High-Throughput Discovery of Mutations in Tef Semi-Dwarfing Genes by Next-Generation Sequencing Analysis

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ABSTRACT Tef (*Eragrostis tef*) is a major cereal crop in Ethiopia. Lodging is the primary constraint to increasing productivity in this allotetraploid species, accounting for losses of ~15–45% in yield each year. As a first step toward identifying semi-dwarf varieties that might have improved lodging resistance, an ~6× fosmid library was constructed and used to identify both homeologues of the *dw3* semi-dwarfing gene of *Sorghum bicolor*. An EMS mutagenized population, consisting of ~21,210 tef plants, was planted and leaf materials were collected into 23 superpools. Two dwarfing candidate genes, homeologues of *dw3* of sorghum and *rht1* of wheat, were sequenced directly from each superpool with 454 technology, and 120 candidate mutations were identified. Out of 10 candidates tested, six independent mutations were validated by Sanger sequencing, including two predicted detrimental mutations in both *dw3* homeologues with a potential to improve lodging resistance in tef through further breeding. This study demonstrates that high-throughput sequencing can identify potentially valuable mutations in under-studied plant species like tef and has provided mutant lines that can now be combined and tested in breeding programs for improved lodging resistance.

TEF [*Eragrostis tef* (Zucc.) Trotter] is an allotetraploid ($2n = 4X = 40$) species that is native to Ethiopia. Tef belongs to the grass subfamily *Chloridoideae*, a lineage that has received very little research attention. Tef has an estimated genome size of 713–733 Mbp (Ayele *et al.* 1996), which is ~60% larger than the rice genome.

As a staple food in Ethiopia, covering more acreage than any other crop in that nation, tef possesses several advantageous characteristics, including excellent storage properties and a very nutritious seed with excellent protein and mineral composition (Stallknecht *et al.* 1993). In addition, tef plants grow well under extreme environmental conditions such as drought and water-logging. However, compared to other crops, the average yield of tef is quite low, at an average of ~700 kg ha^{-1} (Central Statistics Authority 2005). Tef has a tall and tender stem that is susceptible to lodging caused by wind and rain. As a consequence, the yield of tef is reduced ~15–45% each year, depending on the weather and the variety. Furthermore, an increased incidence of lodging is associated with fertilizer application. Using QTL to improve lodging resistance indicated a positive correlation between lodging and yield, such that the most lodging-resistant progeny also had the lowest yield (Yu *et al.* 2007). This is probably because such traits as larger seed size and higher seed number create a heavier panicle that leads to a greater likelihood of lodging.

In other cereal crops (Peng *et al.* 1999; Sasaki *et al.* 2002; Multani *et al.* 2003), semi-dwarf varieties have been found to provide both lodging resistance and higher yields. As shown in the earliest studies (Quinby and Karper 1954; Jennings 1964; Walcott and Laing 1976; Foster and Rutger 1978), semi-dwarf varieties have both strong stalks and a greater carbon partitioning into seed rather than vegetative material. Comparison of genetic-improvement-associated trait changes in tef and wheat revealed that tef harvest index and lodging susceptibility remain unaltered even though plant height and total biomass yield increased in some varieties

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(Assefa *et al.* 2011), further suggesting the importance of developing tef varieties with short stature. However, tef is a tetraploid species with most genes represented by two homeologous copies, thus raising doubts as to the likely effectiveness of the facile use of plant height screens for the outcome of single-gene knockouts.

Numerous studies have been carried out on plant dwarfing genes (Itoh *et al.* 2001; Monna *et al.* 2002; Sasaki *et al.* 2002; Hong *et al.* 2003; Multani *et al.* 2003; Muangprom 2005; Sakamoto *et al.* 2005; Tanabe *et al.* 2005; Zou *et al.* 2006; Asano *et al.* 2009), and mutant alleles of these genes have been seen to have negative effects on plant performance, often by decreasing fertility. However, mutant alleles of a few dwarfing genes have been found to be useful for crop improvement. Among them, two of the most successful applications have been to create the semi-dwarf wheat and rice varieties that led to the Green Revolution in the 1960s. The key genes employed were the reduced height-1 (*Rht-B1b* and *Rht-D1b*) genes in wheat (Peng *et al.* 1999) and the semi-dwarf (*sd1*) gene in rice (Sasaki *et al.* 2002; Spielmeyer 2002; Muangprom 2005). Cloning of these genes revealed that both are involved in the gibberellin (GA) response. The wild-type alleles of *Rht-B1b* and *Rht-D1b* encode DELLA proteins that are important components of the GA signal transduction pathway. Peng *et al.* (1999) found that a point mutation in each of the *Rht-B1b* and *Rht-D1b* alleles introduced a stop codon into the *N*-terminal coding region. The rice *sd1* gene encodes GA20 oxidase, a key enzyme in the GA biosynthesis pathway. The studied *sd1* allele has a 383-bp deletion that results in a frameshift mutation that conditions a greatly lowered GA20 oxidase level (Sasaki *et al.* 2002).

In sorghum, the gene *dwarf3* (*dw3*) (Multani *et al.* 2003) was used for lodging resistance and higher yields in this crop decades before the wheat or rice Green Revolutions were conceptualized (Karper 1932; Quinby and Karper 1954). The maize ortholog of *dw3*, *br2*, has not been used extensively for maize improvement, perhaps because maize lodging is not so much a plant anatomy issue as it is associated with insect and fungal damage to stalks (Munkvold and Hellmich 2000; Multani *et al.* 2003). Multani and his colleagues (Multani *et al.* 2003) discovered that corn *br2* and sorghum *dw3* encode a *p*-glycoprotein whose loss leads to dwarf corn and sorghum plants by interfering with the movement of auxin, thus causing the compact lower stalk internodes characteristic of this mutation.

Mutagenesis has been used to identify the existence and involvement of genes in numerous biological processes (Auerbach and Robson 1946; Rapoport 1946; Ehrenberg and Gustafsson 1957; Natarajan and Ramanna 1966). Chemical mutagens, such as ethyl methanesulfonate (EMS) and *N*-methyl-*N*-nitrosourea (MNU), are applied to induce single nucleotide substitutions and create mutation libraries at the genome level. For instance, the original *sd1* rice mutant was generated by mutagenesis (IRRI annual report for 1966). Recently, mutagenesis has taken a significant step forward by the utilization of reverse genetic approaches that allow the directed investigation of the mutational status of specific genes. TILLING (*t*argeting *i*nduced *l*ocal *l*esions *in* genomes) and EcoTILLING (Comai *et al.* 2004) have been applied to a variety of plants, resulting in identification of mutations in specific target genes in *Arabidopsis* (Greene *et al.* 2003; Till *et al.* 2003; Martin *et al.* 2009), rice (Till *et al.* 2007; Suzuki *et al.* 2008), wheat (Slade *et al.* 2005), maize (Till *et al.* 2004), sorghum (Xin *et al.* 2008), pea (Dalmais *et al.* 2008), soybean (Cooper *et al.* 2008), tomato (Minoia *et al.* 2010), and other plant species. However, TILLING is a time-consuming, expensive, and labor-intensive approach when applied to either large populations or large genomes and might be particularly challenging in a recent tetraploid like tef (Smith *et al.* 2012) where homeologues have not yet differentiated greatly in sequence.

Recent advances in high-throughput sequencing technologies have provided avenues for greatly improved detection of genome-wide variation. For instance, using 454 amplicon sequencing, over 1000 SNPs were detected from a sugarcane mapping population and 93% were found to be valid (Bundock *et al.* 2009). A 454 GS FLX platform based on KeyPoint technology has been used to screen for induced and naturally occurring sequence variation in more than 3000 M2 tomato families and identified two mutants and six haplotypes in the *elF4E* gene (Rigola *et al.* 2009). To enhance this approach, enrichment of a specific gene can be performed before high-throughput sequencing. Array capture and PCR amplification of targets are commonly used (Mamanova *et al.* 2010; Ng *et al.* 2010a). Array-enriched whole-exome sequencing has been widely used to identify candidate causative genes for human disease. For example, SNPs in genes *DHODH* and *MLL2* were respectively identified as causal agents for Miller syndrome and Kabuki syndrome by this approach (Ng *et al.* 2010a,b).

Recently, Smith *et al.* (2012) cloned and sequenced homologs of the *sd1* and *rht1* genes from tef. Two *rht1* homeologues, apparent orthologs of the wheat genes, were sequenced across 31 tef accessions, as were three tef *sd1* homologs of unknown paralogous/orthologous relationship. This study indicated that all accessions contained genes that appeared to be fully functional and that there were both very few haplotypes per gene (one to five) and no clear history of recombination between these haplotypes. Hence, a search for naturally inactivated semi-dwarf genes in the tef germplasm would be an arduous and perhaps fruitless undertaking.

In this study, two dwarfing candidate genes, orthologs of *dw3*, were isolated from a fosmid genomic library containing tef DNA inserts. These two candidate genes, and two tef *rht1* homeologues, were enriched by gene-specific PCR amplification from genomic DNA pools derived from an EMS-mutagenized tef population containing ~21,210 individuals. Candidate mutations were identified, and several were further validated by Sanger sequencing, thereby

demonstrating that high-throughput sequence analysis can be an efficient approach for mutant discovery in a tetraploid orphan crop. Although semi-dwarf phenotypes were not seen in heterozygous or homozygous mutant plants, future crosses to bring these two mutations into the same genetic background may yield progeny with a semi-dwarf phenotype that yields improved lodging resistance and yield.

Materials and Methods

Fosmid genomic library construction

Genomic DNA from tef USDA accession PI524434 was used for fosmid library construction. The genomic DNA was randomly sheared with 40 repetitions using a GeneMachines Hydroshear, and DNA fragments from 35–45 kb were selected. The sheared DNA was end-repaired and purified and then was ligated into the pCC1FOS vector (Epicentre Technologies, WI). For packaging of fosmid clones, MaxPlax Lambda Packaging Extract (Epicentre Technologies) was mixed with 10 μl of ligation reaction and processed according to the manufacturer's instructions. Plates containing ~100 fosmid clones were pooled and stored in deep-well plates. These pools were further combined into superpools for the initial PCR screening to identify clones containing genes of interest.

Fosmid library screening, sequencing, and data analysis

The tef genomic fosmid library was screened for dwarfing candidate gene *dw3/br2* by a PCR-based strategy. This library was assigned to 25 superpools, with 48 pools in each superpool (Supporting Information, Table S1). All 25 superpools were screened by primers specific to putative dwarfing genes. One positive superpool identified in the previous step was selected and all of the 48 pools were screened. Then, one positive pool was selected and 96 clones from this pool were randomly picked and screened. During the entire screening process, tef genomic DNA was used as a positive control for PCR amplification. Finally, the identified positive clones were further verified by complete sequencing to identify a full-length copy of the target gene. The complete clone sequences were submitted to GenBank (accession nos. JN672669–JN672670).

The fosmid sequences were initially annotated by FGENESH (http://www.softberry.com) and GENESCAN (Burge and Karlin 1997). Putative proteins were identified using TBLASN and BLASTP (Altschul *et al.* 1997). Divergence times of the two homeologous *dw3* homologs in the tetraploid tef were estimated by two approaches. One was a molecular clock approach. The divergence time was calculated based on the formula $T = K_s/2r$, where r corresponds to the absolute rate of substitutions/site/year and K_s is the estimated numbers of substitutions per site between homologous sequences. Synonymous substitutions were calculated by the method of Nei–Gojobori (Nei and Gojobori 1986) with the Jukes–Cantor correction. The other dating approach was based on comparative genomics. The number of synonymous mutation (D_s) was calculated for the orthologous *dw3/br2* genes from several grass species, including maize and sorghum. Thus the divergence time of the two tef homeologues was dated using $D_{s(tef)}/T_{tef} = D_{s(AB)}/T_{(AB)}$, where D_s is a measure of the number of synonymous differences when comparing the two tef homeologues; $D_{s(AB)}$ and $T_{(AB)}$ represent the level of synonymous mutation and the divergence time between maize and sorghum, respectively.

Tef mutagenesis and pooling

Tef seed used in the mutagenesis were from cultivar DZ-Cr-37, a released variety that provides early maturing plants that are drought tolerant (Teferra *et al.* 2000). Seed from DZ-Cr-37 were treated with EMS at concentrations of 0, 0.5, 1, and 1.5%. The seeds were soaked in water for 1 hr, then in the EMS solution for 5 hr, and were subsequently washed with water for 3 hr. Mutagenized seeds were planted in the greenhouse in a soilless growing mix. Plant heights measured 3 weeks after planting were used as surrogate scores for the efficacy of different concentrations of EMS. That is, only seeds with good germination efficiency but also showing some obvious detrimental effects of the mutagen were considered good candidates for a high level of mutation. EMS concentrations of 1–1.5% were found to be a good working dose for DZ-Cr-37, so plants grown from these seeds constituted the tef M1 generation, a total of 2,121 M1 plants. M1 plants were self-fertilized, and 10 M2 generation seeds were planted in the greenhouse from each M1 panicle. By definition, an M2 family is derived from a single M1 seed, because it is the self-pollinated seed on the panicle of an M1 plant. Genomic DNAs were isolated from a pool of 10 M2 leaves (one equivalent-size leaf per plant) from each M2 family, using the standard CTAB procedure (Murray and Thompson 1980). A total of 2121 pools were generated, which represented 2121 M2 families. Among them, 934 pools were from the 1.0% EMS treatment and 1187 pools were from the 1.5% EMS treatment. A total of 90–100 pools were combined into each superpool, generating 23 superpools for mutation screening by PCR and 454 sequencing. If a mutation of interest was identified in the superpool, all pools in this superpool were screened by Sanger sequencing. Once the target mutation was identified, 10–16 seeds from this pool were sown to collect M3 single plants for further validation. The seeds from mutationally confirmed M3 plants have been saved for future use.

Phenotypic analysis

The SIFT (sorting intolerant from tolerant; Ng 2003) program was used to predict the severity of the effects of each identified mutation on protein function. The mutagenized tef plants containing damaging mutation predicted by SIFT were selected and mutation phenotypes were further evaluated in the M3 generation. Each M3 plant height was carefully inspected at least three times during the growing season. Plants were grown both under standard greenhouse

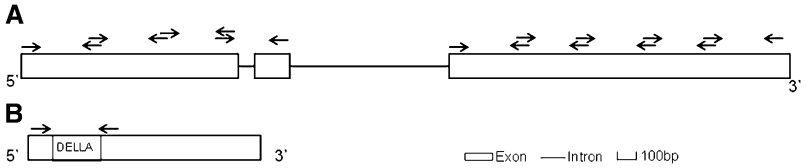

Figure 1 Gene structure for tef dwarfing candidate genes of (A) *dw3/br2* and (B) *rht*. The arrows indicate the primers used to screen the mutagenized populations in the 454 sequencing.

conditions (with high fertilizer inputs like those routinely used for maize) and under conditions where no fertilizer was added after the initial planting.

454 library preparation

Exonic segments of four dwarfing candidate genes, *dw3a*, *dw3b*, *rht1a*, *and rht1b* (Smith *et al.* 2012) were chosen for 454 sequencing. Seven pairs of primers for the *dw3/br2* genes and 1 pair of primers for the *rht1* homeologues were designed to cover the appropriate coding regions (Figure 1) for initial PCR and sequencing. The primer sequences are listed in the supporting information (Table S2). For each primer, an 8- to 10-bp TI-MID tag was added at the 5′ end to differentiate the superpools. These tags differed by at least four nucleotides.

All PCR amplifications were performed in 1× Phusion HF Buffer, 1.5 mM $MgCl_2$, 200 μM of each dNTP, 0.5 μM primer, 3% DMSO, and 2.5 units Phusion high-fidelity DNA Polymerase (New England Biolabs, MA), with 100 ng of genomic DNA in a 50 μl total volume. Amplification was carried out in a PTC-gradient cycler (MJ Research, MA) as follows: 30 sec at 98° followed by 30 cycles of 10 sec at 98°; 20 sec at 72°; and a final extension of 10 min at 72°. PCR products were isolated from 1.5% agarose gels and purified using a Quick gel extraction kit (QIAGEN, Hilden, Germany).

454 titanium sequencing, data processing, and candidate mutation validation

Sequencing was performed at the University of Georgia sequencing facility according to the manufacturer's instructions (Roche Applied Science, IN). Base-called reads were trimmed, filtered for quality, and converted into FASTA format. During preprocessing, the origin of the reads was identified based on the target-specific primer sequences and the 8- to 10-bp TI-MID tags. Single-nucleotide mutations were then identified by mapping the 454 superpool data onto the reference sequences generated by the fosmid sequence analysis. A mutation was considered to be a candidate when it fulfilled the following criteria: (1) it was present in only one of the superpools, and with a frequency of three or more reads in that superpool, and (2) its quality score was >15. The mutation identification was implemented using custom PERL scripts. Candidate mutations identified in the superpools were validated by Sanger sequencing of the individual 10-plant pools, all from the M1 panicle. So the mutation was expected in 50% of the pooled DNA, yielding overlapping reads of ~50% wild type and ~50% mutant at the identified mutant nucleotide. These candidates were further investigated by Sanger sequencing of DNA from single M2 seedlings from the appropriate M1 panicle, indicating M2 progeny that segregated 1:2:1 for the mutant nucleotide. PCR products obtained from the M2 families and individuals were sequenced using the BigDye Terminator v. 3.1 kit on the 3730 DNA analyzer (Applied Biosystems, CA). Once a M2 plant was validated, the M2 seeds were planted again in the greenhouse to collect M3 seed.

Results

Fosmid sequencing and identification of dwarfing candidate genes

A total of 102,191 tef fosmid clones were isolated and transferred to 96-well microtiter plates, corresponding to ~6× genome coverage. The probability of recovering a particular gene in this library is >99.5%, calculated by the formula $N = \ln(1 - P)/\ln(1 - f)$, where P is the calculated probability, f is the proportion of the genome contained in a single clone, and N is the number of fosmid clones. This library was organized into 25 superpools and screened for *dw3/br2* homologs. Two of the positive superpools were chosen to screen all pools and fosmids. Two fosmids containing *dw3* target genes were sequenced to identify the full-length *dw3* sequences. We designated these two genes as *dw3a* and *dw3b* in the allotetraploid tef genome. Annotation of these two fosmids resulted in a total of eight genes. The gene *dw3a* was located in the middle of one fosmid and *dw3b* at the end of the other fosmid (Table 1). Sequence comparison revealed 94.8% and 94.1% identities between *dw3a* and *dw3b* at the nucleotide and protein levels in coding regions, respectively. However, the nucleotide identity dropped to <70% in noncoding regions (Table S3). There were seven PCR positives for *dw3* in the superpool screening of this ~6× library,

Table 1 Two fosmids containing tef *dw3* homologs

Size of fosmid insert (bp)	No. of genes annotated	Target gene	Length of target gene (bp)	Target gene position in the fosmid (bp)
39,729	8	*dw3a*	5,239	10,232–15,470
43,434	8	*dw3b*	5,181	37,617–42,797

thus suggesting that these two genes represent the two homeologous copies from the two diploid ancestors of this allotetraploid. With a molecular clock method, these two *dw3* homeologues were calculated to have shared a common ancestor approximately 9 million years ago (MYA). Similarly, with a comparative genomics approach, this divergence was dated at approximately 10 MYA, when one employs maize and sorghum genome divergences as 12 MYA (Swigonova *et al.* 2004). The gene lengths of the tef *dw3* homeologues were similar to their maize, sorghum, and rice orthologs. However, the tef genes are missing one intron compared to maize and sorghum, and two introns compared to rice.

Generation of EMS mutagenized tef populations and 454 sequencing

EMS is a chemical mutagen that predominantly induces C to T and G to A transitions across the genome (Krieg 1963). In this study, 2121 M2 families were grown for DNA isolation and screening. A total of 934 families were from seed treated with 1% EMS and 1187 families were from seed treated with 1.5% EMS. The M2 seed germination rates for both treatments were >95%, even after 9 years of storage at room temperature. Approximately equal amounts of leaf material were harvested from each of ~10 individual M2 plants that were derived from a single M1 seed. As any mutation occurring in the M1 seed would segregate in the M2 offspring, pooling of leaf material from 10 individual M2 plants meant that the chance of recovery of this mutation was >99%.

A total of 327,696 quality-filtered reads, with a median read length of 393 bp (ranging from 347 to 413 bp), were obtained from half a plate of a 454 Titanium run, producing a total of 123 Mb of raw data (Table S4). This provided 9.7× sequence coverage, on average, for each M2 pool. Of the total reads, 312,786 (95%) could be assigned into one of the 23 superpools and one of the 184 amplicons (23 superpools × 8 primer pairs) to cover most of the coding portion of the *dw3* homeologues and one key region (Peng *et al.* 1999) of the *rht1* homeologues based on the target-specific primer sequences and the 8–10 bp TI-MID tags. For each amplicon, the number of reads from each superpool was fairly consistent (Figure S1). 454 sequencing would be expected to produce more reads for short fragments, and this was observed with our shortest amplicons. The remaining 5% of reads contained one or more deviations in the sample TI-MID tag sequences or were shorter than 100 bp, and thus were excluded from further analysis.

Mutation patterns

The mutagenized population was from cultivar DZ-Cr-37, while the cloned sequences were from accession PI524434, so it was expected that the clone sequence would differ from that of the cultivar that was mutagenized. This observation was confirmed by the amplicon sequencing, which indicated 0.3% nucleotide differences (1/ 3109 bp) in the DZ-Cr-37 amplicons (all from protein-encoding sequence) compared to PI524434. This single-nucleotide change does not cause an amino acid change. Compared to the wild-type sequence of the sequenced exonic DNA of the *dw3* and *rht1* homeologue amplicons from DZ-Cr-37, a total of 120 candidate mutations were identified in the four homeologous genes in the mutagenized population. Many additional sequence variations were observed, but these were low-frequency sequence changes that were likely to have been an outcome of sequencing errors. Of the 120 sequence changes represented three or more times in the amplicon sequence data set, 86 were missense/nonsense mutations (Table 2). The majority (78%) of the mutations detected in the superpools were derived from the 1.5% EMS treatment (Table S5). The mutation frequency, determined by screening 2121 M2 families, was ~8.7 mutations/Mb for *dw3* homeologues and 2.7 mutations/Mb for *rht1* homeologues. Of these candidate mutations, the most abundant mutation types are G/C to A/T transitions, accounting for 69.2% and 84.1% of total mutations in the *dw3a* and *dw3b* genes, respectively. Transversions accounted for <20% of the candidate mutations. However, more detailed analysis of 10 candidate mutations from the set of 120 indicated that only 60% were truly mutant (and the rest were sequencing errors, see below), so the actual rates of sequence change of each type cannot be known unless validation of the entire set of 120 is performed.

For *dw3* homeologues, candidate mutations that were predicted to be silent, missense and nonsense accounted for 31 (27.2%), 80 (70.2%), and 3 (2.6%) of the cases, respectively. This mutation distribution pattern was significantly ($\chi^2 = 16.5$, d.f. = 2, $P < 0.001$) different from the predicted pattern by CODDLE (*c*hoose codons to *o*ptimize the *d*etection of *d*eleterious *le*sions, http://www.proweb.org/coddle/) program. CODDLE predicted ~50% missense, 47% silent, and 3% nonsense mutations (Table S6). For *rht1* genes, only six mutations, including two missense, three silent, and one nonsense mutations, were predicted. In addition, using the SIFT program (Ng 2003), the putative impact of the missense mutations on the protein function of the target genes could be predicted. Fifty (58%) of the candidate missense mutations, 48 from the *dw3* genes, and 2 from the *rht1* genes were predicted as deleterious by SIFT scores <0.05 (Table 2).

Mutant validation and phenotypic characterization

To validate these mutations, 10 candidate mutations were selected and DNA was analyzed from both 10-plant pools and single plants by Sanger sequencing. Six independent mutations, five at *dw3* homeologues, and one at an *rht1* homeologue, were validated in both pools and single plants (Table 2 and Figure 2). The other four, all with abundances less than or equal to four reads in the amplicon data set, were not found in any Sanger sequence, suggesting that they were low-frequency 454 sequencing errors. The mutation density of these validated mutations range from 4 to 19.

Table 2 Candidate dwarfing gene mutation detected by 454 sequencing in a mutagenized tef population

Gene	Mutation[a]	Effect[b]	Frequency[c]	Distribution	Function[d]	SIFT[d]	Validation[e]
dw3a	A89G	E30G	5	sp2	Damaging	0	
	C104T	P35L	7	sp19	Damaging	0	
	A200G	N67S	3	sp11	Damaging	0	
	A238G	S80G	17	sp6	Tolerated	0.36	
	C503T	A168V	19	sp19	Tolerated	1	Yes
	A628T	T210S	6	sp9	Damaging	0.03	
	T677A	V226D	3	sp17	Damaging	0	
	G730A	A244T	3	sp2	Tolerated	0.15	
	G742A	A248T	10	sp19	Damaging	0.02	Yes
	G838A	A280T	3	sp3	Tolerated	0.2	
	G863A	R288Q	9	sp11	Tolerated	0.11	
	C1105T	R369C	6	sp14	Tolerated	0.06	Yes
	G1124A	G375D	11	sp7	Damaging	0	
	C1139T	T380I	4	sp7	Tolerated	0.07	Yes
	C1177T	Q393*	3	sp14	Stop Codon	0	No
	A1189T	S397C	13	sp1	Tolerated	0.23	
	A1228G	K410E	3	sp19	Damaging	0.02	
	G1270A	D424N	4	sp16	Tolerated	0.46	
	A1294G	T432A	3	sp22	Tolerated	0.77	
	C1295T	T432M	8	sp14	Tolerated	0.1	
	G1315A	G439S	5	sp22	Tolerated	0.08	
	T1526A	L509H	10	sp17	Damaging	0	
	G1606A	D536N	4	sp15	Tolerated	0.17	
	G1642A	A548T	7	sp23	Damaging	0.01	
	G1811A	S604N	8	sp9	Damaging	0	
	C1814A	A605E	3	sp15	Damaging	0	
	G1840A	V614M	11	sp16	Damaging	0	
	G1849A	A617T	3	sp2	Damaging	0	
	A1960T	I654F	3	sp7	Tolerated	0.6	
	G1964A	G655E	4	sp23	Damaging	0	
	G1999A	G667S	3	sp5	Tolerated	0.09	
	G2288A	R763K	3	sp17	Tolerated	0.21	
	G2593A	A865T	6	sp14	Damaging	0.01	
	C2855T	A952V	5	sp14	Damaging	0.01	
	C2863T	R955C	3	sp6	Damaging	0	
	C2867T	A956V	5	sp23	Damaging	0.01	
dw3b	C76T	P26S	4	sp18	Damaging	0	
	A95T	H32L	6	sp15	Damaging	0	
	C106T	P36S	4	sp20	Damaging	0	Yes
	C118T	Q40*	3	sp19	Stop Codon	0	No
	G127A	G43R	6	sp21	Tolerated	0.22	
	C230T	S77F	6	sp18	Tolerated	0.05	
	A266G	Q89R	4	sp9	Damaging	0	
	C377T	A126V	9	sp11	Damaging	0	
	C427T	L143F	4	sp7	Damaging	0.01	No
	T499G	Y167D	3	sp2	Tolerated	0.88	
	G517A	A173T	4	sp20	Tolerated	0.08	No
	C530T	A177V	3	sp16	Tolerated	1	
	C689T	A230V	19	sp20	Tolerated	0.08	
	C746T	A249V	8	sp22	Tolerated	0.2	
	T764A	F255Y	4	sp21	Tolerated	0.09	
	G829A	A277T	4	sp22	Damaging	0.04	
	G850A	A284T	8	sp19	Tolerated	0.29	
	A920T	Q307L	8	sp23	Damaging	0	
	C929T	A310V	6	sp1	Tolerated	0.12	
	G1051A	G351S	4	sp21	Damaging	0	
	C1163T	S388F	3	sp14	Damaging	0.02	
	C1273T	L425F	6	sp20	Tolerated	0.06	
	G1318A	V440M	3	sp10	Damaging	0	
	C1322T	T441M	3	sp17	Tolerated	0.1	
	G1354A	A452T	3	sp21	Tolerated	0.17	
	C1460T	T487M	7	sp19	Damaging	0	

(continued)

Table 2, *continued*

Gene	Mutation[a]	Effect[b]	Frequency[c]	Distribution	Function[d]	SIFT[d]	Validation[e]
	G1481A	R494K	11	sp17	Damaging	0	
	G1604A	S535N	3	sp8	Damaging	0.01	
	G1738T	E580*	6	sp14	Stop Codon	0	
	A1739G	E580G	5	sp14	Damaging	0	
	G1760A	G587D	4	sp18	Damaging	0	
	G1831A	A611T	5	sp18	Damaging	0	
	C1841T	A614V	5	sp11	Damaging	0	
	G1867A	V623M	8	sp2	Damaging	0	
	G1984A	E662K	6	sp18	Damaging	0	
	G2077A	A693T	3	sp19	Tolerated	0.66	
	T2210C	F737S	3	sp3	Tolerated	0.29	
	A2257T	M753L	10	sp2	Tolerated	0.72	
	G2284A	A762T	3	sp17	Tolerated	0.57	
	G2287A	G763R	5	sp3	Tolerated	0.57	
	C2387G	A796G	3	sp1	Damaging	0.02	
	C2440T	P814S	7	sp5	Tolerated	0.46	
	G2797A	V933M	3	sp4	Damaging	0.03	
	C2803T	P935S	5	sp23	Damaging	0	
	G3035A	G1012E	5	sp20	Damaging	0	
	C3059T	A1020V	3	sp6	Damaging	0.04	
	C3133T	R1045C	4	sp21	Damaging	0	
rht1a	C358T	P120S	4	sp18	Damaging	0.04	Yes
rht1b	G160A	A54T	3	sp13	Tolerated	0.05	
	C184T	Q62*	3	sp23	Stop Codon	0	

[a] Mutation is named as *X#Y*, where *X* is the wild-type nucleotide, # is the position of the substitution, and *Y* is the mutated nucleotide.
[b] Effect is designated as *X#Y*, where *X* is the wild-type amino acid, # is the position of the substitution, and *Y* is the mutated amino acid.
[c] The mutation density in the total data set.
[d] Function and SIFT represent predicted protein function effect and the scores, respectively. A score value <0.05 is usually predicted as damaging effect.
[e] PCR validation of selected mutations. *Yes* indicates that a mutation was validated by Sanger sequencing. *No* indicates that a mutation was found to be a false positive by Sanger sequencing.

We confirmed a G to A mutation at the 742-nt position (G742A) from the *dw3a* gene (Figure 2, i–iii) and a C to T mutation at the 106-nt position (C106T) from the *dw3b* gene (Figure 2, iv–vi) in the M2 tef lines p893 and p932, respectively. These two mutations encode alanine (nonpolar) to threonine (polar) and proline (nonpolar) to serine (polar) amino acid changes and were predicted to severely affect protein function by SIFT scores of 0.02 and 0.00, respectively, probably by alteration of the folding of the enzyme. Both nucleotides were located in a highly conserved domain of the predicted *dw3* protein. These two amino acids are fully conserved across maize, sorghum, and tef. Sequencing the key region of the *dw3* homeologue in individual M3 tef lines indicated that both G742A and C106T mutants were heterozygous at the target site in the M2 generation. (Figure S2). In an *rht1* homeologue, a C to T mutation at the 358-nt position is also predicted as deleterious (Table 2). This amino acid (proline) is conserved across the grass family except in wheat, where it is a glutamine, while the predicted change in the tef mutation is to a serine.

Possible phenotypic effects of the G742A and C106T mutations were examined in the M3 generations of tef lines p893 and p932. Regardless of high-input or low-input fertilizer regimens, homozygous wild-type plants were not distinguishable from either the heterozygous or homozygous mutations for either gene (Figure S3).

Discussion

The cloning and sequencing of two dw3/br2 homeologues from tef

A fosmid library representing approximately 6× coverage of the *Eragrostis tef* genome was constructed. The quality of this library was confirmed by PCR screening for *dw3* homeologues. This screening identified seven positive clones in the superpools, suggesting that *dw3* homeologues are present as single-copy genes (that is, one copy per diploid genome) in the tetraploid tef genome. This is consistent with previous studies on the diploids maize and sorghum, where *br2* and *dw3*, respectively, are single-copy genes (Multani *et al.* 2003).

From sequence variation between the two homeologous genes, *dw3a* and *dw3b*, an ancestral divergence date of ~9–10 MYA was calculated for the diploid genomes in tetraploid tef. For the two apparent *rht1* homeologues used in this study, Smith and colleagues (Smith *et al.* 2012) calculated a divergence time of ~4–5 MYA. Given the significant variation that exists in the rates of divergence for different plant nuclear genes (Zhang *et al.* 2002), it is clear that many more homeologous pairs need to have their divergence date ascertained in tef, so a relatively robust number can be derived.

Mutagenized tef populations and mutation discovery

For many decades, mutagenesis has been widely used for genetic analysis in plants and animals. Alkylating agents,

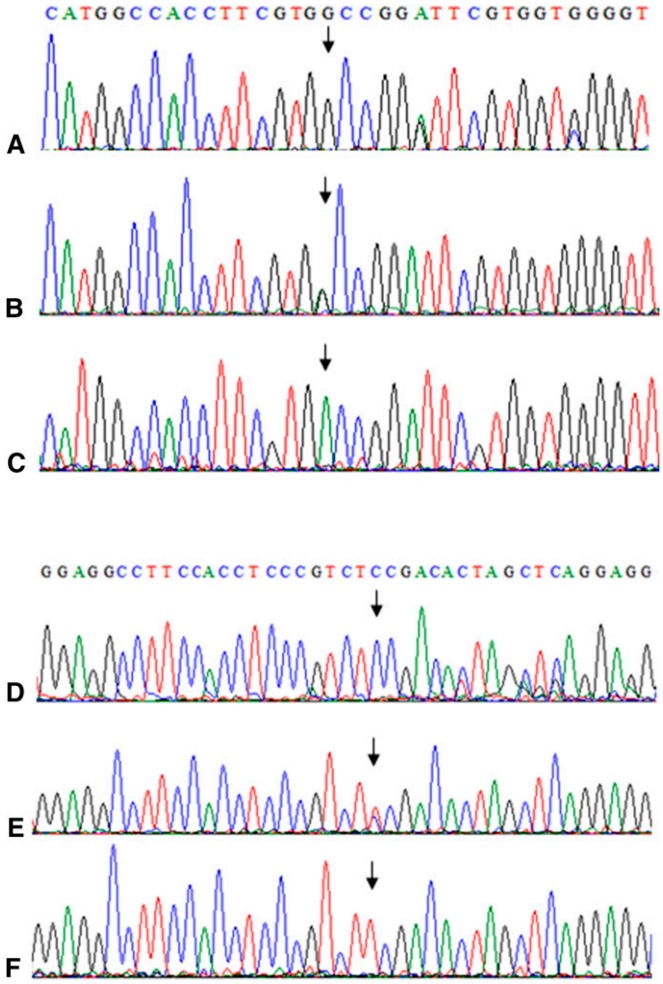

Figure 2 Mutation validation by Sanger sequence analysis. G742A from *dw3a* (A–C) and C106T from *dw3b* (D–F) have been validated in single plants. Mutation position is marked by an arrow. A nucleotide G (wild type) is detected at base 742 from superpool sp19 (A). It is identified as a G/A heterozygote in pool p892 (B) and A in the single plant p893-2 (C). Similarly, A nucleotide C (wild type) is detected as the 106th base in superpool sp20 (D). It is identified as a C/T heterozygote in pool p932 (E) and is confirmed as a T in the single plant p932-4 (F).

including EMS, are particularly well suited for higher plants. One of the most challenging problems is to determine the ideal dosage of the mutagen. The toxicity and mutagenicity of EMS vary tremendously from one species to the next. A low-concentration treatment allows greater survival and less subsequent issues with segregation of desired mutations from undesired events, but requires more screening to identify the targeted mutants. In most crops, the commonly used EMS concentration is 0.25–1.5%. For instance, 1% EMS-treated tomato plants yield ~1.8-fold more mutations per genome than a 0.7% EMS treatment, but the fertility rate was 41% lower than the 0.7% EMS (Minoia *et al.* 2010). In this tef study, seed treatments of up to 1.5% were well tolerated and provided significantly more mutations than 1% treatment. For the genes targeted in this study, the apparent mutation rate was 2.8× higher in the 1.5% treatment than in the 1% treatment, under the conditions employed.

The size of the mutagenized population is also important to ensure that a sufficient number of targeted mutations will be found. For example, in *Arabidopsis*, 18,000 EMS mutagenized lines were needed to find a nonsense mutation in any targeted 1.0-kb coding region with 95% confidence (Greene *et al.* 2003; Parry *et al.* 2009). Based on the apparent mutation frequency at the *dw3* homeologues of tef from prediction, ~2800 mutagenized lines would be required to identify a nonsense mutation with 95% confidence, given a coding region of 4 kb (*dw3*) and nonsense mutations that account for ~3% of the events. In this study, ~3 kb of the coding region was analyzed by amplicon sequencing for the 2,121 mutagenized pools, so the probability of finding a nonsense mutation was estimated to be 83%, assuming a binomial distribution. However, no nonsense mutation was confirmed.

A total of 120 candidate mutations were identified from four candidate genes, two of each of the *dw3/br2* and *rth1* homeologues, in 21,210 mutagenized tef lines. This number (120) was arrived at after low-frequency mutations (less than three reads in the amplicon data set) and low-quality reads were removed as probable false positives. Of the 10 candidate genes that were individually investigated by follow-up Sanger sequencing, the 4 mutations with >4 supporting reads (5–19) in the superpool data were all confirmed as true mutations, while the 2 mutations with only 3 supporting reads were found to be false positives. Of the 4 mutations with 4 supporting reads in the superpool data set, 2 were confirmed and 2 found to be false positives. Hence, if we extrapolate to the entire candidate pool, this suggests that the 61 candidate mutations with at least 5 sequences and half of the sequences with 4 reads in the superpool data (18/2 = 9) are likely to be real. This predicted level of confirmation, 70/120, also suggests that the EMS mutation rate is about ~1.7× lower than our initial calculation. Some mutations may have been missed in this project if they were somewhat underrepresented by mere chance in the bulk sequencing data. A larger redundancy of amplicon reads could have helped solve this problem. Because each pool was derived from a single M1 seed, a mutation in a particular diploid seed embryo would be chimeric in the developing plant and would variably segregate in the M2 generation (due to both heterozygosity and the degree to which the mutated embryonic cell was represented by a clone of cells that made it into the gametophytic lineage). As the sequencing coverage for each pool was estimated at approximately 10×, we should observe 5 reads that harbor this mutation on average. The chance for a *bona fide* mutation to be observed with frequency <3 is 0.0547 (calculated by binomial distribution). Hence, mutations with a frequency <3 were not further considered. On the other hand, previous studies indicated that EMS mutations are predominantly G/C to A/T transitions, given the frequent alkylation of guanine residues by EMS (Krieg 1963). In *Arabidopsis*, maize and wheat, >99% of EMS-induced mutations were G/C to A/T transition (Greene *et al.* 2003; Till *et al.* 2004; Slade *et al.* 2005). In the mutagenized tef populations

generated for this study, 69% of 1% EMS mutants and 82% of 1.5% EMS were found to be G/C to A/T transitions. A few transversions and A/T to G/C transitions were also observed, similar to the mutation spectrum reported in rice (Till *et al.* 2007), barley (Caldwell *et al.* 2004), and tomato (Minoia *et al.* 2010). The transversion mutations might result from EMS treatment or natural mutations in the population. In addition, PCR/sequencing errors may be responsible for some false positives even though we used high-fidelity Taq polymerase (error rate 4.4×10^{-7}). Taq polymerase errors are heavily biased toward A/T to G/C changes (Keohavong and Thilly 1989).

The observed mutation frequency (8.7 mutations/Mbp) for *dw3* genes was found to be higher than the *rht1* genes (2.7 mutations/Mbp). These frequencies are comparable to previous studies on rice, sorghum, tomato, and *Arabidopsis* (2–6 mutations/Mbp) (Greene *et al.* 2003; Xin *et al.* 2008; Minoia *et al.* 2010). These values are notably higher than the 1 mutation/Mb that was found in diploid rice and barley as well as tetraploid peanut (Caldwell *et al.* 2004; Knoll *et al.* 2011), but much lower than the 24–42 mutations/Mbp documented in tetraploid and hexaploid wheat (Slade *et al.* 2005), the only species with a mutation rate >1 mutation/50 kb that has been reported so far. A higher mutation rate will, of course, reduce the population size required for effective screening, but requires tolerance of this mutational rate by the mutagenized lines. The mutagenized tef in this project showed excellent vigor and fertility, so it is likely that an even-higher level of mutagenesis would be tolerated.

Phenotypic analysis

We investigated two predicted deleterious mutations, one in each tef *dw3* homeologue, in the corresponding M3 generations and validated both by Sanger sequence analysis. Plants segregating for these mutations exhibited no obvious height or other morphological differences at either high-fertilizer input conditions like those usually seen in greenhouse studies or under very low fertilizer inputs like those seen in standard Ethiopian tef agriculture. This suggests that each gene can complement mutations at the other locus.

Next steps

As a tetraploid species, it is challenging to discover mutation phenotypes from the tef genome because homeologous alleles are expected to be present even for a single-copy gene. Unless genes have been lost, subfunctionalized or neofunctionalized, it is likely that mutation in a single homeologous copy, even when homozygous, would not yield a phenotype. Fortunately, two mutations that were predicted to be quite deleterious, G742A and C106T, from each of the homeologous *dw3* genes were found in this analysis. These lines will now be crossed to bring these two mutations together and then self or sibling pollinated to generate segregants that are homozygous for mutations in these two *dw3* orthologs. It is hoped that some of the resulting lines will show a semi-dwarf character, lodging resistance and improved grain yield. If so, these *dw3* alleles can be introgressed into a wide variety of locally adapted tef cultivars, thereby removing any undesired mutations in the mutagenized stock and allowing rigorous field experimentation regarding the value of this approach to tef improvement.

The results demonstrate that next-generation sequencing offers a nontransgenic alternative for the rapid identification of targeted genetic variation. In tef, semi-dwarfing mutations were found that might dramatically improve crop yield, once brought together into the same genetic background. This approach is universally applicable and may provide exceptional value to orphan crop species with a very limited research toolkit and/or research community and to agricultural environments where transgenic improvement might not be tolerated.

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Literature Cited

Altschul, S. F., T. L. Madden, A. A. Schäffer, J. Zhang, Z. Zhang *et al.*, 1997 Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res. 25: 3389–3402.

Asano, K., K. Hirano, M. Ueguchi-Tanaka, R. B. Angeles-Shim, T. Komura *et al.*, 2009 Isolation and characterization of dominant dwarf mutants, *Slr*1-d, in rice. Mol. Gen. Genet. 281: 223–231.

Assefa, K., J. K. Yu, M. Zeid, G. Belay, H. Tefera *et al.*, 2011 Breeding tef [*Eragrostis tef* (Zucc.) trotter]: conventional and molecular approaches. Plant Breed. 130: 1–9.

Auerbach, C., and J. M. Robson, 1946 Chemical production of mutations. Nature 157: 302.

Ayele, M., J. Doleže, M. Duren, H. Brunner, and F. Zapata-Arias, 1996 Flow cytometric analysis of nuclear genome of the Ethiopian cereal Tef. [*Eragrostis tef* (Zucc.) Trotter] Genetica 98: 211–215.

Bundock, P. C., F. G. Eliott, G. Ablett, A. D. Benson, R. E. Casu *et al.*, 2009 Targeted single nucleotide polymorphism (SNP) discovery in a highly polyploid plant species using 454 sequencing. Plant Biotechnol. J. 7: 347–354.

Burge, C., and S. Karlin, 1997 Prediction of complete gene structures in human genomic DNA1. J. Mol. Biol. 268: 78–94.

Caldwell, D. G., N. McCallum, P. Shaw, G. J. Muehlbauer, D. F. Marshall *et al.*, 2004 A structured mutant population for forward and reverse genetics in Barley (*Hordeum vulgare* L.). Plant J. 40: 143–150.

Central Statistics Authority, 2005 Agricultural Sample Survey 2004/2005 Report on area and production for major crops, vol. 1. CSA, Addis Ababa, Ethiopia.

Comai, L., K. Young, B. J. Till, S. H. Reynolds, E. A. Greene *et al.*, 2004 Efficient discovery of DNA polymorphisms in natural populations by Ecotilling. Plant J. 37: 778–786.

Cooper, J. L., B. J. Till, R. G. Laport, M. C. Darlow, J. M. Kleffner *et al.*, 2008 TILLING to detect induced mutations in soybean. BMC Plant Biol. 8: 9.

Dalmais, M., J. Schmidt, C. Le Signor, F. Moussy, J. Burstin *et al.*, 2008 UTILLdb, a *Pisum sativum* in silico forward and reverse genetics tool. Genome Biol. 9: R43.

Ehrenberg, L., and A. Gustafsson, 1957 On the mutagenic action of ethylene oxide and di-epoxybutane in barley. Hereditas 43: 595–602.

Foster, K. W., and J. N. Rutger, 1978 Inheritance of semidwarfism in rice, *Oryza sativa* L. Genetics 88: 559–574.

Greene, E. A., C. A. Codomo, N. E. Taylor, J. G. Henikoff, B. J. Till *et al.*, 2003 Spectrum of chemically induced mutations from a large-scale reverse-genetic screen in Arabidopsis. Genetics 164: 731–740.

Hong, Z., M. Ueguchi-Tanaka, K. Umemura, S. Uozu, S. Fujioka *et al.*, 2003 A rice brassinosteroid-deficient mutant, ebisu dwarf (*d2*), is caused by a loss of function of a new member of cytochrome P450. Plant Cell 15: 2900–2910.

Itoh, H., M. Ueguchi-Tanaka, N. Sentoku, H. Kitano, M. Matsuoka *et al.*, 2001 Cloning and functional analysis of two gibberellin 3-hydroxylase genes that are differently expressed during the growth of rice. Proc. Natl. Acad. Sci. USA 98: 8909–8914.

Jennings, P. R., 1964 Plant type as a rice breeding objective. Crop Sci. 4: 13–15.

Karper, R. E., 1932 A dominant mutation of frequent recurrence in sorghum. Am. Nat. 66: 511–529.

Keohavong, P., and W. G. Thilly, 1989 Fidelity of DNA polymerases in DNA amplification. Proc. Natl. Acad. Sci. USA 86: 9253–9257.

Knoll, J. E., M. L. Ramos, Y. Zeng, C. C. Holbrook, M. Chow *et al.*, 2011 TILLING for allergen reduction and improvement of quality traits in peanut (*Arachis hypogaea* L.). BMC Plant Biol. 11: 81.

Krieg, D. R., 1963 Ethyl methanesulfonate-induced reversion of bacteriophage *T4rII* mutants. Genetics 48: 561–580.

Mamanova, L., A. J. Coffey, C. E. Scott, I. Kozarewa, E. H. Turner *et al.*, 2010 Target-enrichment strategies for next-generation sequencing. Nat. Methods 7: 111–118.

Martín, B., M. Ramiro, J. M. Martínez-Zapater, and C. Alonso-Blanco, 2009 A high-density collection of EMS-induced mutations for TILLING in Landsberg erecta genetic background of Arabidopsis. BMC Plant Biol. 9: 147.

Minoia, S., A. Petrozza, O. D'Onofrio, F. Piron, G. Mosca *et al.*, 2010 A new mutant genetic resource for tomato crop improvement by TILLING technology. BMC Res. Notes 3: 69.

Monna, L., N. Kitazawa, R. Yoshino, J. Suzuki, H. Masuda *et al.*, 2002 Positional cloning of rice semidwarfing gene, *sd*-1: rice "green revolution gene" encodes a mutant enzyme involved in gibberellin synthesis. DNA Res. 9: 11–17.

Muangprom, A., 2005 A novel dwarfing mutation in a green revolution gene from *Brassica rapa*. Plant Physiol. 137: 931–938.

Multani, D. S., S. P. Briggs, M. A. Chamberlin, J. J. Blakeslee, A. S. Murphy *et al.*, 2003 Loss of an MDR transporter in compact stalks of maize *br*2 and sorghum *dw*3 mutants. Science 302: 81–84.

Munkvold, G. P., and R. L. Hellmich, 2000 Genetically modified, insect resistant maize: implications for management of ear and stalk diseases. Plant Health Prog., http://www.planthealthprogress.org/current/reviews/maize/ 10.1094/PHP-2000–0912–01-RV.

Murray, M. G., and W. Thompson, 1980 Rapid isolation of high molecular weight plant DNA. Nucleic Acids Res. 8: 4321–4325.

Natarajan, A. T., and M. S. Ramanna, 1966 Modification of relative mutagenic efficiency in barley of mesyloxy esters by different treatments. Nature 211: 1099–1100.

Nei, M., and T. Gojobori, 1986 Simple methods for estimating the numbers of synonymous and nonsynonymous nucleotide substitutions. Mol. Biol. Evol. 3: 418–426.

Ng, P. C., 2003 SIFT: predicting amino acid changes that affect protein function. Nucleic Acids Res. 31: 3812–3814.

Ng, S. B., K. J. Buckingham, C. Lee, A. W. Bigham, H. K. Tabor *et al.*, 2010a Exome sequencing identifies the cause of a mendelian disorder. Nat. Genet. 42: 30–35.

Ng, S. B., A. W. Bigham, K. J. Buckingham, M. C. Hannibal, M. J. McMillin *et al.*, 2010b Exome sequencing identifies *MLL*2 mutations as a cause of Kabuki syndrome. Nat. Genet. 42: 790–793.

Parry, M. A. J., P. J. Madgwick, C. Bayon, K. Tearall, A. Hernandez-Lopez *et al.*, 2009 Mutation discovery for crop improvement. J. Exp. Bot. 60: 2817–2825.

Peng, J., D. E. Richards, N. M. Hartley, G. P. Murphy, K. M. Devos *et al.*, 1999 'Green revolution' genes encode mutant gibberellin response modulators. Science 400: 256–261.

Quinby, J., and R. Karper, 1954 Inheritance of height in sorghum. Agron. J. 46: 211–216.

Rapoport, I. A., 1946 Carbonyl compounds and the chemical mechanism of mutations. Compt. Rend. Acad. Sci. 54: 65–67.

Rigola, D., J. Van Oeveren, A. Janssen, A. Bonné, H. Schneiders *et al.*, 2009 High-throughput detection of induced mutations and natural variation using KeyPoint technology. PLoS ONE 4: e4761.

Sakamoto, T., Y. Morinaka, T. Ohnishi, H. Sunohara, S. Fujioka *et al.*, 2005 Erect leaves caused by brassinosteroid deficiency increase biomass production and grain yield in rice. Nat. Biotechnol. 24: 105–109.

Sasaki, A., M. Ashikari, M. Ueguchi-Tanaka, H. Itoh, A. Nishimura *et al.*, 2002 A mutant gibberellin-synthesis gene in rice. Nature 416: 701–702.

Slade, A. J., S. I. Fuerstenberg, D. Loeffler, M. N. Steine, and D. Facciotti, 2005 A reverse genetic, nontransgenic approach to wheat crop improvement by TILLING. Nat. Biotechnol. 23: 75–81.

Smith, S.M., Y. Yuan, A. N. Doust, and J. L. Bennetzen, 2012 Haplotype analysis and linkage disequilibrium at five loci in Eragrostis tef. G3: Genes Genomes Genetics 2: 407–419.

Spielmeyer, W., 2002 Semidwarf (*sd*-1), "green revolution" rice, contains a defective gibberellin 20-oxidase gene. Proc. Natl. Acad. Sci. USA 99: 9043–9048.

Stallknecht, G. F., K. M. Gilbertson, and J. Eckhoff, 1993 Teff: food crop for humans and animals, pp. 604–615 in *New Crops*, edited by J. Janick and J. E. Simon. Wiley, New York.

Suzuki, T., M. Eiguchi, T. Kumamaru, H. Satoh, H. Matsusaka *et al.*, 2008 MNU-induced mutant pools and high performance TILLING enable finding of any gene mutation in rice. Mol. Gen. Genet. 279: 213–223.

Swigonova, Z., J. Lai, J. Ma, W. Ramakrishna, V. Llaca *et al.*, 2004 On the tetraploid origin of the maize genome. Comp. Funct. Genomics 5: 281–284.

Tanabe, S., M. Ashikari, S. Fujioka, S. Takatsuto, S. Yoshida *et al.*, 2005 A novel cytochrome P450 is implicated in brassinosteroid biosynthesis via the characterization of a rice dwarf mutant, *dwarf*11, with reduced seed length. Plant Cell 17: 776–790.

Teferra, T., H. Tefera, B. Simane, and M. Tuinstra, 2000 The influence of drought stress on yield of tef (*Eragrostis tef*). Trop. Sci. 40: 40–45.

Till, B. J., S. H. Reynolds, E. A. Greene, C. A. Codomo, L. C. Enns *et al.*, 2003 Large-scale discovery of induced point mutations with high-throughput TILLING. Genome Res. 13: 524–530.

Till, B. J., S. H. Reynolds, C. Weil, N. Springer, C. Burtner *et al.*, 2004 Discovery of induced point mutations in maize genes by TILLING. BMC Plant Biol. 4: 12.

Till, B. J., J. Cooper, T. H. Tai, P. Colowit, E. A. Greene *et al.*, 2007 Discovery of chemically induced mutations in rice by TILLING. BMC Plant Biol. 7: 19.

Walcott, J. J., and D. R. Laing, 1976 Some physiological aspects of growth and yield in wheat crops: a comparison of a semidwarf and standard height cultivar. Aust. J. Exp. Agric. Anim. Husb. 16: 578–587.

Xin, Z., L. Wang, N. A. Barkley, G. Burow, C. Franks *et al.*, 2008 Applying genotyping (TILLING) and phenotyping analyses to elucidate gene function in a chemically induced sorghum mutant population. BMC Plant Biol. 8: 103.

Yu, J.-K., E. Graznak, F. Breseghello, H. Tefera, and M. E. Sorrells, 2007 QTL mapping of agronomic traits in tef. [*Eragrostis tef* (Zucc) Trotter] BMC Plant Biol. 7: 30.

Zhang, L., T. J. Vision, and B. S. Gaut, 2002 Patterns of nucleotide substitution among simultaneously duplicated gene pairs in *Arabidopsis thaliana*. Mol. Biol. Evol. 19: 1464.

Zou, J., S. Zhang, W. Zhang, G. Li, Z. Chen *et al.*, 2006 The rice HIGH-TILLERING DWARF1 encoding an ortholog of Arabidopsis *MAX*3 is required for negative regulation of the outgrowth of axillary buds. Plant J. 48: 687–698.

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GENETICS

Supporting Information
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High-Throughput Discovery of Mutations in Tef Semi-Dwarfing Genes by Next-Generation Sequencing Analysis

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DOI: 10.1534/genetics.112.144436

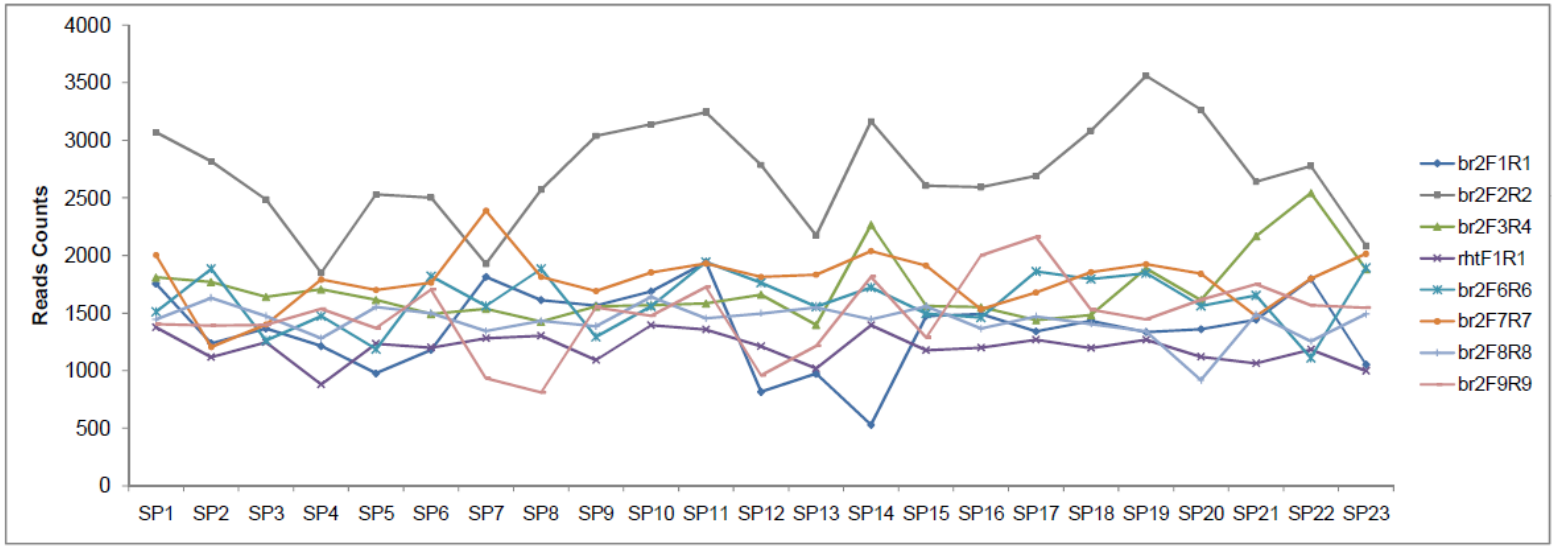
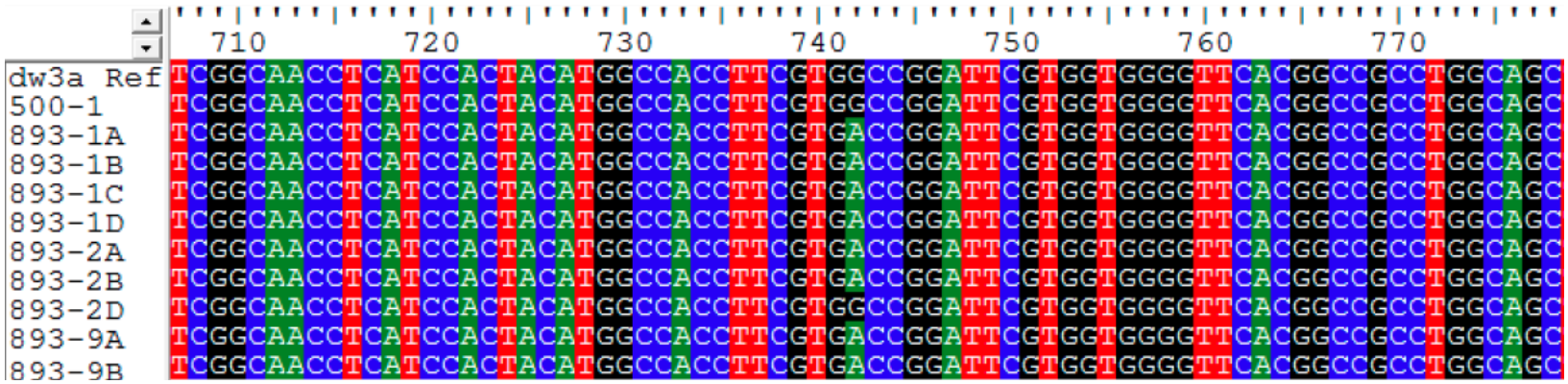
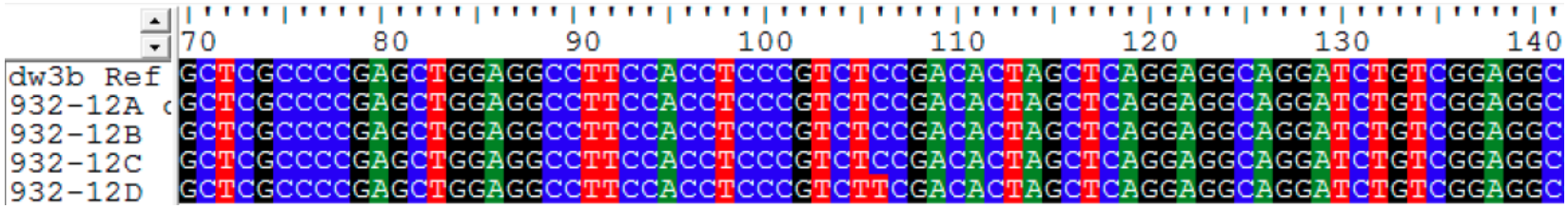


Figure S1 454 read distribution of 8 amplicons in the 23 superpools. X axis represents 23 superpools from the 21,210 EMS mutagenized tef lines. Y axis represents the number of reads in the superpools. 8 different signs represent 8 amplicons. Among them, rhtF1R1 was amplified from *rht*1 genes and the other 7 were amplified from *dw*3 genes



A)



B)

Figure S2 Sanger sequencing validation on M3 generation plants segregating for mutations in a candidate tef dwarfing gene. A) p893 mutant alignment with reference sequence of dw3a gene. The G742A mutation is validated by seven p893 M3 lines; B) p932 mutant alignment with reference sequence of the dw3b gene. The C106T mutation is validated by one p932 M3 line, 932-12D

Figure S3 Phenotypes of *dw3* homologue mutants in M3 lines of tef (500-1: control; 893-1 and 893-9: G742A mutants; 932-12: C106T mutant)

Table S1 Summary of tef genomic fosmid library

Accession number	PI 524434
Source of genomic DNA	1-month-old seedlings
Total number of colony forming units	102,191
Number of superpools	25
Number of pools	1,187
Average insert size	35 kb – 45kb
Haploid genome size	714Mbp
Library coverage	5.0X – 6.4X

Table S2 Primers for 454 sequencing

Primer name	Fusion primer (5'-3')	MID_Tag	PCR primer (5'-3')
dw3E1F1_SP1_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	ACGAGTGC	CTAGYAGCGACCCGGAGGAGAT
dw3E1F1_SP2_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	AGACGCAC	CTAGYAGCGACCCGGAGGAGAT
dw3E1F1_SP3_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	AGCACTGT	CTAGYAGCGACCCGGAGGAGAT
dw3E1F1_SP4_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	CTCGCGTG	CTAGYAGCGACCCGGAGGAGAT
dw3E1F1_SP5_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	TGATACGT	CTAGYAGCGACCCGGAGGAGAT
dw3E1F1_SP6_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	CATAGTAG	CTAGYAGCGACCCGGAGGAGAT
dw3E1F1_SP7_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	CGAGAGAT	CTAGYAGCGACCCGGAGGAGAT
dw3E1F1_SP8_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	CGTCTAGT	CTAGYAGCGACCCGGAGGAGAT
dw3E1F1_SP9_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	TGTACTAC	CTAGYAGCGACCCGGAGGAGAT
dw3E1F1_SP10_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	CGTAGACT	CTAGYAGCGACCCGGAGGAGAT
dw3E1F1_SP11_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	TACGAGTA	CTAGYAGCGACCCGGAGGAGAT
dw3E1F1_SP12_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	TACTCTCG	CTAGYAGCGACCCGGAGGAGAT
dw3E1F1_SP13_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	TAGAGACG	CTAGYAGCGACCCGGAGGAGAT
dw3E1F1_SP14_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	ACTGTACA	CTAGYAGCGACCCGGAGGAGAT
dw3E1F1_SP15_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	ATAGAGTA	CTAGYAGCGACCCGGAGGAGAT
dw3E1F1_SP16_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	CACGCTAC	CTAGYAGCGACCCGGAGGAGAT
dw3E1F1_SP17_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	CAGTAGAC	CTAGYAGCGACCCGGAGGAGAT
dw3E1F1_SP18_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	CGACGTGA	CTAGYAGCGACCCGGAGGAGAT
dw3E1F1_SP19_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	TCGATCAC	CTAGYAGCGACCCGGAGGAGAT
dw3E1F1_SP20_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	TCGCACTA	CTAGYAGCGACCCGGAGGAGAT
dw3E1F1_SP21_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	AGTCGAGA	CTAGYAGCGACCCGGAGGAGAT
dw3E1F1_SP22_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	CGCAGTAC	CTAGYAGCGACCCGGAGGAGAT
dw3E1F1_SP23_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	TATGCTAG	CTAGYAGCGACCCGGAGGAGAT
dw3E1R1a_FB	CCTATCCCCTGTGTGCCTTGGCAGTCTCAG		ACYTGGCCACCAGCCGCACCA
dw3E1R2a_SP1_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	ACGAGTGCGT	TGGCTCCGCGACGACAGCTT
dw3E1R2a_SP2_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	AGACGCACTC	TGGCTCCGCGACGACAGCTT
dw3E1R2a_SP3_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	AGCACTGTAG	TGGCTCCGCGACGACAGCTT
dw3E1R2a_SP4_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	CTCGCGTGTC	TGGCTCCGCGACGACAGCTT
dw3E1R2a_SP5_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	TGATACGTCT	TGGCTCCGCGACGACAGCTT
dw3E1R2a_SP6_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	CATAGTAGTG	TGGCTCCGCGACGACAGCTT
dw3E1R2a_SP7_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	CGAGAGATAC	TGGCTCCGCGACGACAGCTT
dw3E1R2a_SP8_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	CGTCTAGTAC	TGGCTCCGCGACGACAGCTT
dw3E1R2a_SP9_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	TGTACTACTC	TGGCTCCGCGACGACAGCTT
dw3E1R2a_SP10_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	CGTAGACTAG	TGGCTCCGCGACGACAGCTT
dw3E1R2a_SP11_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	TACGAGTATG	TGGCTCCGCGACGACAGCTT
dw3E1R2a_SP12_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	TACTCTCGTG	TGGCTCCGCGACGACAGCTT

dw3E1R2a_SP13_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	TAGAGACGAG	TGGCTCCGCGACGACAGCTT
dw3E1R2a_SP14_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	ACTGTACAGT	TGGCTCCGCGACGACAGCTT
dw3E1R2a_SP15_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	ATAGAGTACT	TGGCTCCGCGACGACAGCTT
dw3E1R2a_SP16_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	CACGCTACGT	TGGCTCCGCGACGACAGCTT
dw3E1R2a_SP17_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	CAGTAGACGT	TGGCTCCGCGACGACAGCTT
dw3E1R2a_SP18_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	CGACGTGACT	TGGCTCCGCGACGACAGCTT
dw3E1R2a_SP19_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	TCGATCACGT	TGGCTCCGCGACGACAGCTT
dw3E1R2a_SP20_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	TCGCACTAGT	TGGCTCCGCGACGACAGCTT
dw3E1R2a_SP21_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	AGTCGAGAGA	TGGCTCCGCGACGACAGCTT
dw3E1R2a_SP22_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	CGCAGTACGA	TGGCTCCGCGACGACAGCTT
dw3E1R2a_SP23_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	TATGCTAGTA	TGGCTCCGCGACGACAGCTT
dw3E1F2a_FB	CCTATCCCCTGTGTGCCTTGGCAGTCTCAG		ATGGTGCGGCTGGTGGCCARG
dw3E1R4_SP1_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	ACGAGTGC	GTGACGGGTAGGCGAAGTCAAC
dw3E1R4_SP2_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	AGACGCAC	GTGACGGGTAGGCGAAGTCAAC
dw3E1R4_SP3_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	AGCACTGT	GTGACGGGTAGGCGAAGTCAAC
dw3E1R4_SP4_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	CTCGCGTG	GTGACGGGTAGGCGAAGTCAAC
dw3E1R4_SP5_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	TGATACGT	GTGACGGGTAGGCGAAGTCAAC
dw3E1R4_SP6_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	CATAGTAG	GTGACGGGTAGGCGAAGTCAAC
dw3E1R4_SP7_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	CGAGAGAT	GTGACGGGTAGGCGAAGTCAAC
dw3E1R4_SP8_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	CGTCTAGT	GTGACGGGTAGGCGAAGTCAAC
dw3E1R4_SP9_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	TGTACTAC	GTGACGGGTAGGCGAAGTCAAC
dw3E1R4_SP10_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	CGTAGACT	GTGACGGGTAGGCGAAGTCAAC
dw3E1R4_SP11_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	TACGAGTA	GTGACGGGTAGGCGAAGTCAAC
dw3E1R4_SP12_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	TACTCTCG	GTGACGGGTAGGCGAAGTCAAC
dw3E1R4_SP13_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	TAGAGACG	GTGACGGGTAGGCGAAGTCAAC
dw3E1R4_SP14_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	ACTGTACA	GTGACGGGTAGGCGAAGTCAAC
dw3E1R4_SP15_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	ATAGAGTA	GTGACGGGTAGGCGAAGTCAAC
dw3E1R4_SP16_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	CACGCTAC	GTGACGGGTAGGCGAAGTCAAC
dw3E1R4_SP17_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	CAGTAGAC	GTGACGGGTAGGCGAAGTCAAC
dw3E1R4_SP18_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	CGACGTGA	GTGACGGGTAGGCGAAGTCAAC
dw3E1R4_SP19_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	TCGATCAC	GTGACGGGTAGGCGAAGTCAAC
dw3E1R4_SP20_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	TCGCACTA	GTGACGGGTAGGCGAAGTCAAC
dw3E1R4_SP21_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	AGTCGAGA	GTGACGGGTAGGCGAAGTCAAC
dw3E1R4_SP22_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	CGCAGTAC	GTGACGGGTAGGCGAAGTCAAC
dw3E1R4_SP23_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	TATGCTAG	GTGACGGGTAGGCGAAGTCAAC
dw3E1F3a_FB	CCTATCCCCTGTGTGCCTTGGCAGTCTCAG		AAGCTGTCGTCGCGGAGCCA
rht1F1_SP1_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	ACGAGTGCGT	AAGGACAAGATGATGGTGGC
rht1F1_SP2_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	AGACGCACTC	AAGGACAAGATGATGGTGGC
rht1F1_SP3_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	AGCACTGTAG	AAGGACAAGATGATGGTGGC

rht1F1_SP4_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	CTCGCGTGTC	AAGGACAAGATGATGGTGGC
rht1F1_SP5_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	TGATACGTCT	AAGGACAAGATGATGGTGGC
rht1F1_SP6_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	CATAGTAGTG	AAGGACAAGATGATGGTGGC
rht1F1_SP7_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	CGAGAGATAC	AAGGACAAGATGATGGTGGC
rht1F1_SP8_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	CGTCTAGTAC	AAGGACAAGATGATGGTGGC
rht1F1_SP9_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	TGTACTACTC	AAGGACAAGATGATGGTGGC
rht1F1_SP10_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	CGTAGACTAG	AAGGACAAGATGATGGTGGC
rht1F1_SP11_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	TACGAGTATG	AAGGACAAGATGATGGTGGC
rht1F1_SP12_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	TACTCTCGTG	AAGGACAAGATGATGGTGGC
rht1F1_SP13_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	TAGAGACGAG	AAGGACAAGATGATGGTGGC
rht1F1_SP14_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	ACTGTACAGT	AAGGACAAGATGATGGTGGC
rht1F1_SP15_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	ATAGAGTACT	AAGGACAAGATGATGGTGGC
rht1F1_SP16_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	CACGCTACGT	AAGGACAAGATGATGGTGGC
rht1F1_SP17_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	CAGTAGACGT	AAGGACAAGATGATGGTGGC
rht1F1_SP18_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	CGACGTGACT	AAGGACAAGATGATGGTGGC
rht1F1_SP19_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	TCGATCACGT	AAGGACAAGATGATGGTGGC
rht1F1_SP20_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	TCGCACTAGT	AAGGACAAGATGATGGTGGC
rht1F1_SP21_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	AGTCGAGAGA	AAGGACAAGATGATGGTGGC
rht1F1_SP22_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	CGCAGTACGA	AAGGACAAGATGATGGTGGC
rht1F1_SP23_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	TATGCTAGTA	AAGGACAAGATGATGGTGGC
rht1R1_FB	CCTATCCCCTGTGTGCCTTGGCAGTCTCAG		GAAGACGAGGACGAGGAAGA
dw3E2R1_SP1_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	ACGAGTGC	GCAGCTTGATGATGAATGAGTG
dw3E2R1_SP2_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	AGACGCAC	GCAGCTTGATGATGAATGAGTG
dw3E2R1_SP3_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	AGCACTGT	GCAGCTTGATGATGAATGAGTG
dw3E2R1_SP4_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	CTCGCGTG	GCAGCTTGATGATGAATGAGTG
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dw3E2R1_SP7_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	CGAGAGAT	GCAGCTTGATGATGAATGAGTG
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dw3E2R1_SP17_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	CAGTAGAC	GCAGCTTGATGATGAATGAGTG
dw3E2R1_SP18_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	CGACGTGA	GCAGCTTGATGATGAATGAGTG

dw3E2R1_SP19_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	TCGATCAC	GCAGCTTGATGATGAATGAGTG
dw3E2R1_SP20_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	TCGCACTA	GCAGCTTGATGATGAATGAGTG
dw3E2R1_SP21_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	AGTCGAGA	GCAGCTTGATGATGAATGAGTG
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dw3E2R1_SP23_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	TATGCTAG	GCAGCTTGATGATGAATGAGTG
dw3E1F6_FB	CCTATCCCCTGTGTGCCTTGGCAGTCTCAG		GTTGACTTCGCCTACCCGTC
dw3E3F9_SP1_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	ACGAGTGCGT	TGAGCGCGGTCTGCAGCTGT
dw3E3F9_SP2_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	AGACGCACTC	TGAGCGCGGTCTGCAGCTGT
dw3E3F9_SP3_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	AGCACTGTAG	TGAGCGCGGTCTGCAGCTGT
dw3E3F9_SP4_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	CTCGCGTGTC	TGAGCGCGGTCTGCAGCTGT
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dw3E3F9_SP6_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	CATAGTAGTG	TGAGCGCGGTCTGCAGCTGT
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dw3E3R1_FB	CCTATCCCCTGTGTGCCTTGGCAGTCTCAG		CGTAGGAGGAGTTGCGCGTC
dw3E3F10_SP1_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	ACGAGTGC	TCCGACTTCTCCAACGCCGACT
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dw3E3F10_SP8_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	CGTCTAGT	TCCGACTTCTCCAACGCCGACT
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dw3E3F10_SP10_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	CGTAGACT	TCCGACTTCTCCAACGCCGACT
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dw3E3F11_SP1_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	ACGAGTGCGT	GCATCTCGGTGATCGTGCAG
dw3E3F11_SP2_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	AGACGCACTC	GCATCTCGGTGATCGTGCAG
dw3E3F11_SP3_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	AGCACTGTAG	GCATCTCGGTGATCGTGCAG
dw3E3F11_SP4_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	CTCGCGTGTC	GCATCTCGGTGATCGTGCAG
dw3E3F11_SP5_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	TGATACGTCT	GCATCTCGGTGATCGTGCAG
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dw3E3F11_SP8_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	CGTCTAGTAC	GCATCTCGGTGATCGTGCAG
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dw3E3F11_SP13_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	TAGAGACGAG	GCATCTCGGTGATCGTGCAG
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dw3E3F11_SP19_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	TCGATCACGT	GCATCTCGGTGATCGTGCAG
dw3E3F11_SP20_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	TCGCACTAGT	GCATCTCGGTGATCGTGCAG
dw3E3F11_SP21_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	AGTCGAGAGA	GCATCTCGGTGATCGTGCAG
dw3E3F11_SP22_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	CGCAGTACGA	GCATCTCGGTGATCGTGCAG
dw3E3F11_SP23_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	TATGCTAGTA	GCATCTCGGTGATCGTGCAG
dw3E3R11_FB	CCTATCCCCTGTGTGCCTTGGCAGTCTCAG		GGAGACCATGAGCACCATGAAG

Table S3 Comparison of two *dw3* homoeologous genes

	Identity
Gene level	0.916
Coding region	0.948
Noncoding region	0.667
Protein level	0.941
dN (nonsynonymous substitution rate)	0.033
dS (synonymous substitution rate)	0.125

Table S4 Summary of 454 reads

Region	PCR length (bp)	Ave length of reads(bp)	Number of reads
dw3E1F1/R1	468	399	31,309
dw3E1F2/R2	416	354	61,602
dw3E1F3/R4	488	413	37,817
dw3E1F6/E2R1	471	347	37,119
dw3E3F9/E3R1	428	359	41,259
dw3E3F10/E3R11	513	371	32,929
dw3E3F11/E3R11	458	380	34,237
rhtF1/R1	521	398	35,954
others			14,910

Table S5 Spectrum of mutations identified in the dwarfing candidate genes

Gene	Freq[a]	Position	WT_Nu[b]	Mu_Nu[c]	WT_AA[d]	Mu_AA[e]	Function[f]	SIFT[f]	Superpool	EMS[g]	Validation[h]
dw3a	5	89	A	G	E	G	missense	0	sp2	1.5%	
dw3a	7	104	C	T	P	L	missense	0	sp19	1.5%	
dw3a	3	200	A	G	N	S	missense	0	sp11	1.5%	
dw3a	17	238	A	G	S	G	missense	0.4	sp6	1.0%	
dw3a	19	503	C	T	A	V	missense	1	sp19	1.5%	YES
dw3a	11	582	C	T	R	R	silent	1	sp14	1.5%	
dw3a	6	628	A	T	T	S	missense	0	sp9	1.0%	
dw3a	3	677	T	A	V	D	missense	0	sp17	1.5%	
dw3a	3	730	G	A	A	T	missense	0.2	sp2	1.5%	
dw3a	10	742	G	A	A	T	missense	0	sp19	1.5%	YES
dw3a	3	838	G	A	A	T	missense	0.2	sp3	1.0%	
dw3a	6	840	G	A	A	A	silent	1	sp19	1.5%	
dw3a	9	863	G	A	R	Q	missense	0.1	sp11	1.5%	
dw3a	3	1023	G	A	G	G	silent	1	sp17	1.5%	
dw3a	6	1105	C	T	R	C	missense	0.1	sp14	1.5%	YES
dw3a	11	1124	G	A	G	D	missense	0	sp7	1.0%	
dw3a	4	1139	C	T	T	I	missense	0.1	sp7	1.0%	YES
dw3a	3	1177	C	T	Q	*	Stop codon	0	sp14	1.5%	NO
dw3a	9	1185	G	A	P	P	silent	1	sp9	1.0%	
dw3a	13	1189	A	T	S	C	missense	0.2	sp1	1.0%	
dw3a	5	1200	C	T	F	F	silent	1	sp14	1.5%	
dw3a	3	1228	A	G	K	E	missense	0	sp19	1.5%	
dw3a	4	1270	G	A	D	N	missense	0.5	sp16	1.5%	
dw3a	3	1294	A	G	T	A	missense	0.8	sp22	1.5%	
dw3a	8	1295	C	T	T	M	missense	0.1	sp14	1.5%	
dw3a	5	1315	G	A	G	S	missense	0.1	sp22	1.5%	
dw3a	10	1526	T	A	L	H	missense	0	sp17	1.5%	
dw3a	5	1564	C	T	L	L	silent	1	sp21	1.5%	
dw3a	9	1594	C	T	L	L	silent	1	sp21	1.5%	
dw3a	4	1606	G	A	D	N	missense	0.2	sp15	1.5%	
dw3a	7	1642	G	A	A	T	missense	0	sp23	1.5%	
dw3a	8	1811	G	A	S	N	missense	0	sp9	1.0%	
dw3a	3	1814	C	A	A	E	missense	0	sp15	1.5%	
dw3a	11	1840	G	A	V	M	missense	0	sp16	1.5%	
dw3a	3	1849	G	A	A	T	missense	0	sp2	1.5%	
dw3a	3	1960	A	T	I	F	missense	0.6	sp7	1.0%	
dw3a	4	1964	G	A	G	E	missense	0	sp23	1.5%	

dw3a	3	1999	G	A	G	S	missense	0.1	sp5	1.0%	
dw3a	3	2288	G	A	R	K	missense	0.2	sp17	1.5%	
dw3a	3	2451	G	A	Y	Y	silent	1	sp12	1.0%	
dw3a	6	2593	G	A	A	T	missense	0	sp14	1.5%	
dw3a	5	2855	C	T	A	V	missense	0	sp14	1.5%	
dw3a	3	2863	C	T	R	C	missense	0	sp6	1.0%	
dw3a	5	2867	C	T	A	V	missense	0	sp23	1.5%	
dw3a	3	2922	C	A	A	A	silent	1	sp10	1.0%	
dw3b	14	48	C	T	G	G	silent	1	sp18	1.5%	
dw3b	4	76	C	T	P	S	missense	0	sp18	1.5%	
dw3b	6	95	A	T	H	L	missense	0	sp15	1.5%	
dw3b	4	106	C	T	P	S	missense	0	sp20	1.5%	YES
dw3b	3	118	C	T	Q	*	Stop codon	0	sp19	1.5%	NO
dw3b	6	127	G	A	G	R	missense	0.2	sp21	1.5%	
dw3b	6	230	C	T	S	F	missense	0.1	sp18	1.5%	
dw3b	4	266	A	G	Q	R	missense	0	sp9	1.0%	
dw3b	9	377	C	T	A	V	missense	0	sp11	1.5%	
dw3b	4	427	C	T	L	F	missense	0	sp7	1.0%	NO
dw3b	3	478	C	T	L	L	silent	1	sp11	1.5%	
dw3b	3	495	G	A	L	L	silent	1	sp2	1.5%	
dw3b	3	499	T	G	Y	D	missense	0.9	sp2	1.5%	
dw3b	11	513	G	A	G	G	silent	1	sp19	1.5%	
dw3b	4	517	G	A	A	T	missense	0.1	sp20	1.5%	NO
dw3b	3	530	C	T	A	V	missense	1	sp16	1.5%	
dw3b	4	642	C	T	T	T	silent	1	sp16	1.5%	
dw3b	19	689	C	T	A	V	missense	0.1	sp20	1.5%	
dw3b	8	746	C	T	A	V	missense	0.2	sp22	1.5%	
dw3b	4	764	T	A	F	Y	missense	0.1	sp21	1.5%	
dw3b	5	813	C	T	V	V	silent	1	sp14	1.5%	
dw3b	4	829	G	A	A	T	missense	0	sp22	1.5%	
dw3b	3	843	C	T	L	L	silent	1	sp10	1.0%	
dw3b	8	850	G	A	A	T	missense	0.3	sp19	1.5%	
dw3b	10	855	G	A	A	A	silent	1	sp19	1.5%	
dw3b	8	920	A	T	Q	L	missense	0	sp23	1.5%	
dw3b	6	929	C	T	A	V	missense	0.1	sp1	1.0%	
dw3b	5	1041	C	T	L	L	silent	1	sp18	1.5%	
dw3b	4	1051	G	A	G	S	missense	0	sp21	1.5%	
dw3b	3	1143	C	T	A	A	silent	1	sp13	1.5%	
dw3b	3	1146	C	T	I	I	silent	1	sp20	1.5%	

dw3b	3	1163	C	T	S	F	missense	0	sp14	1.5%	
dw3b	6	1273	C	T	L	F	missense	0.1	sp20	1.5%	
dw3b	3	1318	G	A	V	M	missense	0	sp10	1.0%	
dw3b	3	1322	C	T	T	M	missense	0.1	sp17	1.5%	
dw3b	3	1354	G	A	A	T	missense	0.2	sp21	1.5%	
dw3b	7	1460	C	T	T	M	missense	0	sp19	1.5%	
dw3b	11	1481	G	A	R	K	missense	0	sp17	1.5%	
dw3b	3	1604	G	A	S	N	missense	0	sp8	1.0%	
dw3b	4	1621	C	T	L	L	silent	1	sp2	1.5%	
dw3b	6	1683	C	T	N	N	silent	1	sp14	1.5%	
dw3b	6	1738	G	T	E	*	Stop codon	0	sp14	1.5%	
dw3b	5	1739	A	G	E	G	missense	0	sp14	1.5%	
dw3b	4	1760	G	A	G	D	missense	0	sp18	1.5%	
dw3b	5	1831	G	A	A	T	missense	0	sp18	1.5%	
dw3b	5	1841	C	T	A	V	missense	0	sp11	1.5%	
dw3b	8	1867	G	A	V	M	missense	0	sp2	1.5%	
dw3b	3	1884	C	T	R	R	silent	1	sp16	1.5%	
dw3b	5	1899	G	A	R	R	silent	1	sp11	1.5%	
dw3b	3	1941	C	T	K	K	silent	1	sp6	1.0%	
dw3b	6	1984	G	A	E	K	missense	0	sp18	1.5%	
dw3b	3	2077	G	A	A	T	missense	0.7	sp19	1.5%	
dw3b	3	2210	T	C	F	S	missense	0.3	sp3	1.0%	
dw3b	14	2238	G	A	E	E	silent	1	sp19	1.5%	
dw3b	10	2257	A	T	M	L	missense	0.7	sp2	1.5%	
dw3b	3	2284	G	A	A	T	missense	0.6	sp17	1.5%	
dw3b	5	2287	G	A	G	R	missense	0.6	sp3	1.0%	
dw3b	11	2289	G	A	A	A	silent	1	sp15	1.5%	
dw3b	3	2387	C	G	A	G	missense	0	sp1	1.0%	
dw3b	7	2440	C	T	P	S	missense	0.5	sp5	1.0%	
dw3b	5	2772	G	A	R	R	silent	1	sp14	1.5%	
dw3b	10	2784	G	A	V	V	silent	1	sp18	1.5%	
dw3b	3	2797	G	A	V	M	missense	0	sp4	1.0%	
dw3b	5	2803	C	T	P	S	missense	0	sp23	1.5%	
dw3b	3	2892	G	A	I	I	silent	1	sp16	1.5%	
dw3b	8	2925	C	T	V	V	silent	1	sp20	1.5%	
dw3b	5	3035	G	A	G	E	missense	0	sp20	1.5%	
dw3b	3	3059	C	T	A	V	missense	0	sp6	1.0%	
dw3b	4	3133	C	T	R	C	missense	0	sp21	1.5%	
rht1a	4	358	C	T	P	S	missense	0	sp18	1.5%	YES

rht1a	3	408	C	T	G	G	silent	1	sp2	1.5%
rht1b	5	108	G	A	V	V	silent	1	sp21	1.5%
rht1b	3	160	G	A	A	T	missense	0.1	sp13	1.5%
rht1b	3	184	C	T	Q	*	Stop codon	0	sp23	1.5%
rht1b	4	219	C	A	A	A	silent	1	sp5	1.0%

[a] The mutation frequency in the total dataset
[b] The wild type nucleotide
[c] The mutated nucleotide
[d] The wild type amino acid
[e] The mutated amino acid
[f] Function and SIFT represent predicted protein function effect and the scores, respectively. A score value < 0.05 is usually predicted as damaging effect
[g] The EMS concentration used to treat the seeds
[h] PCR validation of selected mutations. YES indicates that a mutation was validated by Sanger sequencing. NO indicates that a mutation was found to be a false positive by Sanger sequencing

Table S6 Comparison of expected and observed types of mutations

	dw3		*rht1*	
	Expected	Observed	Expected	Observed
Missense	50.36%	70.18%	57.14%	50.00%
Silent	46.59%	27.19%	41.35%	50.00%
Stop codon	3.05%	2.63%	1.50%	

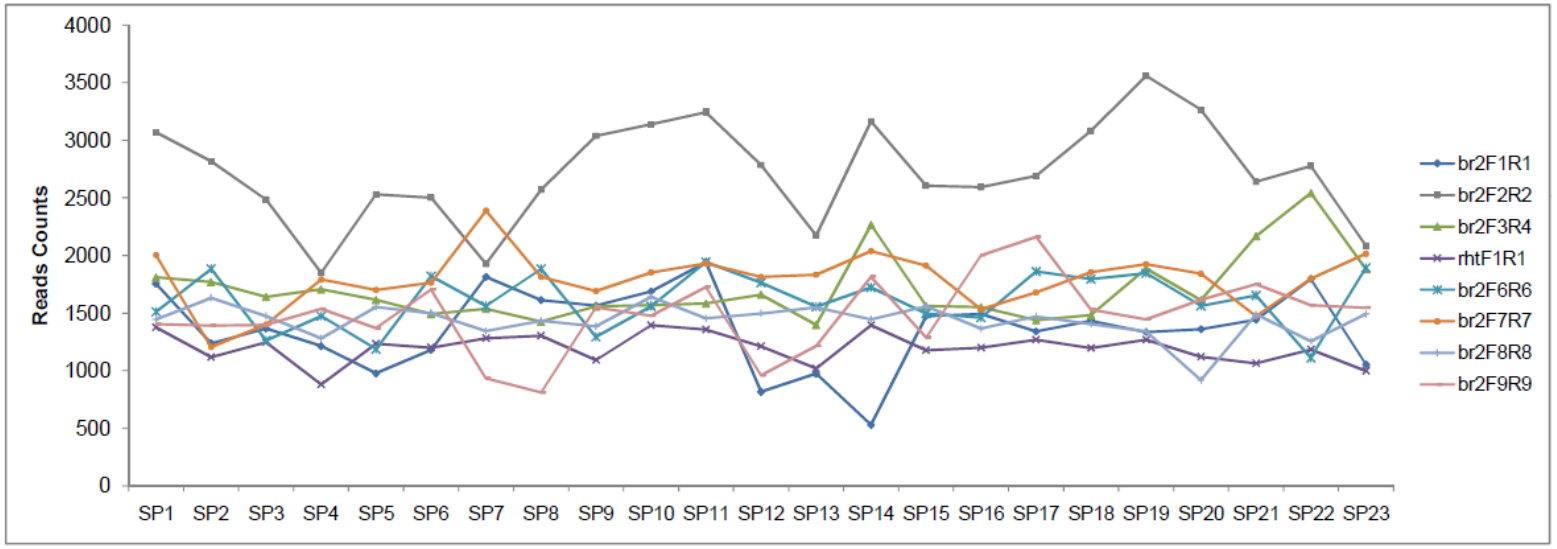
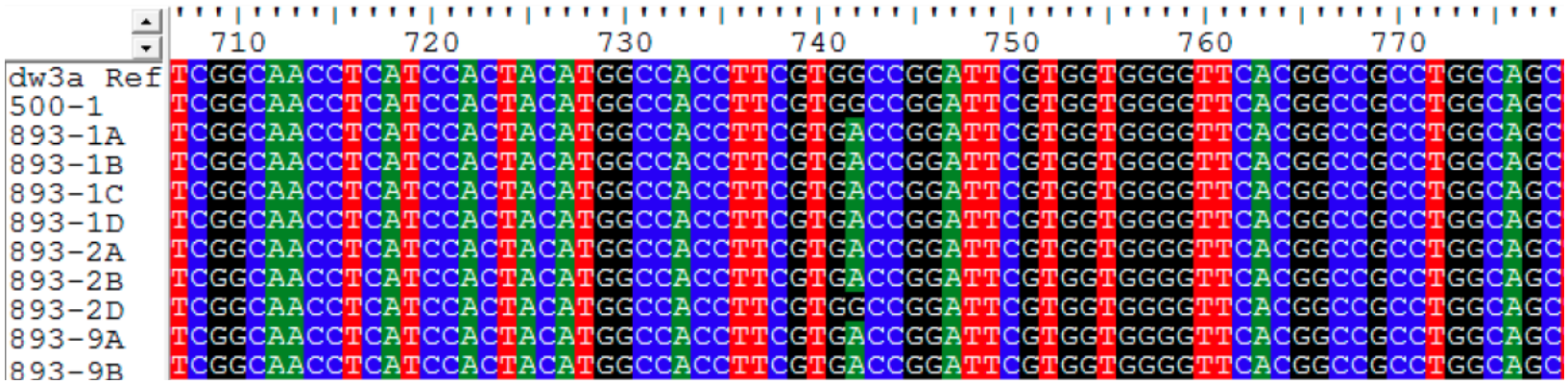
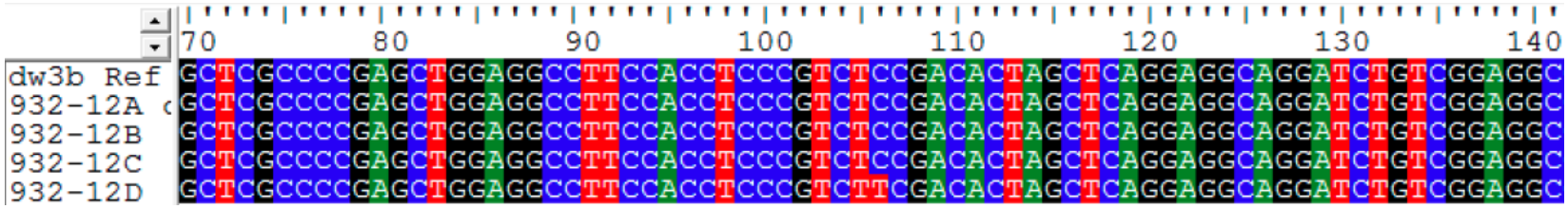


Figure S1 454 read distribution of 8 amplicons in the 23 superpools. X axis represents 23 superpools from the 21,210 EMS mutagenized tef lines. Y axis represents the number of reads in the superpools. 8 different signs represent 8 amplicons. Among them, rhtF1R1 was amplified from *rht*1 genes and the other 7 were amplified from *dw*3 genes



A)



B)

Figure S2 Sanger sequencing validation on M3 generation plants segregating for mutations in a candidate tef dwarfing gene. A) p893 mutant alignment with reference sequence of dw3a gene. The G742A mutation is validated by seven p893 M3 lines; B) p932 mutant alignment with reference sequence of the dw3b gene. The C106T mutation is validated by one p932 M3 line, 932-12D

Figure S3 Phenotypes of *dw3* homologue mutants in M3 lines of tef (500-1: control; 893-1 and 893-9: G742A mutants; 932-12: C106T mutant)

Table S1 Summary of tef genomic fosmid library

Accession number	PI 524434
Source of genomic DNA	1-month-old seedlings
Total number of colony forming units	102,191
Number of superpools	25
Number of pools	1,187
Average insert size	35 kb – 45kb
Haploid genome size	714Mbp
Library coverage	5.0X – 6.4X

Table S2 Primers for 454 sequencing

Primer name	Fusion primer (5'-3')	MID_Tag	PCR primer (5'-3')
dw3E1F1_SP1_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	ACGAGTGC	CTAGYAGCGACCCGGAGGAGAT
dw3E1F1_SP2_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	AGACGCAC	CTAGYAGCGACCCGGAGGAGAT
dw3E1F1_SP3_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	AGCACTGT	CTAGYAGCGACCCGGAGGAGAT
dw3E1F1_SP4_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	CTCGCGTG	CTAGYAGCGACCCGGAGGAGAT
dw3E1F1_SP5_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	TGATACGT	CTAGYAGCGACCCGGAGGAGAT
dw3E1F1_SP6_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	CATAGTAG	CTAGYAGCGACCCGGAGGAGAT
dw3E1F1_SP7_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	CGAGAGAT	CTAGYAGCGACCCGGAGGAGAT
dw3E1F1_SP8_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	CGTCTAGT	CTAGYAGCGACCCGGAGGAGAT
dw3E1F1_SP9_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	TGTACTAC	CTAGYAGCGACCCGGAGGAGAT
dw3E1F1_SP10_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	CGTAGACT	CTAGYAGCGACCCGGAGGAGAT
dw3E1F1_SP11_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	TACGAGTA	CTAGYAGCGACCCGGAGGAGAT
dw3E1F1_SP12_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	TACTCTCG	CTAGYAGCGACCCGGAGGAGAT
dw3E1F1_SP13_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	TAGAGACG	CTAGYAGCGACCCGGAGGAGAT
dw3E1F1_SP14_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	ACTGTACA	CTAGYAGCGACCCGGAGGAGAT
dw3E1F1_SP15_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	ATAGAGTA	CTAGYAGCGACCCGGAGGAGAT
dw3E1F1_SP16_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	CACGCTAC	CTAGYAGCGACCCGGAGGAGAT
dw3E1F1_SP17_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	CAGTAGAC	CTAGYAGCGACCCGGAGGAGAT
dw3E1F1_SP18_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	CGACGTGA	CTAGYAGCGACCCGGAGGAGAT
dw3E1F1_SP19_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	TCGATCAC	CTAGYAGCGACCCGGAGGAGAT
dw3E1F1_SP20_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	TCGCACTA	CTAGYAGCGACCCGGAGGAGAT
dw3E1F1_SP21_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	AGTCGAGA	CTAGYAGCGACCCGGAGGAGAT
dw3E1F1_SP22_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	CGCAGTAC	CTAGYAGCGACCCGGAGGAGAT
dw3E1F1_SP23_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	TATGCTAG	CTAGYAGCGACCCGGAGGAGAT
dw3E1R1a_FB	CCTATCCCCTGTGTGCCTTGGCAGTCTCAG		ACYTGGCCACCAGCCGCACCA
dw3E1R2a_SP1_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	ACGAGTGCGT	TGGCTCCGCGACGACAGCTT
dw3E1R2a_SP2_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	AGACGCACTC	TGGCTCCGCGACGACAGCTT
dw3E1R2a_SP3_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	AGCACTGTAG	TGGCTCCGCGACGACAGCTT
dw3E1R2a_SP4_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	CTCGCGTGTC	TGGCTCCGCGACGACAGCTT
dw3E1R2a_SP5_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	TGATACGTCT	TGGCTCCGCGACGACAGCTT
dw3E1R2a_SP6_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	CATAGTAGTG	TGGCTCCGCGACGACAGCTT
dw3E1R2a_SP7_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	CGAGAGATAC	TGGCTCCGCGACGACAGCTT
dw3E1R2a_SP8_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	CGTCTAGTAC	TGGCTCCGCGACGACAGCTT
dw3E1R2a_SP9_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	TGTACTACTC	TGGCTCCGCGACGACAGCTT
dw3E1R2a_SP10_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	CGTAGACTAG	TGGCTCCGCGACGACAGCTT
dw3E1R2a_SP11_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	TACGAGTATG	TGGCTCCGCGACGACAGCTT
dw3E1R2a_SP12_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	TACTCTCGTG	TGGCTCCGCGACGACAGCTT

dw3E1R2a_SP13_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	TAGAGACGAG	TGGCTCCGCGACGACAGCTT
dw3E1R2a_SP14_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	ACTGTACAGT	TGGCTCCGCGACGACAGCTT
dw3E1R2a_SP15_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	ATAGAGTACT	TGGCTCCGCGACGACAGCTT
dw3E1R2a_SP16_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	CACGCTACGT	TGGCTCCGCGACGACAGCTT
dw3E1R2a_SP17_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	CAGTAGACGT	TGGCTCCGCGACGACAGCTT
dw3E1R2a_SP18_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	CGACGTGACT	TGGCTCCGCGACGACAGCTT
dw3E1R2a_SP19_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	TCGATCACGT	TGGCTCCGCGACGACAGCTT
dw3E1R2a_SP20_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	TCGCACTAGT	TGGCTCCGCGACGACAGCTT
dw3E1R2a_SP21_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	AGTCGAGAGA	TGGCTCCGCGACGACAGCTT
dw3E1R2a_SP22_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	CGCAGTACGA	TGGCTCCGCGACGACAGCTT
dw3E1R2a_SP23_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	TATGCTAGTA	TGGCTCCGCGACGACAGCTT
dw3E1F2a_FB	CCTATCCCCTGTGTGCCTTGGCAGTCTCAG		ATGGTGCGGCTGGTGGCCARG
dw3E1R4_SP1_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	ACGAGTGC	GTGACGGGTAGGCGAAGTCAAC
dw3E1R4_SP2_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	AGACGCAC	GTGACGGGTAGGCGAAGTCAAC
dw3E1R4_SP3_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	AGCACTGT	GTGACGGGTAGGCGAAGTCAAC
dw3E1R4_SP4_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	CTCGCGTG	GTGACGGGTAGGCGAAGTCAAC
dw3E1R4_SP5_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	TGATACGT	GTGACGGGTAGGCGAAGTCAAC
dw3E1R4_SP6_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	CATAGTAG	GTGACGGGTAGGCGAAGTCAAC
dw3E1R4_SP7_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	CGAGAGAT	GTGACGGGTAGGCGAAGTCAAC
dw3E1R4_SP8_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	CGTCTAGT	GTGACGGGTAGGCGAAGTCAAC
dw3E1R4_SP9_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	TGTACTAC	GTGACGGGTAGGCGAAGTCAAC
dw3E1R4_SP10_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	CGTAGACT	GTGACGGGTAGGCGAAGTCAAC
dw3E1R4_SP11_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	TACGAGTA	GTGACGGGTAGGCGAAGTCAAC
dw3E1R4_SP12_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	TACTCTCG	GTGACGGGTAGGCGAAGTCAAC
dw3E1R4_SP13_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	TAGAGACG	GTGACGGGTAGGCGAAGTCAAC
dw3E1R4_SP14_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	ACTGTACA	GTGACGGGTAGGCGAAGTCAAC
dw3E1R4_SP15_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	ATAGAGTA	GTGACGGGTAGGCGAAGTCAAC
dw3E1R4_SP16_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	CACGCTAC	GTGACGGGTAGGCGAAGTCAAC
dw3E1R4_SP17_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	CAGTAGAC	GTGACGGGTAGGCGAAGTCAAC
dw3E1R4_SP18_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	CGACGTGA	GTGACGGGTAGGCGAAGTCAAC
dw3E1R4_SP19_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	TCGATCAC	GTGACGGGTAGGCGAAGTCAAC
dw3E1R4_SP20_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	TCGCACTA	GTGACGGGTAGGCGAAGTCAAC
dw3E1R4_SP21_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	AGTCGAGA	GTGACGGGTAGGCGAAGTCAAC
dw3E1R4_SP22_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	CGCAGTAC	GTGACGGGTAGGCGAAGTCAAC
dw3E1R4_SP23_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	TATGCTAG	GTGACGGGTAGGCGAAGTCAAC
dw3E1F3a_FB	CCTATCCCCTGTGTGCCTTGGCAGTCTCAG		AAGCTGTCGTCGCGGAGCCA
rht1F1_SP1_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	ACGAGTGCGT	AAGGACAAGATGATGGTGGC
rht1F1_SP2_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	AGACGCACTC	AAGGACAAGATGATGGTGGC
rht1F1_SP3_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	AGCACTGTAG	AAGGACAAGATGATGGTGGC

rht1F1_SP4_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	CTCGCGTGTC	AAGGACAAGATGATGGTGGC
rht1F1_SP5_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	TGATACGTCT	AAGGACAAGATGATGGTGGC
rht1F1_SP6_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	CATAGTAGTG	AAGGACAAGATGATGGTGGC
rht1F1_SP7_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	CGAGAGATAC	AAGGACAAGATGATGGTGGC
rht1F1_SP8_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	CGTCTAGTAC	AAGGACAAGATGATGGTGGC
rht1F1_SP9_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	TGTACTACTC	AAGGACAAGATGATGGTGGC
rht1F1_SP10_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	CGTAGACTAG	AAGGACAAGATGATGGTGGC
rht1F1_SP11_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	TACGAGTATG	AAGGACAAGATGATGGTGGC
rht1F1_SP12_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	TACTCTCGTG	AAGGACAAGATGATGGTGGC
rht1F1_SP13_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	TAGAGACGAG	AAGGACAAGATGATGGTGGC
rht1F1_SP14_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	ACTGTACAGT	AAGGACAAGATGATGGTGGC
rht1F1_SP15_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	ATAGAGTACT	AAGGACAAGATGATGGTGGC
rht1F1_SP16_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	CACGCTACGT	AAGGACAAGATGATGGTGGC
rht1F1_SP17_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	CAGTAGACGT	AAGGACAAGATGATGGTGGC
rht1F1_SP18_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	CGACGTGACT	AAGGACAAGATGATGGTGGC
rht1F1_SP19_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	TCGATCACGT	AAGGACAAGATGATGGTGGC
rht1F1_SP20_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	TCGCACTAGT	AAGGACAAGATGATGGTGGC
rht1F1_SP21_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	AGTCGAGAGA	AAGGACAAGATGATGGTGGC
rht1F1_SP22_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	CGCAGTACGA	AAGGACAAGATGATGGTGGC
rht1F1_SP23_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	TATGCTAGTA	AAGGACAAGATGATGGTGGC
rht1R1_FB	CCTATCCCCTGTGTGCCTTGGCAGTCTCAG		GAAGACGAGGACGAGGAAGA
dw3E2R1_SP1_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	ACGAGTGC	GCAGCTTGATGATGAATGAGTG
dw3E2R1_SP2_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	AGACGCAC	GCAGCTTGATGATGAATGAGTG
dw3E2R1_SP3_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	AGCACTGT	GCAGCTTGATGATGAATGAGTG
dw3E2R1_SP4_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	CTCGCGTG	GCAGCTTGATGATGAATGAGTG
dw3E2R1_SP5_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	TGATACGT	GCAGCTTGATGATGAATGAGTG
dw3E2R1_SP6_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	CATAGTAG	GCAGCTTGATGATGAATGAGTG
dw3E2R1_SP7_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	CGAGAGAT	GCAGCTTGATGATGAATGAGTG
dw3E2R1_SP8_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	CGTCTAGT	GCAGCTTGATGATGAATGAGTG
dw3E2R1_SP9_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	TGTACTAC	GCAGCTTGATGATGAATGAGTG
dw3E2R1_SP10_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	CGTAGACT	GCAGCTTGATGATGAATGAGTG
dw3E2R1_SP11_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	TACGAGTA	GCAGCTTGATGATGAATGAGTG
dw3E2R1_SP12_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	TACTCTCG	GCAGCTTGATGATGAATGAGTG
dw3E2R1_SP13_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	TAGAGACG	GCAGCTTGATGATGAATGAGTG
dw3E2R1_SP14_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	ACTGTACA	GCAGCTTGATGATGAATGAGTG
dw3E2R1_SP15_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	ATAGAGTA	GCAGCTTGATGATGAATGAGTG
dw3E2R1_SP16_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	CACGCTAC	GCAGCTTGATGATGAATGAGTG
dw3E2R1_SP17_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	CAGTAGAC	GCAGCTTGATGATGAATGAGTG
dw3E2R1_SP18_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	CGACGTGA	GCAGCTTGATGATGAATGAGTG

dw3E2R1_SP19_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	TCGATCAC	GCAGCTTGATGATGAATGAGTG
dw3E2R1_SP20_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	TCGCACTA	GCAGCTTGATGATGAATGAGTG
dw3E2R1_SP21_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	AGTCGAGA	GCAGCTTGATGATGAATGAGTG
dw3E2R1_SP22_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	CGCAGTAC	GCAGCTTGATGATGAATGAGTG
dw3E2R1_SP23_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	TATGCTAG	GCAGCTTGATGATGAATGAGTG
dw3E1F6_FB	CCTATCCCCTGTGTGCCTTGGCAGTCTCAG		GTTGACTTCGCCTACCCGTC
dw3E3F9_SP1_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	ACGAGTGCGT	TGAGCGCGGTCTGCAGCTGT
dw3E3F9_SP2_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	AGACGCACTC	TGAGCGCGGTCTGCAGCTGT
dw3E3F9_SP3_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	AGCACTGTAG	TGAGCGCGGTCTGCAGCTGT
dw3E3F9_SP4_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	CTCGCGTGTC	TGAGCGCGGTCTGCAGCTGT
dw3E3F9_SP5_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	TGATACGTCT	TGAGCGCGGTCTGCAGCTGT
dw3E3F9_SP6_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	CATAGTAGTG	TGAGCGCGGTCTGCAGCTGT
dw3E3F9_SP7_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	CGAGAGATAC	TGAGCGCGGTCTGCAGCTGT
dw3E3F9_SP8_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	CGTCTAGTAC	TGAGCGCGGTCTGCAGCTGT
dw3E3F9_SP9_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	TGTACTACTC	TGAGCGCGGTCTGCAGCTGT
dw3E3F9_SP10_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	CGTAGACTAG	TGAGCGCGGTCTGCAGCTGT
dw3E3F9_SP11_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	TACGAGTATG	TGAGCGCGGTCTGCAGCTGT
dw3E3F9_SP12_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	TACTCTCGTG	TGAGCGCGGTCTGCAGCTGT
dw3E3F9_SP13_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	TAGAGACGAG	TGAGCGCGGTCTGCAGCTGT
dw3E3F9_SP14_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	ACTGTACAGT	TGAGCGCGGTCTGCAGCTGT
dw3E3F9_SP15_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	ATAGAGTACT	TGAGCGCGGTCTGCAGCTGT
dw3E3F9_SP16_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	CACGCTACGT	TGAGCGCGGTCTGCAGCTGT
dw3E3F9_SP17_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	CAGTAGACGT	TGAGCGCGGTCTGCAGCTGT
dw3E3F9_SP18_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	CGACGTGACT	TGAGCGCGGTCTGCAGCTGT
dw3E3F9_SP19_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	TCGATCACGT	TGAGCGCGGTCTGCAGCTGT
dw3E3F9_SP20_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	TCGCACTAGT	TGAGCGCGGTCTGCAGCTGT
dw3E3F9_SP21_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	AGTCGAGAGA	TGAGCGCGGTCTGCAGCTGT
dw3E3F9_SP22_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	CGCAGTACGA	TGAGCGCGGTCTGCAGCTGT
dw3E3F9_SP23_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	TATGCTAGTA	TGAGCGCGGTCTGCAGCTGT
dw3E3R1_FB	CCTATCCCCTGTGTGCCTTGGCAGTCTCAG		CGTAGGAGGAGTTGCGCGTC
dw3E3F10_SP1_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	ACGAGTGC	TCCGACTTCTCCAACGCCGACT
dw3E3F10_SP2_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	AGACGCAC	TCCGACTTCTCCAACGCCGACT
dw3E3F10_SP3_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	AGCACTGT	TCCGACTTCTCCAACGCCGACT
dw3E3F10_SP4_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	CTCGCGTG	TCCGACTTCTCCAACGCCGACT
dw3E3F10_SP5_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	TGATACGT	TCCGACTTCTCCAACGCCGACT
dw3E3F10_SP6_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	CATAGTAG	TCCGACTTCTCCAACGCCGACT
dw3E3F10_SP7_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	CGAGAGAT	TCCGACTTCTCCAACGCCGACT
dw3E3F10_SP8_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	CGTCTAGT	TCCGACTTCTCCAACGCCGACT
dw3E3F10_SP9_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	TGTACTAC	TCCGACTTCTCCAACGCCGACT

dw3E3F10_SP10_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	CGTAGACT	TCCGACTTCTCCAACGCCGACT
dw3E3F10_SP11_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	TACGAGTA	TCCGACTTCTCCAACGCCGACT
dw3E3F10_SP12_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	TACTCTCG	TCCGACTTCTCCAACGCCGACT
dw3E3F10_SP13_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	TAGAGACG	TCCGACTTCTCCAACGCCGACT
dw3E3F10_SP14_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	ACTGTACA	TCCGACTTCTCCAACGCCGACT
dw3E3F10_SP15_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	ATAGAGTA	TCCGACTTCTCCAACGCCGACT
dw3E3F10_SP16_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	CACGCTAC	TCCGACTTCTCCAACGCCGACT
dw3E3F10_SP17_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	CAGTAGAC	TCCGACTTCTCCAACGCCGACT
dw3E3F10_SP18_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	CGACGTGA	TCCGACTTCTCCAACGCCGACT
dw3E3F10_SP19_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	TCGATCAC	TCCGACTTCTCCAACGCCGACT
dw3E3F10_SP20_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	TCGCACTA	TCCGACTTCTCCAACGCCGACT
dw3E3F10_SP21_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	AGTCGAGA	TCCGACTTCTCCAACGCCGACT
dw3E3F10_SP22_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	CGCAGTAC	TCCGACTTCTCCAACGCCGACT
dw3E3F10_SP23_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	TATGCTAG	TCCGACTTCTCCAACGCCGACT
dw3E3R10_FB	CCTATCCCCTGTGTGCCTTGGCAGTCTCAG		GATGGCGGARCGCACGTTCT
dw3E3F11_SP1_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	ACGAGTGCGT	GCATCTCGGTGATCGTGCAG
dw3E3F11_SP2_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	AGACGCACTC	GCATCTCGGTGATCGTGCAG
dw3E3F11_SP3_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	AGCACTGTAG	GCATCTCGGTGATCGTGCAG
dw3E3F11_SP4_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	CTCGCGTGTC	GCATCTCGGTGATCGTGCAG
dw3E3F11_SP5_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	TGATACGTCT	GCATCTCGGTGATCGTGCAG
dw3E3F11_SP6_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	CATAGTAGTG	GCATCTCGGTGATCGTGCAG
dw3E3F11_SP7_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	CGAGAGATAC	GCATCTCGGTGATCGTGCAG
dw3E3F11_SP8_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	CGTCTAGTAC	GCATCTCGGTGATCGTGCAG
dw3E3F11_SP9_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	TGTACTACTC	GCATCTCGGTGATCGTGCAG
dw3E3F11_SP10_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	CGTAGACTAG	GCATCTCGGTGATCGTGCAG
dw3E3F11_SP11_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	TACGAGTATG	GCATCTCGGTGATCGTGCAG
dw3E3F11_SP12_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	TACTCTCGTG	GCATCTCGGTGATCGTGCAG
dw3E3F11_SP13_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	TAGAGACGAG	GCATCTCGGTGATCGTGCAG
dw3E3F11_SP14_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	ACTGTACAGT	GCATCTCGGTGATCGTGCAG
dw3E3F11_SP15_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	ATAGAGTACT	GCATCTCGGTGATCGTGCAG
dw3E3F11_SP16_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	CACGCTACGT	GCATCTCGGTGATCGTGCAG
dw3E3F11_SP17_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	CAGTAGACGT	GCATCTCGGTGATCGTGCAG
dw3E3F11_SP18_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	CGACGTGACT	GCATCTCGGTGATCGTGCAG
dw3E3F11_SP19_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	TCGATCACGT	GCATCTCGGTGATCGTGCAG
dw3E3F11_SP20_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	TCGCACTAGT	GCATCTCGGTGATCGTGCAG
dw3E3F11_SP21_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	AGTCGAGAGA	GCATCTCGGTGATCGTGCAG
dw3E3F11_SP22_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	CGCAGTACGA	GCATCTCGGTGATCGTGCAG
dw3E3F11_SP23_FA	CCATCTCATCCCTGCGTGTCTCCGACTCAG	TATGCTAGTA	GCATCTCGGTGATCGTGCAG
dw3E3R11_FB	CCTATCCCCTGTGTGCCTTGGCAGTCTCAG		GGAGACCATGAGCACCATGAAG

Table S3 Comparison of two *dw3* homoeologous genes

	Identity
Gene level	0.916
Coding region	0.948
Noncoding region	0.667
Protein level	0.941
dN (nonsynonymous substitution rate)	0.033
dS (synonymous substitution rate)	0.125

Table S4 Summary of 454 reads

Region	PCR length (bp)	Ave length of reads(bp)	Number of reads
dw3E1F1/R1	468	399	31,309
dw3E1F2/R2	416	354	61,602
dw3E1F3/R4	488	413	37,817
dw3E1F6/E2R1	471	347	37,119
dw3E3F9/E3R1	428	359	41,259
dw3E3F10/E3R11	513	371	32,929
dw3E3F11/E3R11	458	380	34,237
rhtF1/R1	521	398	35,954
others			14,910

Table S5 Spectrum of mutations identified in the dwarfing candidate genes

Gene	Freq[a]	Position	WT_Nu[b]	Mu_Nu[c]	WT_AA[d]	Mu_AA[e]	Function[f]	SIFT[f]	Superpool	EMS[g]	Validation[h]
dw3a	5	89	A	G	E	G	missense	0	sp2	1.5%	
dw3a	7	104	C	T	P	L	missense	0	sp19	1.5%	
dw3a	3	200	A	G	N	S	missense	0	sp11	1.5%	
dw3a	17	238	A	G	S	G	missense	0.4	sp6	1.0%	
dw3a	19	503	C	T	A	V	missense	1	sp19	1.5%	YES
dw3a	11	582	C	T	R	R	silent	1	sp14	1.5%	
dw3a	6	628	A	T	T	S	missense	0	sp9	1.0%	
dw3a	3	677	T	A	V	D	missense	0	sp17	1.5%	
dw3a	3	730	G	A	A	T	missense	0.2	sp2	1.5%	
dw3a	10	742	G	A	A	T	missense	0	sp19	1.5%	YES
dw3a	3	838	G	A	A	T	missense	0.2	sp3	1.0%	
dw3a	6	840	G	A	A	A	silent	1	sp19	1.5%	
dw3a	9	863	G	A	R	Q	missense	0.1	sp11	1.5%	
dw3a	3	1023	G	A	G	G	silent	1	sp17	1.5%	
dw3a	6	1105	C	T	R	C	missense	0.1	sp14	1.5%	YES
dw3a	11	1124	G	A	G	D	missense	0	sp7	1.0%	
dw3a	4	1139	C	T	T	I	missense	0.1	sp7	1.0%	YES
dw3a	3	1177	C	T	Q	*	Stop codon	0	sp14	1.5%	NO
dw3a	9	1185	G	A	P	P	silent	1	sp9	1.0%	
dw3a	13	1189	A	T	S	C	missense	0.2	sp1	1.0%	
dw3a	5	1200	C	T	F	F	silent	1	sp14	1.5%	
dw3a	3	1228	A	G	K	E	missense	0	sp19	1.5%	
dw3a	4	1270	G	A	D	N	missense	0.5	sp16	1.5%	
dw3a	3	1294	A	G	T	A	missense	0.8	sp22	1.5%	
dw3a	8	1295	C	T	T	M	missense	0.1	sp14	1.5%	
dw3a	5	1315	G	A	G	S	missense	0.1	sp22	1.5%	
dw3a	10	1526	T	A	L	H	missense	0	sp17	1.5%	
dw3a	5	1564	C	T	L	L	silent	1	sp21	1.5%	
dw3a	9	1594	C	T	L	L	silent	1	sp21	1.5%	
dw3a	4	1606	G	A	D	N	missense	0.2	sp15	1.5%	
dw3a	7	1642	G	A	A	T	missense	0	sp23	1.5%	
dw3a	8	1811	G	A	S	N	missense	0	sp9	1.0%	
dw3a	3	1814	C	A	A	E	missense	0	sp15	1.5%	
dw3a	11	1840	G	A	V	M	missense	0	sp16	1.5%	
dw3a	3	1849	G	A	A	T	missense	0	sp2	1.5%	
dw3a	3	1960	A	T	I	F	missense	0.6	sp7	1.0%	
dw3a	4	1964	G	A	G	E	missense	0	sp23	1.5%	

dw3a	3	1999	G	A	G	S	missense	0.1	sp5	1.0%	
dw3a	3	2288	G	A	R	K	missense	0.2	sp17	1.5%	
dw3a	3	2451	G	A	Y	Y	silent	1	sp12	1.0%	
dw3a	6	2593	G	A	A	T	missense	0	sp14	1.5%	
dw3a	5	2855	C	T	A	V	missense	0	sp14	1.5%	
dw3a	3	2863	C	T	R	C	missense	0	sp6	1.0%	
dw3a	5	2867	C	T	A	V	missense	0	sp23	1.5%	
dw3a	3	2922	C	A	A	A	silent	1	sp10	1.0%	
dw3b	14	48	C	T	G	G	silent	1	sp18	1.5%	
dw3b	4	76	C	T	P	S	missense	0	sp18	1.5%	
dw3b	6	95	A	T	H	L	missense	0	sp15	1.5%	
dw3b	4	106	C	T	P	S	missense	0	sp20	1.5%	YES
dw3b	3	118	C	T	Q	*	Stop codon	0	sp19	1.5%	NO
dw3b	6	127	G	A	G	R	missense	0.2	sp21	1.5%	
dw3b	6	230	C	T	S	F	missense	0.1	sp18	1.5%	
dw3b	4	266	A	G	Q	R	missense	0	sp9	1.0%	
dw3b	9	377	C	T	A	V	missense	0	sp11	1.5%	
dw3b	4	427	C	T	L	F	missense	0	sp7	1.0%	NO
dw3b	3	478	C	T	L	L	silent	1	sp11	1.5%	
dw3b	3	495	G	A	L	L	silent	1	sp2	1.5%	
dw3b	3	499	T	G	Y	D	missense	0.9	sp2	1.5%	
dw3b	11	513	G	A	G	G	silent	1	sp19	1.5%	
dw3b	4	517	G	A	A	T	missense	0.1	sp20	1.5%	NO
dw3b	3	530	C	T	A	V	missense	1	sp16	1.5%	
dw3b	4	642	C	T	T	T	silent	1	sp16	1.5%	
dw3b	19	689	C	T	A	V	missense	0.1	sp20	1.5%	
dw3b	8	746	C	T	A	V	missense	0.2	sp22	1.5%	
dw3b	4	764	T	A	F	Y	missense	0.1	sp21	1.5%	
dw3b	5	813	C	T	V	V	silent	1	sp14	1.5%	
dw3b	4	829	G	A	A	T	missense	0	sp22	1.5%	
dw3b	3	843	C	T	L	L	silent	1	sp10	1.0%	
dw3b	8	850	G	A	A	T	missense	0.3	sp19	1.5%	
dw3b	10	855	G	A	A	A	silent	1	sp19	1.5%	
dw3b	8	920	A	T	Q	L	missense	0	sp23	1.5%	
dw3b	6	929	C	T	A	V	missense	0.1	sp1	1.0%	
dw3b	5	1041	C	T	L	L	silent	1	sp18	1.5%	
dw3b	4	1051	G	A	G	S	missense	0	sp21	1.5%	
dw3b	3	1143	C	T	A	A	silent	1	sp13	1.5%	
dw3b	3	1146	C	T	I	I	silent	1	sp20	1.5%	

dw3b	3	1163	C	T	S	F	missense	0	sp14	1.5%	
dw3b	6	1273	C	T	L	F	missense	0.1	sp20	1.5%	
dw3b	3	1318	G	A	V	M	missense	0	sp10	1.0%	
dw3b	3	1322	C	T	T	M	missense	0.1	sp17	1.5%	
dw3b	3	1354	G	A	A	T	missense	0.2	sp21	1.5%	
dw3b	7	1460	C	T	T	M	missense	0	sp19	1.5%	
dw3b	11	1481	G	A	R	K	missense	0	sp17	1.5%	
dw3b	3	1604	G	A	S	N	missense	0	sp8	1.0%	
dw3b	4	1621	C	T	L	L	silent	1	sp2	1.5%	
dw3b	6	1683	C	T	N	N	silent	1	sp14	1.5%	
dw3b	6	1738	G	T	E	*	Stop codon	0	sp14	1.5%	
dw3b	5	1739	A	G	E	G	missense	0	sp14	1.5%	
dw3b	4	1760	G	A	G	D	missense	0	sp18	1.5%	
dw3b	5	1831	G	A	A	T	missense	0	sp18	1.5%	
dw3b	5	1841	C	T	A	V	missense	0	sp11	1.5%	
dw3b	8	1867	G	A	V	M	missense	0	sp2	1.5%	
dw3b	3	1884	C	T	R	R	silent	1	sp16	1.5%	
dw3b	5	1899	G	A	R	R	silent	1	sp11	1.5%	
dw3b	3	1941	C	T	K	K	silent	1	sp6	1.0%	
dw3b	6	1984	G	A	E	K	missense	0	sp18	1.5%	
dw3b	3	2077	G	A	A	T	missense	0.7	sp19	1.5%	
dw3b	3	2210	T	C	F	S	missense	0.3	sp3	1.0%	
dw3b	14	2238	G	A	E	E	silent	1	sp19	1.5%	
dw3b	10	2257	A	T	M	L	missense	0.7	sp2	1.5%	
dw3b	3	2284	G	A	A	T	missense	0.6	sp17	1.5%	
dw3b	5	2287	G	A	G	R	missense	0.6	sp3	1.0%	
dw3b	11	2289	G	A	A	A	silent	1	sp15	1.5%	
dw3b	3	2387	C	G	A	G	missense	0	sp1	1.0%	
dw3b	7	2440	C	T	P	S	missense	0.5	sp5	1.0%	
dw3b	5	2772	G	A	R	R	silent	1	sp14	1.5%	
dw3b	10	2784	G	A	V	V	silent	1	sp18	1.5%	
dw3b	3	2797	G	A	V	M	missense	0	sp4	1.0%	
dw3b	5	2803	C	T	P	S	missense	0	sp23	1.5%	
dw3b	3	2892	G	A	I	I	silent	1	sp16	1.5%	
dw3b	8	2925	C	T	V	V	silent	1	sp20	1.5%	
dw3b	5	3035	G	A	G	E	missense	0	sp20	1.5%	
dw3b	3	3059	C	T	A	V	missense	0	sp6	1.0%	
dw3b	4	3133	C	T	R	C	missense	0	sp21	1.5%	
rht1a	4	358	C	T	P	S	missense	0	sp18	1.5%	YES

rht1a	3	408	C	T	G	G	silent	1	sp2	1.5%
rht1b	5	108	G	A	V	V	silent	1	sp21	1.5%
rht1b	3	160	G	A	A	T	missense	0.1	sp13	1.5%
rht1b	3	184	C	T	Q	*	Stop codon	0	sp23	1.5%
rht1b	4	219	C	A	A	A	silent	1	sp5	1.0%

[a] The mutation frequency in the total dataset
[b] The wild type nucleotide
[c] The mutated nucleotide
[d] The wild type amino acid
[e] The mutated amino acid
[f] Function and SIFT represent predicted protein function effect and the scores, respectively. A score value < 0.05 is usually predicted as damaging effect
[g] The EMS concentration used to treat the seeds
[h] PCR validation of selected mutations. YES indicates that a mutation was validated by Sanger sequencing. NO indicates that a mutation was found to be a false positive by Sanger sequencing

Table S6 Comparison of expected and observed types of mutations

	dw3		*rht1*	
	Expected	Observed	Expected	Observed
Missense	50.36%	70.18%	57.14%	50.00%
Silent	46.59%	27.19%	41.35%	50.00%
Stop codon	3.05%	2.63%	1.50%	