7 vectors for use as bait and prey, respectively. The bait and prey colonies were identified using PCR with gene-specific primers and restriction analysis by *Hin*dIII digestion. The yeast strain AH109 was transformed with the bait and prey plasmids for each gene, and the yeast cells were plated on SC (synthetic complete) medium lacking tryptophan (-W) and leucine (-L) before incubation at 30°C for two days. To test the protein interactions, a single clone from the SC-L-W plates was streaked on dropout SC medium lacking leucine, tryptophan, histidine, and adenine (SC-L-W-A-H). The interaction between WAP2:pGBKT7 (bait vectors) and empty pGADT7 (prey vector) were used as negative interactions on the dropout medium. To compare the protein interaction strength and homodimer ability of the *WAP2A*^{Q}, *WAP2A*^{q}, and *WAP2D* genes, a single clone from the SC-L-W plates was selected and inoculated in 2 ml of YPAD medium and then incubated at 30°C with a shaker until the yeast culture yielded to approximately 0.8 at OD_{600}. After adjusting all the yeast cultures at the same OD_{600}, 100 μl of each culture was mixed with 900 μl of water and diluted over 6 steps to obtain the concentration of 1/10 to $1/10^{6}$ and were then plated on SC-L-W-A-H and SC-L-W-H containing 10-mM 3-amino-1,2,4-triazole (3AT) plates. The plates were then incubated at 30°C for 48 h.

Results

Sequence comparison of WAP2 cDNAs

The sequences of the *WAP2* genes used in the study were compared, along with the *Arabidopsis APETALA2* gene (Figure 1), *WAP2A*^{Q} from the A genome of Chinese Spring wheat (CS), *WAP2A*^{q} from the A genome of *T. spelta*, and *WAP2D* from the D genome of CS. Since *WAP2B* from the B genome of CS harbors 531 nucleotides deletion containing the first to fourth exons (unpublished; Zang et al. 2011), *WAP2B* should be inactive. Thus, we omitted *WAP2B* from the present analyses. Earlier sequence analysis revealed several conserved features that might be important to AP2 protein structure or function (Jofuku et al. 1994; Riechmann and Meyerowitz 1998). First, AP2 contains a 37-amino acid serine-rich acidic domain (amino acids 14–50) that is known to act as an activation domain in a number of RNA polymerase II transcription factors (Mitchell and Tjian 1989). Second, AP2 has a highly basic 10-amino acid domain (amino acids 119–128) that includes a putative nuclear localization sequence, KKSR. Finally, the central core of the AP2 polypeptide (amino acids 129–288) contains two copies of a 68-amino acid direct repeat that are referred to as AP2 domains. These two copies of this repeat, designated AP2-R1 and AP2-R2, share 53% amino acid identity and 69% amino acid homology. Both domains are capable of forming amphipathic α-helical structures that may participate in protein–protein interactions and DNA binding (Jofuku et al. 1994; Rienchmann and Myerowitz 1998). Comparison of the AP2-predicted proteins from *Arabidopsis thaliana* and wheat AP2-like genes does not show much similarity in the serine-rich domain, whereas the KKSR sequence and AP2 domains are highly conserved between wheat and *Arabidopsis*. *WAP2A*^{q} differs from *WAP2A*^{Q} and *WAP2D* in a single amino acid at position 108 in the KKSR sequence, whereas WAP2A^{Q} and WAP2D possess lysine, and WAP2A^{q} confers glutamic acid (Figure 1). Two AP2 domains are identical among the wheat three genotypes, and almost identical with that of *Arabidopsis*. *Arabidopsis* AP2-R1 has 5 different single amino acids from those of wheat AP2-R1, whereas AP2-R2 of *Arabidopsis* differs from those of wheat in 11 amino acids. The three *WAP2A*^{Q}, *WAP2A*^{q}, and *WAP2D* genotypes used in this study differed in amino acid at position 329: *WAP2A*^{Q} and *WAP2A*^{q} possess isoleucine and valine, respectively, whereas *WAP2D* and *AP2* harbor leucine (Figure 1). In addition, *WAP2D* have two insertions-deletions at the N-terminus region and 4 more substitutions at the C-terminus region. An *AP2* mutant, *ap2-5*, carries two amino acid substitutions, that is, glycine to glutamic acid at position 159, which is positioned inside AP2-R1, and glutamine to glutamic acid at position 420 (Figure 1). In the phylogenetic relationships of *AP2s*, *WAP2A*^{q} is more closely related to *WAP2*^{Q} than *WAP2D* in the maize *ids1* cluster (Figure 2).

Overexpression of the WAP2 genes distinctly recovered flower phenotypes in the Arabidopsis AP2 mutant

To investigate the function(s) of wheat *WAP2* genes in

Figure 1. Sequence alignment of *APETALA2* of *Arabidopsis thaliana* and 3 wheat *APETALA2*-like products (WAP2A^{Q}, WAP2A^{q}, and WAP2D). The boxes indicate the AP2 domain-repeated motif AP2-R1 and AP2-R2. A highly acidic, serine-rich region and a basic region that contains a putative nuclear localization signal sequence, KKSR, are underlined in single and dotted lines, respectively. The arrows indicate the position of the different amino acids between WAP2A^{Q} and WAP2A^{q}. The arrow heads indicate the position of the *ap2-5* mutation.

the heterologous background of *Arabidopsis*, three wheat cDNAs, namely *WAP2A*^{Q}, *WAP2A*^{q}, and *WAP2D*, were overexpressed in the *Arabidopsis ap2-5* mutant (Kunst et al. 1989) under the 35S promoter control. The wild-type flowers of *Arabidopsis* consist of 4 sepals surrounding and alternating with 4 white petals. Within the whorl of the petals there are 6 stamens (4 long medial and 2 short lateral ones) and a pistil with two carpels. All the organs are arranged in concentric circles (Figure 3; Bowman et al. 1989). The *ap2-5* mutant reveals homoeotic transformation of median sepals to carpels and of petals to stamens (Figure 3).

Two homozygous T_2 lines conferring *35S:WAP2A*^{Q}, *35S:WAP2A*^{q}, and *35S:WAP2D* were characterized. Integrations of these cDNAs and their expressions were confirmed by PCR and/or RT-PCR using specific primers for their constructs (Figure 4). Because the strong promoter (35S) had driven their expression, ectopic expressions of three cDNAs were found in all of the transformed lines (Figure 4). Once the flowers of the T_2 plants were fully opened, their phenotypes were compared with those of the wild type.

The *35S:WAP2A*^{Q} in the *ap2-5* background completely rescued their phenotype and showed the wild *Arabidopsis* phenotype (Figures 3C and D). In contrast, the *35S:WAP2D* partially rescued their flower phenotypes (Figures 3E and F): petals were found as petal and sepal mosaics. Furthermore, white petal tissues in the outer margin of the organ and green tissues at the bottom were found. Although 4 sepals were observed, they did

Figure 2. Phylogenetic relationships of the *AP2* transcription factor among 14 members from 8 plant species. The phylogenetic tree was constructed using MEGA4 (Tamura et al. 2007) with the Neighbor-Joining method. Bar=0.1 amino acid substitution/site. Accession numbers: *Triticum aestivum* (WAp2A^{Q}, AB697000; and WAP2D, AB697002), *T. spelta* (WAP2A^{q}, AB697001), *T. urartu* (WAP2A (*T. urartu*), AAU94924), *Aegilops tauschii* (WAP2D (*Ae. tauschii*), ABY53104), *Hordeum vulgare* (AP2-like (Barley), AAL50205; and Cly 1 (Barley), cleistogamy 1, ACY29532), *Zea mays* (ids1 (Maize), indeterminate spikelet 1, NP_001104904; and sid1 (Maize), sister of intederminate spikelet 1, NP_001139539), *Oryza sativa* (AP2-like, Os03g0818800; and AP2-like, Os7g0235800), and *Arabidopsis thaliana* (APETALA2, AT4G36920; RAP2.7, Related to AP2.7, AT2G28550; RAP2.7, and AINTEGUMENTA, AT4G37750).

not properly cover the buds compared with those of the wild type and the *35S:WAP2A*^{Q} gene. *35S:WAP2A*^{q} did not recover the mutant flower phenotype. Although most *35S:WAP2A*^{q} plants showed similar phenotypes to that of the *ap2-5* line (Figure 3G and H), a few flowers had only one white petal each. The sepals were not as curved as the mutant flowers, but they still did not cover the buds. The stamens were shorter than those of the wild type. Thus, the three overexpressors revealed distinct flower phenotypes in terms of recovery abilities of the *AP2* mutant phenotype.

Homodimer formation activity of the three WAP2s in yeast

To examine and compare the potential of homodimer formation for three WAP2 proteins, a yeast two-hybrid system was used. The full-length cDNAs of *WAP2A*^{Q}, *WAP2A*^{q}, and *WAP2D* were cloned into the expression vector as bait and prey to form homodimers. All three transformants were grown on the SC medium lacking leucine, tryptophan, histidine, and adenine, indicating that the three WAP2s could form active homodimers (Figure 5A). Furthermore, the relative growth among them was compared by culture dilution. Figure 5B clearly shows that the growth of WAP2A^{Q} was high, that of WAP2A^{q} was medium, and that of WAP2D was relatively poor. These findings suggest distinct functional activities of the three WAP2s due to interactive homodimer formations.

Discussion

The *Q* gene of polyploid wheat pleiotropically governs spike characteristics. The *Q* had been cloned as an *APETALA2*-like gene of *Arabidopsis* (Simons et al. 2006). In *Arabidopsis*, the AP2 having two plant-specific AP2 domains is a floral homoerotic gene controlling flower developments (Bowman et al. 1989, 1993; Kunst et al. 1989; Jofuku et al. 1994). An EMS-induced *AP2* mutant

Figure 3. Overexpressions of the three wheat *AP2s* differently recover the flower phenotype of the *Arabidopsis ap2-5* mutant. The flower organs of Col (wild type) (A), *ap2-5* mutant (B), and overexpressors of *35S:WAP2A*^{Q} (C, *WAP2A*^{Q}*-6*; D, *WAP2A*^{Q}*-7*), *35S:WAP2D* (E, *WAP2D-1*; F, *WAP2D-11*), and *35S:WAP2A*^{q} (G, *WAP2A*^{q}*-17*; H, *WAP2A*^{q}*-18*).

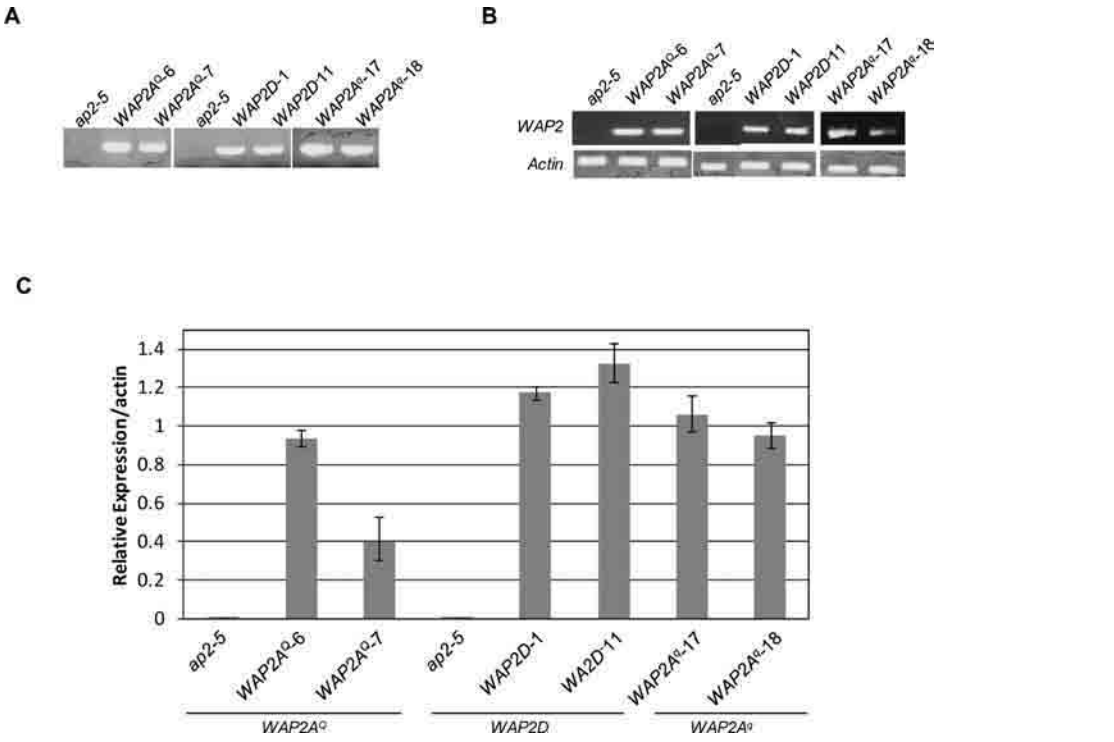

Figure 4. Ectopic expression of three wheat *AP2* genes. (A) Genomic polymerase chain reaction (PCR) to check the insert in the T_2 lines. (B) Reverse transcription PCR used to check the *WAP2s* expression in the T_2 lines. (C) Relative expressions of the *WAP2* genes compared with that of *ACTIN*. For line Nos. and their phenotypes, see Figure 3.

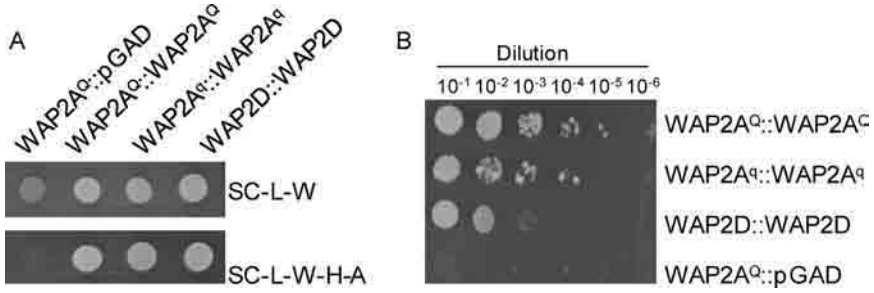

Figure 5. Homodimer formations of the three WAP2 proteins in yeast two-hybrid analysis. (A) The interactions to form the homodimers were examined between WAP2:pGBKT7 (bait) and WAP2:pGADT7 (prey) in the SC medium lacking leucine, tryptophan, histidine, and adenine (SC-L-W-H-A). (B) Comparison of the homodimer-forming ability among the three WAP2 proteins. The transformants were grown on the medium, diluted to 1/10–1/10^6, and were plated on the SC-L-W-H medium containing aminotriazole.

(*ap2-5*) shows homoeotic transformation of median sepals to carpels, and petals to stamens, although the androecium and gynoecium of the mutant remained normal (Kunst et al. 1989). In this study, we cloned wheat full-length cDNAs from the *Q* (*WAP2A*^{Q}), *q* (*WAP2A*^{q}), and homoeoallele of the D genome (*WAP2D*), and characterized their functions by overexpressing in the *AP2* mutant of *Arabidopsis* (*ap2-5*; Kunst et al. 1989) and yeast two-hybrid analyses. The amino acid comparison between the *AP2*-like genes indicates that *WAP2A*^{Q} and *WAP2A*^{q} were closely related and *WAP2D* was relatively divergent (Figure 2). Wheat *WAP2*s were grouped into the *indeterminate spikelet 1* (*ids1*) of maize (Chuck et al. 1998). Actually, only two amino acid changes between WAP2A^Q and WAP2A^q were found: one was located at position 108 (K to E in WAP2A^q), and the other was found at position 329 (I to V in WAP2A^q). In contrast, 7 amino acid changes of WAP2D were detected (Figure 1).

Overexpression of three *WAP2*-related cDNAs showed characteristic recoveries of the flower phenotype in the *ap2-5* mutant. The *35S:WAP2A*^{Q} recovered flower organs similar to the wild type (Figures 3C, D). The *35S:WAP2D* partially recovered the flower phenotypes (Figures 3E, F), and the *35S:WAP2A*^{q} least rescued homoeotic transformation (Figures 3G, H). Because the expression levels were not correlated with the flower phenotypes (Figure 4), the distinct recovery rates of each overexpressor could be attributed to the structural

alteration(s) of the AP2 proteins resulting from the amino acid substitutions.

The homodimerization rates of each protein were highest in WAP2^{Q}, medium in WAP2A^{q}, and lowest in WAP2D (Figure 7). This order was not consistent with the recovery rate of the flower phenotypes. It is well accepted that dimer formation is essential for transcription factor activation. Common amino acid changes among the three WAP2s were found only at position 329 (Figure 1). Although all three amino acids at this position were neutral α-polar ones (Ile in WAP2A^{Q}, Val in WAP2A^{q}, and Leu in WAP2D), the amino acid changes at this position might contribute to efficiencies of WAP2 homodimer formation efficiencies (Simons et al. 2006). However, it should be pointed out that in the AP2 of wild-type *Arabidopsis thaliana*, Col confers Leu at this position and a 12-amino acid deletion is seen just downstream of the position compared with WAP2As. Taking this into account, it can be said that protein structure(s) based on the primary arrangements around this region play important roles in dimer formation.

The *Arabidopsis* mutant (*ap2-5*) carries AP2 amino acid changes at the 159 (Gly to Glu) and 420 (Gln to Glu) positions. Both substitutions introduced polar changes of the target amino acids (neutral to acidic). The former change was located in the AP2-R1 domain, whereas the latter was detected around the C-terminus. Therefore, the amino acid change in the AP2-R1 domain may bring about abnormal function for flower development. On the other hand, wheat WAP2s did not harbor the amino acid changes in both AP2 domains. The *WAP2A^{q}* was least able to recover the flower formation in the *ap2-5* mutant of *Arabidopsis* among the three WAP2s. However, the dimer formation of WAP2A^{q} was better than that of WAP2D. This finding cannot be explained by the dimer formation alone owing to the amino acid change at the 319 position of WAP2A. The other substitution (Lys to Glu) between WAP2A^{Q} and WAP2A^{q} was found at position 108 (Figure 1). This substitution occurred at the first amino acid of the nuclear localization signal and introduced a drastic charge change (basic to acidic amino acid). This amino acid change might have disturbed nuclear localization of the WAP2A^{q} from the cytosol, although dimer formation was still well done. These less abilities of nuclear localization and dimer formation of WAP2A^{q} could be explained in part dosage effects of the *q* gene on spike morphology (Muramatsu 1963). Because *WAP2A^{q}* was believed to be an ancestral type of wheat domestication (Feldman 2001), this is a back mutation that is shared by most wheat species. On the other hand, the amino acid sequences of WAP2D were identical to those of WAP2A^{Q} in the regions of the two AP2 domains and nuclear localization signal: two insertions-deletions were found around the N-terminus, whereas 5 amino acid substitutions were situated around the C-terminus. This finding strongly suggests that amino acid changes at the N- and C-terminus regions brought about less dimer formation and that this structure decreased the recovery of flower development to some extent. Consequently, it can be concluded that WAP2A^{Q} functions appropriately to recover complete flower organs in *Arabidopsis*. Genetic resources and systems to analyze gene functions in *Arabidopsis* can provide powerful tools for understanding the characteristic genes of foreign plants such as wheat.

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