CGTCCTGCAGAAGATGTTCATGAAGGGCTTCTCGGGGGGACCTGGAGGCCGCGCACGCCACGCCACGCA GATCGCGGGCGAGGCCGTCGCCAACCTGCGCACCGTGGCGGCGTTCAACGCGGAGCGCAAGATCACGG<mark>G</mark> GCTCTTCGAGGCCAACCTTCGCGGCCCGCTCCGGCGCTTCTGGAAGGGGCAGATCGCCGGGAGCG GCTACGGCGTGGCGCAGTTCCTGCTGTACGCGTCCTACGCGCTGGGGCTCTGGTACGCCGCGTGGCTAG^T GAAGCACGGCGTCTCCGACTTCTCGCGCACCATCCGCGTGTTCATGGTGCTCATGGTGTCCGCCAACGGC **GACCATCGACCGGAAAACGGAGGTGGAGCCCGACGACGTGGACGCGGCGCGCGGTGCCGGAGCGGCCC** AAGGGCGAGGTGGAGCTGAAGCACGTGGACTTCTCGTACCCGTCGCGGCCGGACATCCAGGTGTTCCGC GACCTGAGCCTCCGGGCGCGCGCGGGAAGACGCTGGCGCTGGTGGGTCCGAGCGGGTGCGGCAAGA GCTCGGTGCTGGCGCTGCAGCGGTTCTACGAGCCCACGTCCGGGCGCGTGCTCCTGGACGGCAAG GACGTGCGCAAGTACAACCTGCGGGCGCTGCGGCGCGTGGTGGCGGTGCCGCAGGAGCCGTTCCT GTTCGCGGCGAGCATCCACGACAACATCGCGTACGGGCGCGAGGGCGCGACGGAGGCGGAGGTGGTGG AGGCGGCGACGCAGCGAACGCGCACCGGTTCATCTCGGCGCTGCCGGAGGGCTACGGGACGCAGGTG AGCAGGCGGCCATCATGCTGCTGGACGAGGCGACCAGCGCGCTGGACGCCGAGTCGGAGCGGT<mark>GGCTC</mark> TTCGAGGCCAACCTTCGCGGCCCGCTCCGGCGCTGCTTCTGGAAGGGGCAGATCGCCGGGAGCCGCTAC GGCGTGGCGCAGTTCCTGCTGTACGCGTCCTACGCGCGTGGGCTCTGGTACGCCGCGTGGCTAGTGAAGC ACGGCGTCTCCGACTTCTCGCGCACCATCCGCGTGTTCATGGTGCTCATGGTGTCCGCCAACGGCGCCGC CGACCGGAAAACGGAGGTGGAGCCCGACGACGTGGACGCGGCGCCCGGTGCCGGAGCGGCCCAAGGGCG AGGTGGAGCTGAAGCACGTGGACTTCTCGTACCCGTCGCGGCCGGACATCCAGGTGTTCCGCGACCTGAG CCTCCGGGCGCGCGCGGAAGACGCTGGCGCTGGTGGGTCCGAGCGGGTGCGGCAAGAGCTCGGTGC TGGCGCTGGTGCAGCGGTTCTACGAGCCCACGTCCGGGCGCGTGCTCCTGGACGGCAAGGACGTGCGCA AGTACAACCTGCGGGCGCTGCGGCGCGTGGTGGCGGTGCCGCAGGAGCCGTTCCTGTTCGCGGCGA GCATCCACGACAACATCGCGTACGGGCGCGAGGGCGCGACGGAGGCGGAGGTGGTGGAGGCGGCGACG CAGGCGAACGCGCACCGGTTCATCTCGGCGCTGCCGGAGGGCTACGGGACGCAGGTGGGCGAGCGCGG **TCATGCTGCTGGACGAGGCGACCAGCGCGCTGGACGCCGAGTCGGAGCGGT**GCGTGCAGGAGGCGCTG GAGCGCGCGGGAACGGCCGCACCACCATCGTGGTGGCGCAC

Sequence amplified to genotype dw3/Dw3. Within exon V of Sb07g023730.1 chromosome_7: 58613050 – 58611091. The forward and reverse primers' positions underlined in sequence above, respectively, were designed by Barrero Farfan et. al. (2012). Specifically, wirh respect to Sbi1 (Paterson et al 2009) the forward primer was 5'-CGT CCT GCA GAA GAT GTT CAT GAA GG-3' for the negative strand on chromosome_7: 58613025 – 58613050 and the reverse primer was 5'-GTG CGC CAC CAC GAT GGT GGT GC-3' for the positive strand on chromosome_7: 58611091 – 58611113. The 882 bp tandem repeat that makes many dw3 alleles in sorghum unstable is highlighted in black and in yellow, flanked by the primers. There is a sequence_amp.txt file of just the sequence displayed in the in the folder containing this .pdf as well.

PCR solution	1x (50 ul)	
Promega PCR master mix	25.0	https://www.promega.com/products/pcr/routine-pcr/pcr-master-mix/
forward primer (10 uM)	2.0	https://www.crops.org/publications/cs/pdfs/52/5/2063
reverse primer (10 uM)	2.0	https://www.crops.org/publications/cs/pdfs/52/5/2063
template DNA (100ng/21ul)	21.0	
	50.0	=

PCR in Applied Biosystems Thermal Cycler

GeneAmp© PCR System 2700

(avoid using red wells they don't seem to hold temperature well, see table ->)

		2	25 cycles			
temperature (Celsius)	95	95	68	72	72	4
time (minutes seconds)	05:00	00.45	00.45	02:00	07:00	(hold)

Run PCR product on 0.7% agarose gel.

