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Gibberellin deficiency pleiotropically induces culm bending in sorghum: an insight into sorghum semi-dwarf breeding

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Regulation of symmetrical cell growth in the culm is important for proper culm development. So far, the involvement of gibberellin (GA) in this process has not yet been demonstrated in sorghum. Here, we show that GA deficiency resulting from any loss-of-function mutation in four genes (*SbCPS1*, *SbKS1*, *SbKO1*, *SbKAO1*) involved in the early steps of GA biosynthesis, not only results in severe dwarfism but also in abnormal culm bending. Histological analysis of the bent culm revealed that the intrinsic bending was due to an uneven cell proliferation between the lower and upper sides of culm internodes. GA treatment alleviated the bending and dwarfism in mutants, whereas the GA biosynthesis inhibitor, uniconazole, induced such phenotypes in wild-type plants— both in a concentration-dependent manner, indicating an important role of GA in controlling erectness of the sorghum culm. Finally, we propose that because of the tight relationship between GA deficiency-induced dwarfism and culm bending in sorghum, GA-related mutations have unlikely been selected in the history of sorghum breeding, as could be inferred from previous QTL and association studies on sorghum plant height that did not pinpoint GA-related genes.

Dwarfism in plants is brought about by an irregularity in one or more of the various growth-related mechanisms, and may involve physical defects in some cellular growth processes, or problems in the production and action of phytohormones. So far, aberrant cellular division or elongation has already been reported to cause dwarfism in some mutants such as the rice *d6*[1], *bent uppermost internode 1* (*bui1*)[2], *jmj703*[3] and *dwarf and gladius leaf* (*dgl-1*)[4], and the *Arabidopsis lue1*[5]. However, the relationship between dwarfism and phytohormones has been more widely studied using various kinds of plant species. In the case of auxin, some of the reported dwarf mutants include the maize *brachytic 2* (*br2*)[6,7] and the sorghum *dwarf 3* (*dw3*)[6] which both have semi-dwarfism due to a defect in auxin transport; the rice *small organ 1* (*smos1*), which has a defective transcription factor that disrupts auxin signaling[8], and the auxin-deficient *tdd1*[9]; and the *Arabidopsis sax1* which shows high sensitivity to auxin[10], and *bud1* which has a defect in auxin metabolism[11]. Another growth-promoting hormone is brassinosteroid (BR). Mutants that have a defect in BR biosynthesis and signalling show severe dwarf phenotypes as in the case of the *Arabidopsis* BR-deficient *de-etiolated 2* (*det2*)[12] and *seuss-1* (*seu-1*)[13], and the brassinosteroid-insensitive *bri1*[14]; the rice BR signalling mutants, *d61-4*[15] and *erect leaf 1* (*elf1-1*)[16], and the BR-deficient *ebisu dwarf* (*d2*)[17]; and the tomato *br6ox* (BR biosynthesis) mutant "Micro-Tom", a commercial cultivar[18]. BR-related mutations can also give rise to semi-dwarfism, and among them, a barley mutant, *uzu*, was used for producing a lodging-resistant variety[19]. Recently, strigolactones (SLs), a group of newly identified plant hormones that control plant shoot branching, have also been implicated for dwarfism in high-tillering dwarf mutants as in the case of the SL-deficient *d10* and the SL-insensitive *d53* and *d14* mutants of rice[20,21].

Among the phytohormones, gibberellin (GA) is the most well known to be involved in controlling stem elongation, and a deficiency or insensitivity to GA could easily result in severe dwarfism as reported in many different kinds of plant species such as the rice mutants independently mutated in any of the six GA biosynthetic enzymes, ent-copalyl diphosphate synthase (CPS), ent-kaurene synthase (KS), ent-kaurene oxidase (KO), ent-kaurenoic acid oxidase (KAO), GA 20 oxidase (GA20ox), and GA 3-oxidase (GA3ox)[22]; the pea GA-deficient *na-1*[23]; the *Arabidopsis* GA-insensitive *short internodes* (*shi*)[24] and *ga-insensitive* (*gai*)[25], and the GA-deficient *ga1 – 3* mutants[26]; the barley GA-deficient *grd2c* mutant[27], and the potato *andigena* (*adg*) mutant[28]. Across different

species, GA-related mutants with severe dwarfism show several consensus phenotypes such as small dark-green leaves, delayed germination, defective flowering, reduced seed production and male sterility[22,28–34], whereas, semi-dwarf mutants only show mild height reduction with no adverse effects on overall morphology or agronomical traits. This is the reason why GA-related mutants with semi-dwarfism were widely used in the history of crop production. Actually, the rice *semi-dwarf1* (*sd1*) and wheat *Reduced height-1* (*Rht-1*) mutants became the highlights of the Green Revolution, which avoided the imminent food shortage of the mid-20th century[35]. Later on, the *SD1* gene was characterized as a GA 20-oxidase[36,37], which catalyses the penultimate step of GA biosynthesis, while *Rht-1* gene was found to encode a constitutively active repressor of GA signaling[38]. Further, another GA-deficient mutant defective in KO, *Tan-ginbozu/d35*, also contributed to rice crop productivity due to its lodging resistance[39].

In contrast to the other plant species above, there are still no other reports of dwarfing mechanisms (including those relating to GA) in sorghum aside from the auxin-related *dw3* mutant mentioned earlier. Until now, even the underlying mechanisms behind the major dwarfing QTL genes (*dw1*, *dw2* and *dw4*) used in the history of sorghum breeding have remained unknown[40–42]. Aside from these classical dwarfing genes, increasing the number of available dwarfing mechanisms for sorghum should be important not only for breeding but also for basic research. Thus, in this study, we mutagenized sweet sorghum to create a mutant library, and searched it for plants with varying degrees of dwarfism. In the process, we found five severe dwarf mutants showing identical unusually bent culms which have never been reported in sorghum. We hereby attempted to isolate and analyse these mutants namely, *bending dwarf 1* (*bdw1*), *bdw2*, *bdw3-1*, *bdw3-2* and *bdw4*. We found that the mutants were mutated in four GA biosynthetic genes encoding enzymes catalysing the earlier steps of GA biosynthesis (Supplementary Fig. S1), and that their loss of function directly promotes culm bending. Thus, we reveal for the first time that GA-deficiency can pleiotropically induce culm bending or prostrate growth in sorghum. We also hypothesize that this could possibly be related to why GA-related mutations have not been utilized for sorghum breeding, unlike the case of rice and wheat.

Results

The *bdw* mutants show an abnormal bent culm. In order to increase the diversity of sorghum dwarf mutants and to further our knowledge of sorghum dwarfing mechanisms, we produced a mutant library through gamma-ray irradiation. During the process of screening for dwarf phenotypes, we isolated several unusual dwarf mutants, which developed bent culms. Succeeding analyses revealed that they were mutated in four different loci, thus we named them as *bending dwarf* (*bdw*)1 - *bdw4*, with *bdw3* having two different alleles (*bdw3-1* and -*2*). At the seedling stage, the bending culm phenotype was not evident in the mutants (Fig. 1a) but severe inhibition of internode elongation was observed (Fig. 1b). At about three and a half months after sowing, the bent culm phenotype was clearly observed (Fig. 1c). The mutants bent in a prostrate manner with leaves extending from the sides of the culm (rightmost in Fig. 1c).

In order to study the underlying reason for the bending, we carried out a histological analysis of the *bdw3-1* culm (Fig. 1d). The cell length at the upper side of the bent region was significantly shorter than that of the lower side (Fig. 1d, e), suggesting that there is a faster rate of cell proliferation at the upper side. Actually, the cell number at the upper side was more than two-folds higher than that at the lower side (Fig. 1f). These observations suggest that the bending was due to a difference in cell proliferation rates between the upper and lower sides of the bent culm.

The *bdw* mutants have reduced gravitropic response. Since we suspected that the gravitropic perception of the mutants might be

defective, we directly examined the response of the *bdw1* culm to gravity at two stages, seedling and vegetative (Fig. 2). The aerial part of *bdw1* at the seedling stage responded positively to gravistimulation, but with a much weaker response compared to the WT (Fig. 2a), possibly due to its naturally inhibited culm elongation. Such weak gravitropic response was also observed at the late vegetative stage (Fig. 2b). Aside from the culm, the roots of *bdw1* also responded positively but much more slowly to gravistimulation at the seedling stage (Fig. 2a), as observed in another *bdw* mutant, *bdw3-1* (Supplementary Fig. S2), strongly suggesting that the *bdw* mutants have diminished ability to respond to gravity.

A loss of function of GA biosynthetic genes causes the bending dwarf phenotype. To further characterize the *bdw* mutants, we carried out positional cloning of the *BDW3* gene since there was a pair of allelic mutants for this gene among the five *bdw* mutants. We used F_2 plants derived from the cross between *bdw3-1* and the cultivar bmr-6. These plants segregated into two phenotypic groups, tall and dwarf, with a segregation ratio of 3 : 1 (P<0.05), respectively. By genotyping 249 F_2 plants, we narrowed down the locus of *BDW3* to approximately 4.0-Mb region between the markers Sb5370 and SSR5_5423 (Fig. 3a). This region contains 283 genes annotated in the sorghum genomic DNA sequence database (http://www.phytozome.net/), including *Sobic.010G172700* (Supplementary Table S1), a gene which is homologous to the rice *ent-kaurene oxidase* (*KO*) that encodes a GA biosynthetic enzyme[22]. In rice and *Arabidopsis*, the loss of function of KO causes a severe dwarf phenotype with small dark-green leaves[22,43], which was also evident in *bdw3-1* except that it had an added bent culm trait (Fig. 1c). The deduced amino acid sequence of *Sobic.010G172700* showed a high similarity to the entire sequence of rice KO (67%) and also to that of *Arabidopsis* KO (56%) (Supplementary Fig. S3c)[22,43]. We searched for mutations in the genomic DNA sequence encompassing the *SbKO* gene by PCR, and obtained no PCR products from the genome of *bdw3-1* (upright red triangles, Fig. 3b), whereas the same primers produced PCR products from the WT genome at the same condition. By using primers covering the flanking sequences of the *SbKO1* gene, we successfully located sequences that yielded PCR products (upright blue triangles KO1, KO2, KO18 and KO19, Fig. 3b) and predicted the deletion around the *SbKO1* gene to be at about 45 kb from KO3 to KO17. We also performed the same experiment on another *bdw3* mutant, *bdw3-2*, and found that it contained a shorter deletion (~17 kb) involving all portions of its *SbKO1* gene (inverted red or blue triangles in Fig. 3b). A genomic DNA blotting analysis using the promoter sequence of the *SbKO1* gene as probe also confirmed the large deletion in the corresponding region of the *bdw3-1* genome (Supplementary Fig. S4c, d).

The above results suggest that mutation in the *SbKO1* gene could be the reason for the severe dwarfism in the *bdw3* mutants. To determine whether such loss of function of GA biosynthetic genes is behind the phenotype of all the *bdw* mutants, we examined the sequences of the other GA biosynthetic genes (*CPS*, *KS*, *KAO*, *GA20ox*, and *GA3ox*) in the remaining mutants. As expected, the genome of *bdw1* contained an approximately 16-kb deletion involving the entire genomic region of *SbCPS1* (*Sobic.001G248600*) (Fig. 3c; Supplementary Fig. S4a, b). That of *bdw2* had a one-nucleotide deletion in the exon 3 of *SbKS1* (*Sobic.006G211500*) (Fig. 3d), while *bdw4* contained a five-nucleotide deletion in the exon 2 of *SbKAO1* (*Sobic.010G007700*) (Fig. 3e). These demonstrate that all of the five *bdw* mutants have defects in GA biosynthetic genes.

To confirm that the concerned GA biosynthetic genes in sorghum really function *in vivo*, we used them to transform corresponding GA-deficient mutants of rice, namely, *oscps1-1*, *osks1-1*, *osko2-1* and *oskao-1*[22]. We introduced a 7,545-bp sorghum genomic DNA frag-
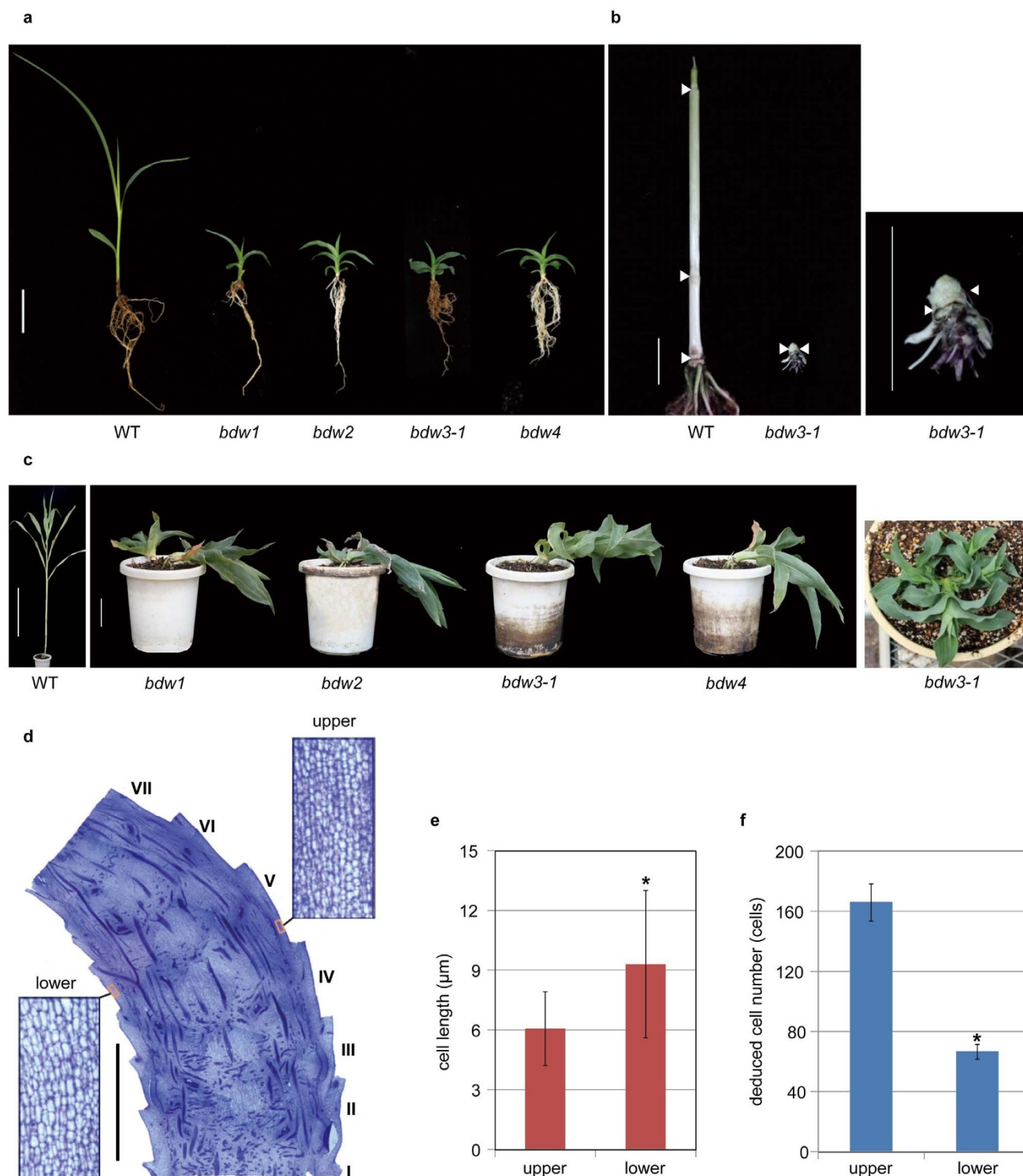
Figure 1 | Phenotypic analysis of the *bdw* mutants. (a) The *bdw* and WT plants at the seedling stage (30 days after germination, DAS). Bar = 4 cm. (b) Culm structure of WT (left) and *bdw3* (center and right) at the vegetative stage. Bars = 2 cm. Nodes are indicated by arrowheads. (c) WT and the bent *bdw* mutants at the vegetative stage (110 DAS). Top view of two-month old *bdw3* is shown at the rightmost panel. Bars: 50 cm, WT; 4 cm, mutants. (d) Longitudinal section of *bdw3* culm. Roman numerals at the right side indicate internode position from bottom to top. Bar = 5 mm. (e–f) The length and number of cells at the upper and lower sides of the fifth internode in the bent region shown in panel (d). Error bars for cell length represent the standard deviation calculated from approximately 100 cells. Error bars for cell number represent the standard deviation calculated from 5 cell files (see Methods). Asterisks indicate significant differences at 0.1% (P<0.001).

ment containing the entire *SbKO1* sequence into rice *osko2-1* via *Agrobacterium tumefaciens*-mediated transformation and found that it completely rescued the severe dwarfism of the mutant (Fig. 4). Similarly, introduction of *SbCPS1*, *SbKS1*, and *SbKAO1* genes res-

cued the dwarfism of corresponding rice mutants (Fig. 4). These observations confirmed that the isolated sorghum genes have biological activity in rice, and therefore the dwarf phenotype of the *bdw* mutants should be caused by the loss of function of these genes.
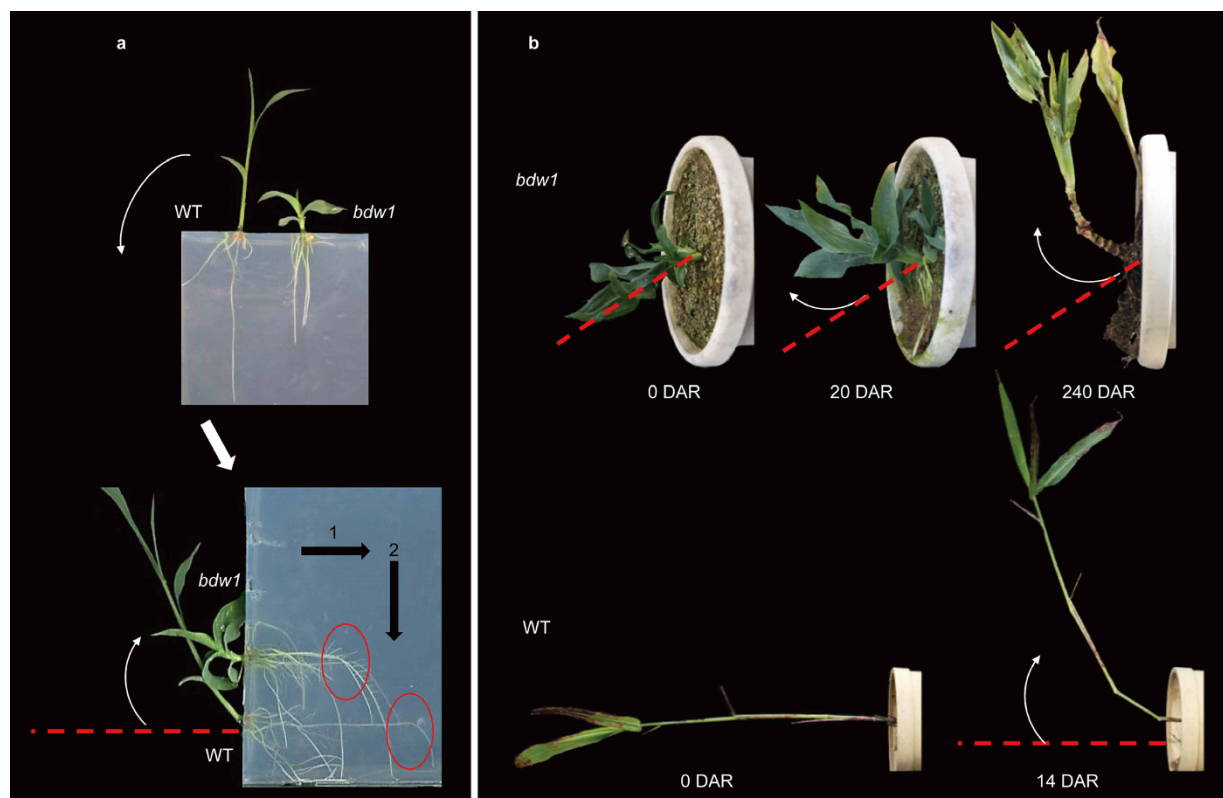
Figure 2 | Gravitropism test of *bdw1*. (a) The gravitropic response at the seedling stage. Top, plants at 4 days after germination (DAG) in upright position before gravistimulation; bottom, 4-DAG plants were rotated 90 degrees and further grown for 4 days after rotation (DAR). The horizontal "1" and vertical "2" arrows indicate the gravity directions before and after rotation, respectively. (b) The gravitropic response of *bdw1* (top) and WT (bottom) at the vegetative stage. Red dashed lines indicate the original direction of main culm before rotation. White arrows indicate the direction of culm movement.

GA rescues the bending culm phenotype, whereas, uniconazole induces bending in WT plants. The results described above indicate that loss of function of GA biosynthetic genes causes dwarfism accompanied by unusual culm bending in the mutants. We further confirmed that GA deficiency causes the culm bending by using two approaches. First, we treated the *bdw3-1* mutant with different concentrations of GA_3 for 4 months. Both the dwarf phenotype and the bending culm phenotype were rescued in a dose-dependent manner by GA_3, and were almost normal at 10^{-4} M GA_3 (Fig. 5). At 10^{-6} M GA_3, the dwarfism was partially rescued to about 60% but the culm remained at a significantly bent state (Fig. 5). Secondly, we treated WT with various concentrations of uniconazole (Fig. 6), an inhibitor of GA biosynthesis. As a result, treatment with 10^{-5} M uniconazole induced WT to bend in a similar manner observed in the mutants (Fig. 6a). At 3×10^{-6} M uniconazole, the plant height was reduced to about 25% while the bending was significantly induced from 0 to about 5 degrees against the vertical axis. These results show that the alleviation of the dwarf and bent phenotype of the mutants by GA was exactly opposite to the effect of uniconazole on WT plants, and that dwarfism and bending are tightly correlated (Fig. 5a, 6a).

Discussion

In this study, we were able to isolate five dwarf mutants (*bdw1*, *2*, *3-1*, *3-2*, and *4*) with abnormal culm bending from a mutant population of sweet sorghum (Fig. 1). Each of the *bdw* mutants had a defect in a specific GA biosynthetic gene, namely, *SbCPS1*, *SbKS1*, *SbKO1* and *SbKAO1* (Fig. 3; Supplementary Fig. S1)— the genes involved in the early part of GA biosynthesis. Further analyses of these mutants revealed that both dwarfism and culm bending were caused by GA deficiency (Fig. 5, 6).

Although previous studies in maize, rice, and *Arabidopsis* have shown that loss-of-function mutations in GA biosynthetic genes result in the suppression of internode elongation and dwarfism[22,43,44], there was no mention of culm bending or GA being involved in such a phenomenon. In barley, however, the loss of function of GA3ox1, which is involved in the last step of GA biosynthesis, resulted not only in dwarfism but also prostrate growth in the *grd2c* mutant[27]. Also in wheat, a strong allele of *Rht-B1*, *Rht-B1c*, induced severe dwarfism that correlated with an increase in tiller angle[45]. These indicate that culm bending (prostrate growth) is one of the pleiotropic effects of GA-related gene mutations and such event appears to be species-dependent. Our conclusion that GA deficiency causes culm bending in sorghum was supported by three experimental evidences. First, the independent loss-of-function mutants defective in four different GA biosynthetic genes were found to have bent culms (Fig. 1, 3). Second, the bent culm phenotype was reverted by exogenous GA treatment in a dose-dependent manner (Fig. 5). Lastly, uniconazole treatment of WT mimicked the bending of the mutants also in a dose-dependent manner (Fig. 6).

Although the exact molecular mechanism controlling bending in sorghum under GA deficiency is still unclear, there was a strong indication that the bending in the *bdw3* mutants was due to the asymmetric growth of culm internodes (Fig. 1d, f), which could be partially due to the reduced gravitropic response of the mutants as compared to the WT (Fig. 2; Supplementary Fig. S2). A comparable situation was observed in the GA-deficient barley *grd2c* mutant[27], which showed a slower pulvinar gravitropic response than the WT, and to a greater extent, to *sln1c*, a GA-hypersensitive mutant. The evidence indicated that gravity-induced auxin asymmetry leads to an asymmetry of GA distribution, and that GAs play a role in facilitating faster gravitropic response by inducing rapid cell elongation on the
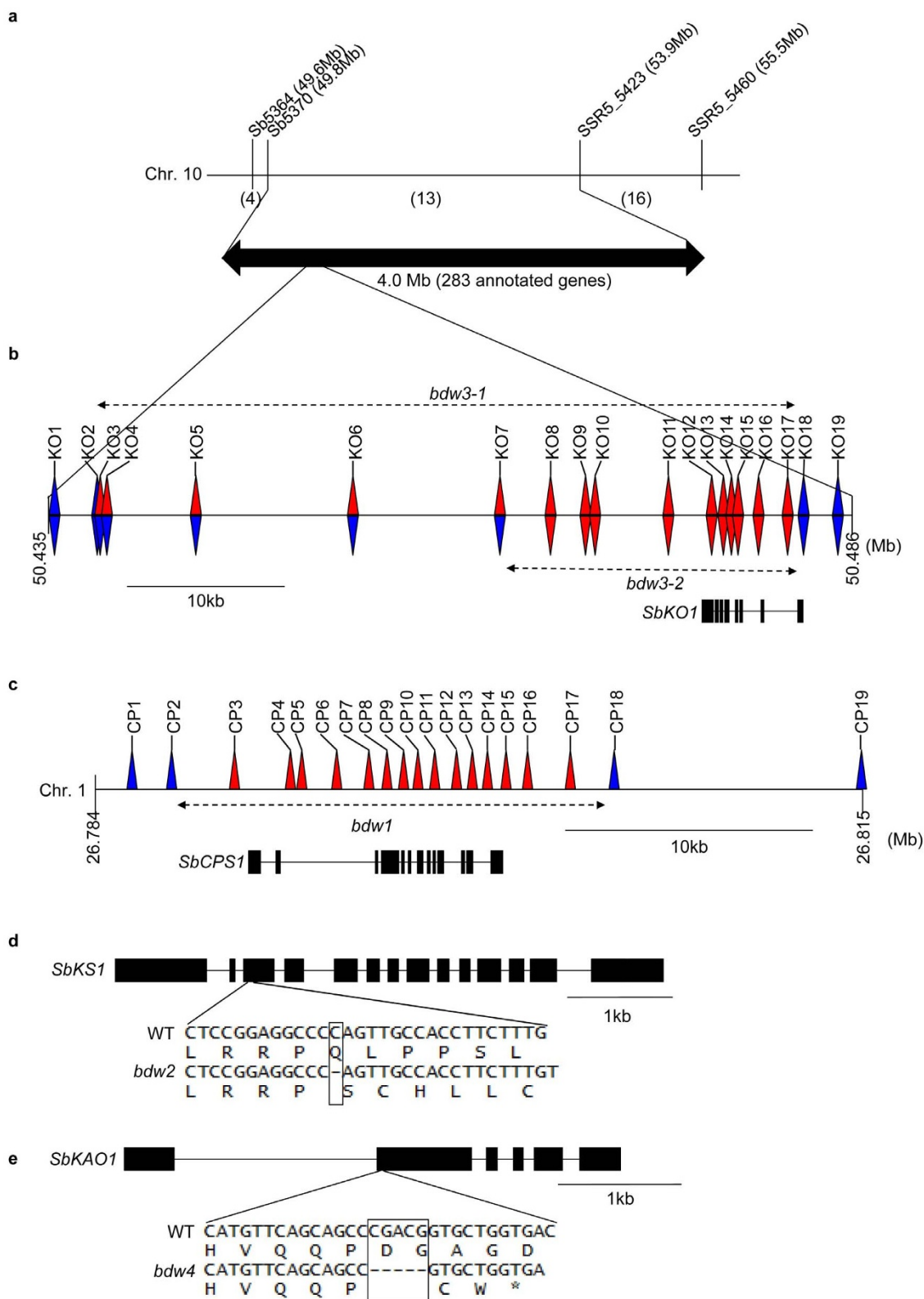
Figure 3 | Identification of the causal genes of the *bdw* mutants. (a) A physical map of the candidate region of *bdw3*. The top line shows the position of markers around the target region of chromosome 10. The numbers in parentheses indicate the number of recombinant plants between the markers. The *bdw3* mutation locates between Sb5370 and SSR5_5423. (b) Deletion in the *bdw3-1* and *bdw3-2* genome as detected by PCR analysis. Presence or absence of PCR products is indicated by blue and red triangles, respectively, whereas all PCR products were successfully obtained from the WT genome. Upright and inverted triangles correspond to the *bdw3-1* and *bdw3-2* genomes, respectively. Dashed arrows at the top and beneath the triangles show estimated deletions around the *SbKO1* gene. The *SbKO1* structure is shown at the bottom, where boxes and lines between them indicate exons and introns, respectively. (c) Deletion in the *bdw1* genome as detected by PCR analysis. The presentation is the same as in panel (b). (d) One-nucleotide deletion in the *SbKS1* gene of *bdw2*. The presentation is the same as in panel (b). (e) Five-nucleotide deletion in *SbKAO1* gene of *bdw4*. The presentation is the same as in panel (b).

lower side of the pulvini, following an asymmetric localization of auxin which serves as an initial trigger for bending. Further, just like the *bdw* mutants, the barley *grd2c* mutant was also reported to have a prostrate growth habit and GA appears to be affecting the gravitropic set-point angle (GSA) of its lateral shoots (tillers)[27]. Recently, using pea and *Arabidopsis*, GSA values were found to be dynamically specified by auxin throughout development by regulating the magnitude of the anti-gravitropic offset component via TIR1/AFB-Aux/IAA-ARF-dependent auxin signalling within the gravity-sensing cells of the root and shoot[46], suggesting that the bending in the *bdw* mutants could also be auxin-related. Our results show that GA deficiency triggers prostrate growth habit in sorghum in the presence of auxin, which is consistent with the case of barley *grd2c*[27], strongly indicating that GA also has a crucial role in controlling the GSA of the culm, at least in these species.

Mutations involving partial GA-deficiency and semi-dominant GA-insensitivity have long been harnessed in breeding programs to induce the favourable semi-dwarf trait made famous by the Green Revolution rice (*sd1*) and wheat (*Rht-B1b* and *Rht-D1b*), respectively[36,38]. In such instances, semi-dwarfism led to increased lodging resistance and high yield. However, the present study strongly suggests that the use of GA-related genes for sorghum breeding remains to be a big challenge because of the bending side-effect linked with GA deficiency. Actually, results presented in Fig. 5 and 6 demonstrated that even semi-dwarf sorghum plants show a potentially destabilizing (e.g. lodging-prone) culm curvature under reduced GA levels. Our assumptions are also being upheld by the fact that there are still no reports of GA-related genes used for sorghum semi-dwarf breeding to date.

So far, it is widely known that four major classical dwarfing genes (*dw1* - *dw4*) had been introgressed into elite varieties in the early history of sorghum breeding[47–51]. Among these dwarf genes, only the causal gene of *dw3* had been identified, namely, a gene encoding a phosphoglycoprotein involved in auxin transport[6]. Because the loca-tion of *dw1*, *dw2* and *dw4* have also been identified[40–42,52] (Supplementary Table S2), we compared them against the loci of GA-related genes (Fig. 7 and Supplementary Table S1) and found that the loci of *dw2*, *dw3* and *dw4* do not coincide with mapped positions of known GA-related genes. On the other hand, *dw1* is in close proximity to a *GA 2-oxidase*-like gene (Fig. 7), *Sobic.009G230800* (referred to as *GA2ox5* in Supplementary Table S1), which was previously discussed by two independent groups to be the possible causal gene of *dw1*[40,41]. However, based on our experiments, it is unlikely that a *GA2ox* is behind the phenotype of *dw1* because in that case, bending would become inevitable. Since GA2ox is involved in GA catabolism, its effect on sorghum should be similar to that of a GA synthesis inhibitor, uniconazole. To investigate whether *dw1* is the same as *GA2ox5*, we directly compared the DNA sequence and expression level of the gene in a tall (*Dw1Dw1*) and dwarf (*dw1dw1*) isogenic sorghum cultivar carrying gain-of-function and loss-of-function alleles of *Dw1*, respectively. As expected, there was no difference in the genome sequence and expression level of *GA2ox5* between the two cultivars (Supplementary Fig. S5). This result supports the above idea that a *GA2ox* is unlikely to be the causal gene of *dw1*.

As mentioned earlier, in wheat, the use of a strong allele of *Rht-B1* caused severe dwarfism and bending[45], and such traits were further aggravated by overexpression of a GA-inactivating enzyme, GA2ox[29,53]. In contrast, the weak *Rht-B1* mutation used in the Green Revolution only resulted in semi-dwarfism, with no obvious bending[38]. On the other hand, the Green Revolution rice and even the severe dwarf rice mutants carrying null alleles for GA biosynthetic or signalling genes do not show culm bending at all[22,36,54]. These observations suggest that, because of the exceptionally tight relationship between GA deficiency-induced dwarfism and bending in sorghum, the use of GA-related mutations to induce semi-dwarfism, without compromising the straightness and lodging resistance of the culm, may not be possible. Taken altogether, our results strongly suggest

Figure 4 | Introduction of sorghum GA biosynthetic genes rescued the corresponding mutants of rice, *oscps1-1*, *osks1-1*, *osko2-1*, and *oskao-1*. Scale bar = 4 cm. Plants at the left and right in each set were transformed with the complementary WT genes from sorghum and the vector control, respectively.
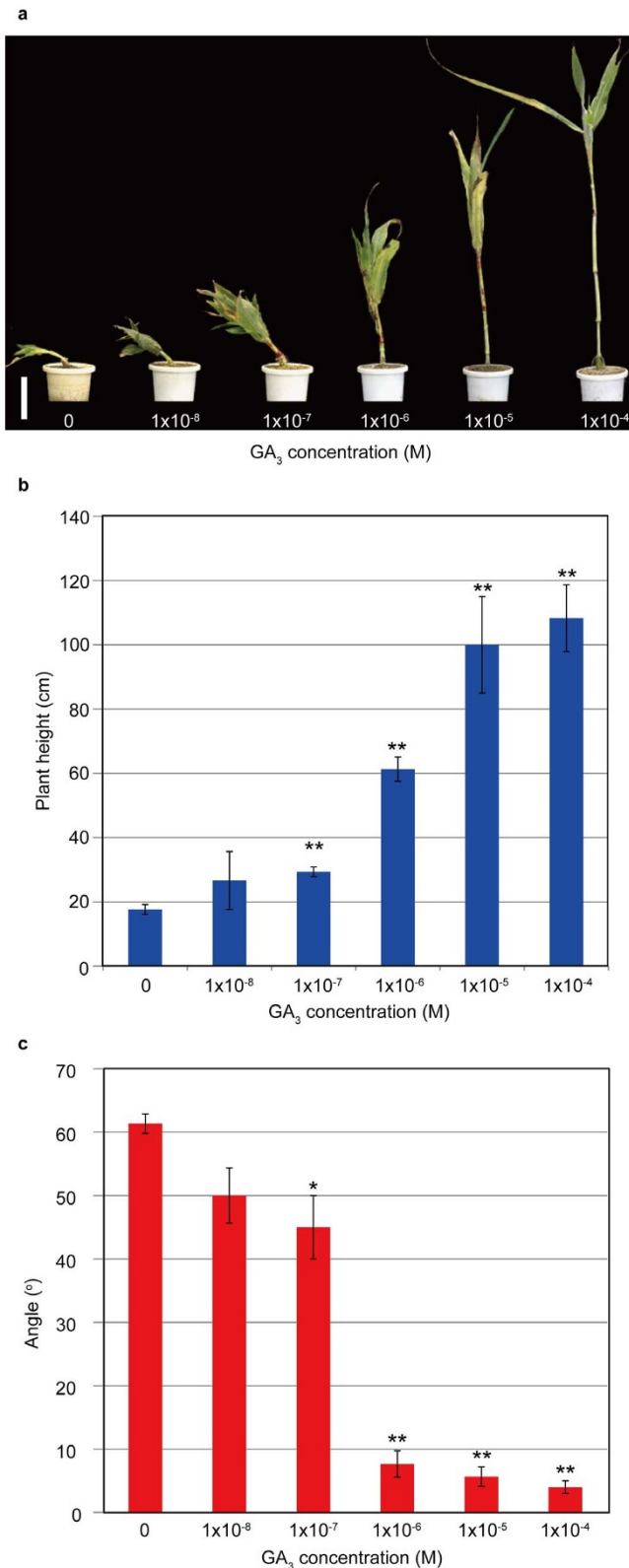
Figure 5 | Dose-dependent recovery of the mutant phenotypes by exogenous GA treatment. (a) The *bdw3-1* plants grown in different concentrations of GA_3 for four months. Bar = 10 cm. (b) Dose-dependent recovery of plant height. (c) Dose-dependent recovery of the bending culm phenotype, which was determined by measuring the angle between the main culm and the vertical axis. Error bars represent the standard deviation calculated from 3 replicates. Single (*) and double asterisks (**) indicate significant difference with the mock (0 M GA_3) at 0.5% (P<0.005) and 0.1% (P<0.001) levels of significance, respectively, as determined by t-test.
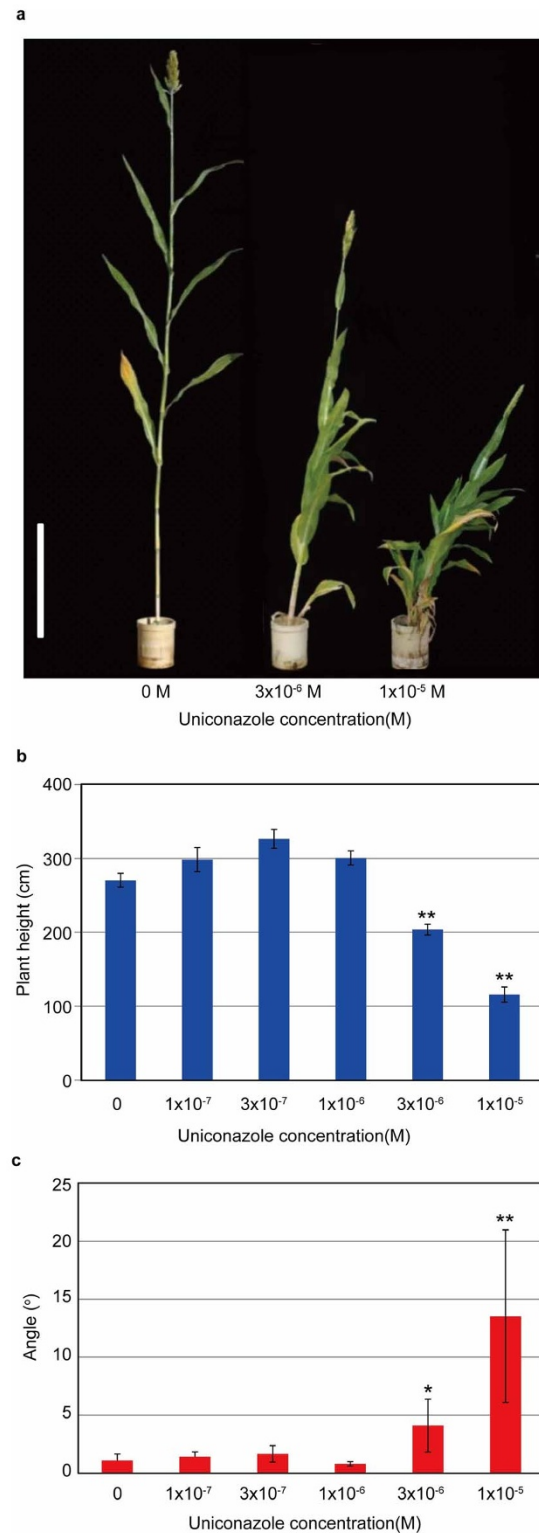
Figure 6 | Effect of uniconazole on the plant height and culm bending of WT plants. (a) Gross morphology of the uniconazole-treated plants. The plants at the left, center, and right were treated with 0, 3×10^{-6} and 1×10^{-5} M uniconazole, respectively. Bar = 50 cm. Dose-dependent inhibition of plant height (b) and induction of culm bending (c) in WT plants. Error bars represent the standard deviation calculated from 5 replicates. Single (*) and double asterisks (**) indicate significant difference between the mock and 3×10^{-6} and 1×10^{-5} M uniconazole at 5% (P<0.05) and 1% (P<0.01) levels of significance, respectively, as determined by t-test.

that the classical dwarfing genes that have been utilized for sorghum breeding in the past may not be related to GA. As in the case of $dw3$[6], the isolation and characterization of $dw1$, along with those of $dw2$ and $dw4$ will reveal novel mechanism(s) for semi-dwarfism in sorghum. Once identified, these genes can also be exploited in rice and other crops as additional or alternative options for lodging resistance breeding.

Methods

Plant materials and mutant library construction. We used *Sorghum bicolor* cv. SIL-05 (sweet sorghum) for constructing a mutant library. Mutagenesis was carried out by irradiating seeds with gamma-ray (175–250 Gray (Gy)). The resulting M_1 seeds were sown in seedling trays and were grown inside the greenhouse at 25°C for more than 3 weeks before planting in the field. About 5,000 of such plants were self-pollinated to obtain M_2 lines which were screened for dwarfism at the seedling (greenhouse) and vegetative (field) stages. Identified mutants and all plants for analysis were grown in pots inside the greenhouse under ambient lighting conditions.

Microscopic analysis. A culm longitudinal section of the *bdw3-1* mutant was made with a cryotome. For staining, hematoxylin and eosin stains were used. Photographs were taken using an Olympus photomicroscope connected to a CCD camera. Resulting photographs were stitched to create a panoramic image (Photostitch, Canon). In order to understand the underlying cause of the bending in the *bdw3-1* mutant, the length and number of the cells at both the upper side and lower side of the fifth internode, where the greatest degree of bending took place, was quantified. This was done by measuring the length and number of cells in 5 cell files within a 500-μm long region (orange rectangles, Fig. 1d) at both the upper and lower sides of the fifth internode and then estimating the total number of cells in the internode.

Gravitropism test. Seeds of the *bdw1* and *bdw3-1* mutants (as representatives of the phenotypically identical *bdw* mutants) and WT were disinfected with 0.5% benlate overnight at 4°C and sowed on MS medium with 0.9% agar in plastic rectangular culture plates. Plants were grown for 4 days after germination (DAG) in an upright position and then rotated 90 degrees and examined 4 days after rotation (DAR). The

culture plates were imaged by using a flatbed digital scanner. Also, bent *bdw1* plants at the vegetative stage were rotated 90 degrees following the direction of culm bending and compared their response with the WT.

Genome mapping of the *bdw3* locus and sequence analysis. To carry out gene mapping, we crossed heterozygous siblings of the *bdw3-1* mutant (sweet sorghum (SIL-05) background) with bmr-6 (grain sorghum cultivar) to produce F_1 plants that were progeny-tested to obtain the desired F_2 population for analysis. Segregation ratio of the tall and dwarf phenotypes was observed and analysed by chi-square test.

We screened about 1,500 Simple Sequence Repeats (SSR) markers[55] and selected 162 polymorphic markers. For rough mapping of the candidate region, we used the SSR markers on 16 F_2 seedlings with tall phenotype. For the fine mapping, we expanded the analysis to 249 F_2 tall plants.

We used the amino acid sequences of known GA biosynthetic genes of rice as queries for the TBLASTN program (http://www.phytozome.net/search.php?show=blast&org=Org_Sbicolor_v2.1) to identify homologous sequences in the sorghum genome. PCR analysis of the identified genes, namely, *SbCPS1* (*Sobic.001G248600*), *SbKS1* (*Sobic.006G211500*), *SbKO1* (*Sobic.010G172700*), *SbKAO1* (*Sobic.010G007700*), *SbGA20ox2* (*Sobic.003G379500*) and *SbGA3ox2* (*Sobic.003G045900*) was done on all the *bdw* mutants and the WT. PCR primers used are listed in Supplementary Table S3. DNA sequencing was done with the ABI Prism 310-10 sequencer following the manufacturer's protocol.

Amino acid sequences of sorghum CPS1, KS1, KO1 and KAO1 were compared with those of other species by aligning with ClustalW (http://www.genome.jp/tools/clustalw/) using default settings (slow/accurate) and then manually adjusted to optimize alignments. The unrooted phylogenetic tree with branch length (N-J) tree file output was used to create a phylogenetic tree with FigTree v1.4.0 (http://tree.bio.ed.ac.uk/software/figtree/) using default settings for the rectangular tree layout.

For DNA gel blot analysis of *SbCPS1* and *SbKO1*, 1 μg genomic DNAs from *bdw1*, *bdw3-1* and WT were analysed as previously described[56]. Primers used to amplify the probes are listed in Supplementary Table S3.

Complementation of rice GA-deficient mutants with WT GA biosynthetic genes from sorghum. To examine the bioactivities of the identified sorghum GA biosynthetic genes, we first cloned 11,287, 6,938, 7,628, and 5,909 bp of sorghum genomic DNA fragments containing entire *SbCPS1*, *SbKS1*, *SbKO1* and *SbKAO1*
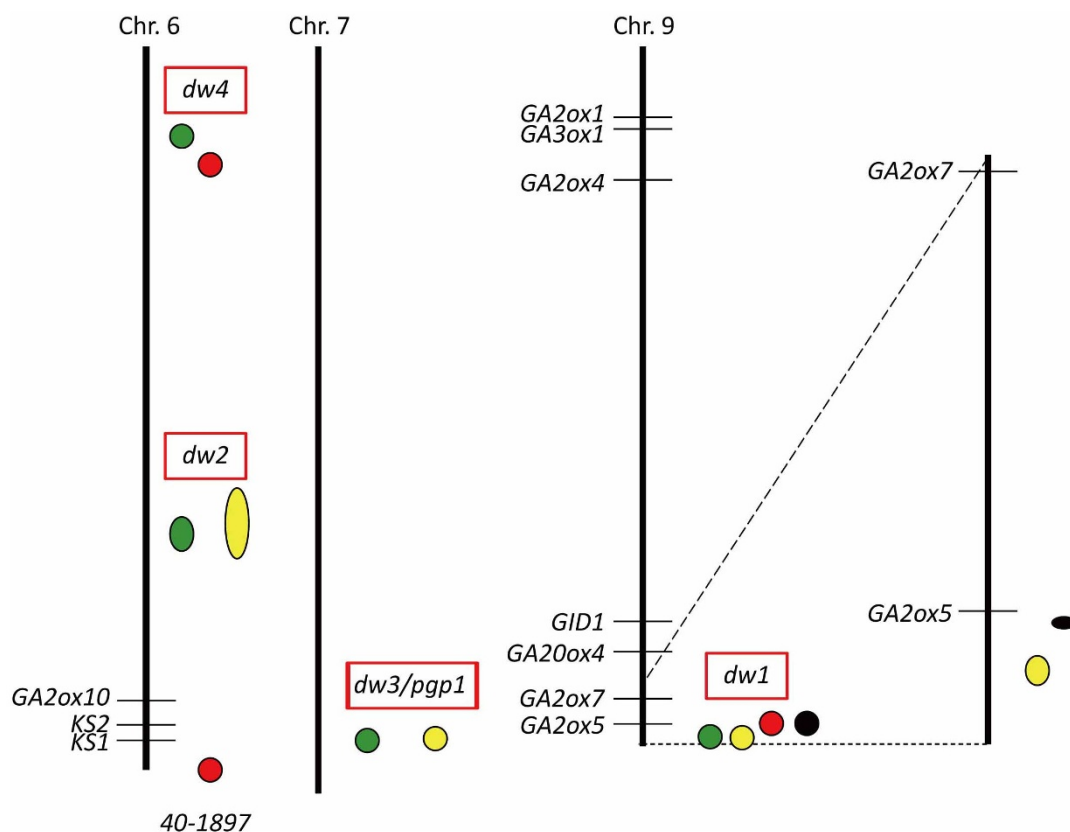
Figure 7 | Comparison between the map positions of previously reported dwarfing QTL genes and the GA-related genes of sorghum. The chromosomes carrying the dwarfing QTL genes previously reported are presented. Ovals and circles at the right side of each chromosome indicate the positions of respective previously reported dwarfing QTLs (red rectangles), where green, yellow, red and black shades represent the mapping work of Morris *et al.* (2013), Thurber *et al.* (2013), Wang *et al.* (2012) and Brown *et al.* (2008), respectively. The positions of GA-related genes are presented at the left side of each chromosome with their corresponding names as presented in Supplementary Table S1.

genes along with their native promoters into a pCAMBIA1300 binary vector, respectively. These chimeric plasmids were introduced into corresponding mutants of rice, oscps1-1, osks1-1, osko2-1 and oskao-1, through *Agrobacterium*-mediated transformation[22,57]. The transformants were screened on selection medium with hygromycin and the regenerated plants were documented.

Exogenous gibberellin treatment and uniconazole treatment. For GA treatment, two-week old *bdw3* seedlings were transplanted into pots. A set of three pots (with holes in the bottom) were placed in a square plastic vat containing a designated concentration of GA_3 (0, 1×10^{-8}, 1×10^{-7}, 1×10^{-6}, 1×10^{-5} and 1×10^{-4} M) and were grown continuously in the solution for four months inside the greenhouse. For uniconazole treatment, a set of five 4-week-old WT plants (SIL-05) were grown continuously for 2 months in a vat containing specified concentrations of uniconazole (0, 1×10^{-7}, 3×10^{-7}, 1×10^{-6}, 3×10^{-6} and 1×10^{-5} M). In both GA and uniconazole experiments, the vats were regularly checked and augmented with water whenever necessary to guard against evaporation and fluctuation in the concentration of the original solution until the completion of the experiment. Measurement of plant height was done with a rope by tracing the contour of the main culm from the base to the longest tip. Culm angle was taken by measuring the angle formed by the culm against the vertical axis.

DNA and RNA-seq analysis by Next Generation Sequencer (NGS). Two isogenic varieties, tall white sooner milo (*Dw1Dw1*) and dwarf white milo (*dw1dw1*) carrying gain-of-function and loss-of-function alleles of *Dw1*, respectively, were utilized for genomic DNA sequence analysis using NGS. Sequencing was performed at Hokkaido System Science Co. Ltd. (Sapporo, Japan). Library for sequencing was prepared using TruSeq DNA/RNA Sample Prep Kit (Illumina, Inc.) according to the manufacturer's protocol. The resultant library was sequenced (2×101 cycles, paired-end) on HiSeq 2500/2000 instrument using TruSeq PE Cluster Kit and TruSeq SBS Kit (Illumina, Inc.) to obtain approximately 40 million raw sequence reads for analysis. Sequences were mapped to the BTx623 sorghum reference genome[58] by using BWA version 0.6.1-r104 (http://bio-bwa.sourceforge.net/), and SNPs were called with the SAMtools version 0.1.18 (http://samtools.sourceforge.net/). The two varieties were also subjected to RNA-seq analysis by using elongating internode total RNAs isolated by a modified Trizol method[59]. Total RNAs were utilized for RNA-seq experiment as described previously[60].

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Author contributions

R.L.O., Y.I., S.K., H.K., M.M. and T.S. designed the experiments and wrote the manuscript. R.L.O. conducted almost all of the molecular analyses and plant assays. T.S. and T.T. grew the M1 and M2 populations. A.H. and Y.I. conducted the uniconazole test and mutant phenotyping in the field. K.O.S. made the construct for *SbKO1* and assisted in genetic transformation, while H.M. did the RNA-seq analysis. And H.M. did the RNA-seq analysis.

Additional information

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