Registration of BTx623<sub>dw5</sub>, a New Sorghum Dwarf Mutant

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Abstract
The USDA-ARS has released a new dwarf sorghum (<i>Sorghum bicolor</i> (L.) Moench) mutant BTx623<sub>dw5</sub> (Reg. No. GS-787, PI 688506). Dwarf genes have been an important driving force in breeding since the Green Revolution. Single dwarf locus is used to breed high-yielding semidwarf wheat (<i>Triticum aestivum</i> L.) and rice (<i>Oryza sativa</i> L.) cultivars. However, in sorghum, any of the known dwarf loci alone are insufficient to breed semidwarf grain sorghum fitting for modern farm practices. Therefore, four dwarf loci have been traditionally used collectively in combinations to breed sorghum cultivars of the desired plant height for machine harvesting. Here we register a new sorghum dwarf mutant isolated from a mutagenized BTx623 mutant library, which is genetically different from those currently known controlling the dwarf phenotype in sorghum. Therefore, we designated this newly identified dwarf genotype as dwarf 5 (<i>dw5</i>). The dwarf phenotype of <i>dw5</i> was evaluated in two environments: the Puerto Rico winter nursery with short days and mild temperature conditions and the Lubbock, TX, summer field with long days and high temperature conditions. The <i>dw5</i> mutation reduced the plant height of BTx623 from 155 to 74 cm in Puerto Rico and from 113 to 72 cm in Lubbock. The dwarf phenotype in <i>dw5</i> mutant is caused by a single nuclear gene mutation and is inherited in a recessive manner. It can be easily identified and bred into other sorghum lines through recurrent backcrossing. The <i>dw5</i> mutation provides a new way to control sorghum height.

Dwarf genes have played a key role in plant breeding since the Green Revolution (Hedden, 2003; Khush, 2001; Peng et al., 1999). The reduced plant height due to the dwarf gene confers lodging resistance to plant varieties and allows the application of ample irrigation and fertilizers, which significantly boosts grain yield and successfully averted the forecasted famine due to increased population and reduced farmland availability in the 1970s (Khush, 2001). The Green Revolution began with wheat (<i>Triticum aestivum</i> L.) and rice (<i>Oryza sativa</i> L.), for which only one dwarf locus is required to breed semidwarf varieties. The reduced height gene 1 (<i>rht1</i>) in wheat is a dominant mutation in a gibberellic acid (GA) receptor (Ueguchi-Tanaka et al., 2005), whereas the dwarf locus used in rice is GA20 oxidase, a key gene involved in the biosynthesis of bioactive GAs (Sasaki et al., 2002).

Semidwarf is an important breeding trait in grain sorghum (<i>Sorghum bicolor</i> (L.) Moench), which contributes to the increased grain yield and allows the utilization of combine in grain harvesting. Four genetic loci, <i>Dw1</i>, <i>Dw2</i>, <i>Dw3</i>, and <i>Dw4</i>, have been used to control the plant height in sorghum (Quinby and Karper, 1954). None of these dwarf loci alone, however, can sufficiently reduce sorghum plant height to meet the requirement for machine harvesting. Therefore, breeders have traditionally incorporated three of the four dwarving loci into grain sorghum breeding lines to produce semidwarf sorghum cultivars and two dwarf loci for breeding tall biomass sorghum. The <i>Dw1</i> gene has been mapped to chromosome 9, <i>Dw2</i> to chromosome 6, <i>Dw3</i> to chromosome 7, and <i>Dw4</i> to chromosome 4 (Li et al., 2015). The exact location of <i>Dw4</i> is still debatable, probably, on the lower arm of chromosome 4 (Li et al., 2015). For hybrid production, dwarfing alleles at these loci are required to introduce into both male and female parents. As a result, the semidwarf sorghum hybrids all contain multiple identical chromosome segments flanking the dwarf loci, causing homozygosity in large chromosome regions around these dwarf loci (Thurber et al., 2013).

The genes for three of the four dwarf loci in sorghum have been identified (Hilley et al., 2017; Hirano et al., 2017; Multani...
et al., 2003; Yamaguchi et al., 2016). However, unlike the ones in wheat and rice, none of these three dwarf genes is involved in the GA signaling or biosynthesis pathway. The Dw1 gene encodes a protein of unknown function, possibly involved in brassinolide signaling (Hirano et al., 2017; Yamaguchi et al., 2016). The Dw2 gene encodes a protein kinase (Hilley et al., 2017). The Dw3 gene encodes an auxin transporter (Multani et al., 2003). The Dw4 gene has been mapped to a chromosome region on chromosome 4, but the causal gene for dw4 locus has not been identified yet. Nevertheless, the mapped dw4 chromosome region does not contain any gene involved in GA signaling or GA biosynthesis pathway (Higgins et al., 2014; Upadhyaya et al., 2012; Zhang et al., 2015). Thus, there is a great need to identify new dwarf loci that enable breeding for semidwarf sorghum effectively using a single dwarf locus.

We isolated a number of dwarf mutants from a pedigreed sorghum mutant library in a leading inbred line BTx623 treated with ethyl methane sulfonate (EMS) (Jiao et al., 2016; Xin et al., 2008). Here, we report the characterization of the first dwarf mutant, BTx623 , (Reg. No. GS-787, PI 688506), which is genetically different from the four currently known controlling the dwarf phenotype in sorghum. The dwarf phenotype is caused by a recessive mutation in a single nuclear gene and may have potential use in breeding semidwarf sorghum cultivars.

**Methods**

**Plant Material and Field Management**

The dw5 mutant was isolated in 2015 from the M1 mutant library of EMS-treated BTx623 seeds as described previously (Xin et al., 2008). Seed from a single dwarf plant was harvested and used for subsequent characterization. The dwarf phenotype of the mutant plants was confirmed in M2 progenies and F1 segregation population under field condition at the USDA-ARS Cropping Systems Research Laboratory in Lubbock, TX (33.58° N, 101.85° W, elevation 976 m). In 2017, the seed of original wild-type BTx623, the male sterile near-isogenic line (NIL) of BTx623 (BTx623 ,) (Xin et al., 2018) used as female for backcrossing, the dw5, and backcrossed F1, were planted in the winter nursery field in Guayanilla, Puerto Rico (18.0373° N, 66.7963° W, elevation 49 m), on 11 Dec. 2017. The plot size for all plantings at both Lubbock and Puerto Rico locations was 4.6 m long with 1.02 m row spacing. About 60 sorghum seeds per row were planted per plot, and the planting depth was about 3 cm. The major agronomic traits and plant height data were collected from six randomly selected plants per plot. Plant height was measured from the soil surface to the tip of the main panicle. Panicle length was measured from the first node to the tip of the panicle. Exsertion was measured from the flag leaf base to the first node of the panicle. The panicles from six plants were harvested at maturation and dried for 4 d in a forced air oven at 42°C before threshing. The 1000 seed weight was determined after drying the seeds for 2 d in 65°C oven. In 2018, the same lines along with backcrossed F2s were planted in Lubbock field on 11 May as three replicates per line, with two plots as a replicate for F1s (segregation population) and a single plot as a replicate for the rest of the lines (homozygous). Numbers of plants segregating for wild-type plant height and dwarf phenotypes were recorded. Data for agronomic traits were collected from six randomly selected plants per replicate the same way as those described above. For each replicate of F2s, data for major agronomic traits were collected from six randomly selected dwarf and six randomly selected wild-type height plants, respectively. Days to flower were recorded as the number of days from planting to mid-anthesis. Plant height was measured from the ground to the tip of the main panicle. Number of leaves was counted from the first green leaf (round) to the flag leaf. For accurate leaf number counting, the leaf number was marked on every odd-numbered leaf after it was fully expanded.

The experiment fields at both locations were managed by routine farm practices. One week before planting, all plots were furrow irrigated. The surface soil was gently loosed by a rolling cultivator prior to planting. Field irrigation started 2 wk after germination and ended at grain maturation. At the Lubbock location, plots were irrigated via an automated subsurface drip system at 3 mm per day, while the winter nursery plots at Puerto Rico were also watered daily via surface drip lines. At both locations, preplanting N–P–K fertilizer at a rate about 34 kg ha$^{-1}$ (30 lb acre$^{-1}$) was applied to the experiment field. In addition, nitrogen fertilizer at a rate about 90 kg ha$^{-1}$ (80 lb acre$^{-1}$) was applied at midseason through irrigation lines. Other activities such as weed control and pest control were managed by routine farm practices.

**Genetic Characteristics**

The dw5 mutant was backcrossed to the male sterile NIL of BTx623, BTx623 , (Xin et al., 2018) and the backcrossed F1 and F2 populations were used to examine the genetic control of dwarf phenotype in dw5 mutant. The results of plant height segregation are provided in Table 1, and the plant height measurement data are provided in Table 2. The average height of wild-type parent (BTx623 ) minus two times of standard deviation data are given in Table 2. The average height of wild-type parent (BTx623 ) minus two times of standard deviation data are given in Table 2.

![Table 1. Plant height segregation of dwarf phenotype in BTx623 , × dw5 backcrossed F1 and F2 populations.](image)

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Environment†</th>
<th>Wild-type plant no</th>
<th>Dwarf plant no</th>
<th>Total plant no</th>
<th>WT/Dwf ratio</th>
<th>Chi$^2$</th>
<th>Note</th>
</tr>
</thead>
<tbody>
<tr>
<td>BTx623 ,</td>
<td>PR17</td>
<td>100</td>
<td>0</td>
<td>100</td>
<td>1.0:0.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BTx623 ,</td>
<td>LB18</td>
<td>145</td>
<td>0</td>
<td>145</td>
<td>1.0:0.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>dw5</td>
<td>PR17</td>
<td>0</td>
<td>96</td>
<td>96</td>
<td>0.0:1.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>dw5</td>
<td>LB18</td>
<td>0</td>
<td>136</td>
<td>136</td>
<td>0.0:1.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>F1,</td>
<td>PR17</td>
<td>107</td>
<td>0</td>
<td>107</td>
<td>1.0:0.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>F1,</td>
<td>LB18</td>
<td>158</td>
<td>0</td>
<td>158</td>
<td>1.0:0.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>F2,</td>
<td>LB18</td>
<td>218</td>
<td>69</td>
<td>287</td>
<td>3.1:1.0</td>
<td>0.71</td>
<td></td>
</tr>
</tbody>
</table>

† LB18, Lubbock, TX, in 2018; PR17, Puerto Rico in 2017.
The dwarf phenotype of the dwarf 5 (dw5) mutant provides a useful source for breeding dwarf sorghum hybrid. It contains a mutation in a new genetic locus from the three dwarf genes residing in BTx623 and differs from the genetic control of the dwarf phenotype of the known dwarf genes (Miller, 1977). Therefore, the dw5 mutation provides a new locus to breed semidwarf sorghum lines.
Availability

Seed for BTx623 \textsubscript{dw5} mutant has been deposited in the USDA-ARS National Plant Germplasm System (NPGS) and will be available immediately for research purposes. Small amounts of seed will be distributed on request to breeders and geneticists from the corresponding author, Junping Chen, USDA–ARS, 3810 Fourth St., Lubbock, TX. It is requested that appropriate recognition of source be given and this registration to be cited when referring to this material and when this material contributes to marker development, research publications, or development of improved line, cultivar, or hybrid.

Acknowledgments

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References

Miller, F.R. 1977. Release of A & B Tx 622, 623 and 624—Female grain sorghum lines and their maintainers. Texas A&M University, College Station, TX.