Gene Deletion in Barley Mediated by LTR-retrotransposon BARE

Yi Shang1,*, Fei Yang2,3,*, Alan H. Schulman4,5, Jinghuan Zhu1, Yong Jia1, Junmei Wang1, Xiao-Qi Zhang3, Qiaojun Jia4, Wei Hua1, Jianming Yang1 & Chengdao Li1,3

Received: 23 August 2016
Accepted: 27 January 2017
Published: 02 March 2017

A poly-row branched spike (prbs) barley mutant was obtained from soaking a two-rowed barley inflorescence in a solution of maize genomic DNA. Positional cloning and sequencing demonstrated that the prbs mutant resulted from a 28 kb deletion including the inflorescence architecture gene HvRA2. Sequence annotation revealed that the HvRA2 gene is flanked by two LTR (long terminal repeat) retrotransposons (BARE) sharing 89% sequence identity. A recombination between the integrase (IN) gene regions of the two BARE copies resulted in the formation of an intact BARE and loss of HvRA2. No maize DNA was detected in the recombination region although the flanking sequences of HvRA2 gene showed over 73% of sequence identity with repetitive sequences on 10 maize chromosomes. It is still unknown whether the interaction of retrotransposons between barley and maize has resulted in the recombination observed in the present study.

The architecture of branched inflorescences in grasses depends on the developmental fate of primordia and axis orientation1. The rice (Oryza sativa L.) panicle generates several primary and secondary branches on which spikelets are produced. Sorghum and maize male inflorescences share a structure similar to that of rice. In barley (Hordeum vulgare L.) spikes, however, spikelets are borne directly on the main axis, the rachis, and there are no pedicels. A diagnostic feature of barley is the possession of three one-flowered spikelets at each rachis node2,3. Based on lateral spikelet size and fertility, barley is classified into two-rowed and six-rowed types. Two-rowed barley only has a central fertile spikelet with small and infertile lateral spikelets while the six-rowed barley has three fully-developed fertile spikelets.

The major genes that control row-type variation in barley are Vrs14, Int-c5 and Vrs46. The barley domestication gene Vrs1, located on the long arm of chromosome 2H, encodes a homeodomain-leucine zipper (HD-Zip) transcription factor that suppresses the development of lateral spikelets in two-rowed barley. Mutant vrs1 results in a well-developed six-rowed phenotype5. Int-c, located on chromosome 4H, is an ortholog of the maize (Zea mays. L.) domestication gene, Teosinte branched (TB1), a member of the TCP gene family encoding putative basic helix-loop-helix DNA-binding proteins6. The Int-c gene modifies lateral spikelet fertility in barley, and can influence the phenotypic effect of the Vrs1 locus.

Vrs4 controls row-type and spikelet determinacy in barley; an induced mutation, vrs4, can convert the two-rowed to a six-rowed phenotype5,6. Vrs4 is an ortholog of the maize inflorescence architecture gene RAMOSA2 (RA2), which encodes a transcriptional regulator that contains the lateral organ boundaries (LOB) domain. Expression analyses by mRNA in situ hybridization and microarray approaches showed that Vrs4 is expressed very early during inflorescence development and controls the row-type pathway through Vrs1 by negatively regulating the lateral spikelet fertility in barley. Moreover, the Vrs4 gene is an important modifier of inflorescence development. Here, we report on a new mutant, poly-row and branched spike (prbs) obtained by soaking a two-rowed barley inflorescence in maize genomic DNA from a single cross hybrid7,8, and characterize its genetics, report its positional cloning, and analyze its origin.

1National Barley Improvement Centre, Institute of Crop and Nuclear Technology Utilization, Zhejiang Academy of Agricultural Science, Hangzhou 310021, China. 2Department of Genetics and Cell Biology, Yangtze University, Jingzhou, Hubei 434023, China. 3Western Barley Genetics Alliance, Murdoch University, 90 South Street, Murdoch WA 6150, Australia. 4Luke/BI Plant Genomics Lab, Institute of Biotechnology and Viikki Plant Science Centre, University of Helsinki, FIN-00014 Helsinki, Finland. 5Green Technology, Natural Resources Institute Finland (Luke), Viikinkaari 1, FIN-00790 Helsinki, Finland. 6College of Life Sciences, Zhejiang Sci-Tech University, Hangzhou 310018, China. *These authors contributed equally to this work. Correspondence and requests for materials should be addressed to J.Y. (email: jmyang@163.com) or C.L. (email: c.li@murdoch.edu.au)
Results

Mutant prbs resulted from deletion of the Vrs4 gene. The poly-row and branched spike (prbs) barley mutant was obtained by soaking a two-rowed barley inflorescence in maize genomic DNA solution. The mutant prbs not only changes two-rowed barley into a poly-rowed form but also adds a spikelet row, forming irregular poly-row and branched spikes (Fig. 1). Genetic analysis indicated that the mutant phenotype was caused by a recessive gene, which has an epistatic effect on Vrs1. The prbs was initially mapped to the centromere of the short arm of chromosome 3H, a location similar to that of vrs4. Furthermore, the immature spikes of the prbs mutant under stereoscope are akin to the scanning electron microscopy images of the Vrs4 immature spikes. Three molecular markers (DQ327702, Cbic43, and Cbic44), closely linked with the Vrs4 gene, co-segregated with the prbs gene in the prbs/Kunlun12 RIL and prbs/Zangqing 320 F2 populations (Supplementary Fig. 1). No fragment was amplified in the mutant plants using these three molecular markers. Similarly, three other primers covering the Vrs4 gene sequence also failed to amplify a specific DNA fragment from either the prbs mutant or its progeny 11R258-95. Expression analyses revealed that the expression of Vrs4 was not detected and the expression of Vrs1 was significantly down-regulated in immature spikes at lemma primordium stage of the prbs mutant (Fig. 2). These results indicated that the prbs mutant may have resulted from a large deletion around the Vrs4 gene.

Identification of deletion region in prbs mutant. To identify the deletion region in the prbs mutant, a Morex BAC clone was identified that contains the Vrs4 gene. PCR primers were designed at 2 kb intervals from 14 kb upstream to 22 kb downstream of the Vrs4 gene and were tested on the prbs mutant, 11R258-95, Pudamai-2, and wild parent Pudamai-2.
and Morex. PCR primers located in the region from 3 kb upstream to 10 kb downstream of the \( Vrs4 \) gene failed to amplify a specific DNA fragment in the \( prbs \) mutant and 11R258-95 but amplified a single band in Pudamai-2 and Morex instead. Sequencing revealed that amplicons represented a single product in Pudamai-2 and Morex. Primers designed from 3 to 13 kb upstream and 10 to 21 kb downstream of the \( Vrs4 \) gene amplified a single band in all tested plants, but the amplicons represented multiple products when sequenced. These results did not support these regions arising from a single deletion event in the \( prbs \) mutant. However, an additional primer pair, Cbic123, matching a site 14 kb upstream of \( Vrs4 \), amplified a single band in all tested plants; the PCR product had 100% sequence identity among the \( prbs \) mutant, 11R258-95, Pudamai-2, and Morex. Another primer pair Cbic119, 22 kb downstream of \( Vrs4 \), also amplified a single band in all tested plants (sequencing was identical in all tested lines). These results revealed that the deletion sequence was from ~13 to 36 kb in the \( prbs \) mutant.

After failure to amplify a single DNA fragment using many PCR primers in the target region, long-range PCR was used to isolate the sequence covering the \( prbs \) mutation. Based on the above PCR test results, PCR primers Cbic131 and Cbic132 were designed for this purpose; the forward primer was near the site of primer Cbic123 and the reverse primer near the site of primer Cbic119, as both have been confirmed to amplify a single copy of DNA from the control varieties and mutants. A 15 kb fragment was successfully amplified from both the \( prbs \) mutant and 11R258-95 (Fig. 3), whereas the control PCRs using DNA from Pudamai-2 and Morex as a template failed to amplify. The amplification product from \( prbs \) was 14,715 bp (accession number KU758926).

We identified a 48,951 bp sequence from Morex using the 14,715 bp \( prbs \) sequence as query in BLASTN searches of the Morex genome database (http://webblast.ipk-gatersleben.de/barley/viroblast.php). Alignment of these two sequences showed that a 27,804 bp sequence in Morex, extending from nt 15,680 to nt 43,769 and containing the entire \( Vrs4 \) gene, is deleted in \( prbs \) (Fig. 4). Sequence analysis demonstrated that the \( Vrs4 \) gene in Morex is flanked by two long terminal repeat (LTR) retrotransposons located, respectively, at nt 10,967 to 19,828 upstream and nt 38,791 to 47,684 downstream (Fig. 4). A search of the Triticeae Repetitive Elements (TREP)
database revealed high similarity to the retrotransposon RLC BARE1 B consensus-1 (TREPACC = TREP3133) with 90% and 98% of sequence identity, respectively, for the two elements. We refer to the retrotransposon upstream of Vrs4 as BARE up, and the one downstream of Vrs4 as BARE down. The two share 90% identity, are bound by 1.8 kb LTRs, and contain the full-length open reading frame encoding Gag, aspartic proteinase (AP), integrase (IN), reverse transcriptase (RT), and RNaseH in Morex. The prbs mutant has 28 kb deletion including the entire Vrs4 gene and parts of the two retrotransposons. The non-retrotransposon sequence in the wild parent Pudamai 2 share 100% of sequence identity with Morex.

Recombination between the integrase genes of two BAREs formed prbs. Sequence analysis of the 15 kb region from prbs identified a single BARE element of the canonical 8.9 kb in length. Alignment revealed that the 5′ part of the prbs (nt 1 to 4,714) BARE was identical to BARE up, whereas the 3′ part (4,972 to 8,918 bp) was the same as BARE down (Fig. 5). The joint between the two halves is between nt 4,715 and nt 4,971 in the
Table 1. Sequence alignment of the Morex deletion with the maize genome sequence.

| Morex-start | Morex-end | Length | Highest percent identity | Highest e value | Hit number | Detail alignment results |
|------------|-----------|--------|--------------------------|----------------|------------|-------------------------|
| 1 | 15345 | 16193 | 848 | 73.01 | 1.484e-75 | 926 | Table S2, Fig. S3 |
| 43155 | 45887 | 732 | | | | |
| 2 | 16423 | 18000 | 1577 | 72.61 | 2.035e-121 | 1019 | Table S3, Fig. S4 |
| 44227 | 45804 | 1577 | | | | |
| 3 | 18046 | 18087 | 41 | 97.62 | 3.38E-12 | 879 | Table S4, Fig. S5 |
| 45850 | 45891 | 41 | | | | |
| 4 | 27390 | 27543 | 153 | 81.1 | 5.543e-169 | 35 | Table S5, Fig. S6 |
| 5 | 25868 | 26648 | 780 | 88.2 | 3.106e-45 | 1 | Table S6, Fig. S7 |

The prbs BARE (Fig. 5), within the integrase (IN) domain, corresponding to position nt 9,402–9,690 in the cloned prbs fragment (accession number KU758926). Retrotransposon integration generates a direct-repeat target-site duplication (TSD) flanking the individual element, as a consequence of repair of the staggered cut made by the integrase\(^{16}\). BARE up is flanked by imperfect CCAAG TSDs and BARE down by a perfect pair of CTGAA motifs. The BARE in prbs is flanked by CCAAG and CTGAA, supporting the origin of this BARE by recombination. Moreover, the upstream and downstream sequences surrounding the single BARE in prbs correspond, respectively, to that upstream of BARE up and downstream of BARE down. Thus, the HvRA2 gene, flanked by two BARE elements in Pudamai-2, was deleted by recombination between them, thereby generating the prbs mutation.

Search the maize genome for sequence similarity. To investigate the possible role of maize DNA in forming the prbs mutation, we used the region spanning the recombination zone in the BARE elements (9,402–9,690 bp) to carry out a BLASTn search of the MaizeGDB B73 reference genome sequence. No maize-specific sequence was found in the region. Hence, it appears that no maize DNA has been inserted into the prbs region and that the prbs phenotype results solely from the Vrs4 gene deletion.

As an alternative to insertion, the maize DNA may have played a role through sequence similarity at the recombination point. The region of recombination in prbs corresponds to the most conserved part of the integrase gene, which is the core domain that includes the D-D-35-E active site motif\(^ {16}\). The recombination itself took place in the region between the second Asp and the Glu of the active site; a BLASTn search of this region against the maize genome, which comprises ~425–485 Mb of the maize genome\(^ {17}\), the universal presence of integrase Integrase\(^ {22}\). Genome-wide analyses show that the average half-life of a retrotransposon in the genome\(^ {21}\), the rate of solo LTR formation varies considerably between retrotransposon families and also between chromosomes and regions\(^ {20,23,24}\). Analyses of the frequency of recombination events between internal retrotransposon domains, such as the generated prbs reported here, have not been made and are difficult to identify in the absence of novel phenotypes.

Recombination between the LTRs of two different elements can generate a concatenated structure comprising two internal domains flanking a single, recombinant LTR, which results in the loss of the intervening genomic sequence, including any gene that happens to be there. A quantitative PCR survey of the barley genome for such structures with three LTRs and two internal domains showed that their presence in about 4.3 × 10\(^3\) copies per haploid genome\(^ {23}\). While this indicates the potential for gene loss through recombination of retrotransposons flanking a gene, especially given that the gene islands\(^ {25}\) are flanked by retrotransposon “seas” which increases the
intervening distance between recombining retrotransposon sequences and appears to be correlated with decreasing recombination frequency. The question arises as to whether the maize DNA soaking procedure is connected to the recombination that generated the prbs mutant. Our procedure and the other methods introduce foreign DNA into the megagametophyte before fertilization. Whether or not any foreign DNA is integrated, the presence of extra chromosomal or cytoplasmic DNA triggers a range of defense responses in animals, mediated by DNA recognition by proteins including STING (also called MITA, MPYS, TMEM173, or ERIS), specific toll-like receptors (TLRs), Z-DNA binding proteins (ZBP-1, DLM-1, or DAI), and Mre11 (meiotic recombination 11). Mre11 is particularly intriguing because, together with RAD50 and NBS1, is a part of the MRN complex and has been shown to play a vital role in double-strand break (DSB) repair in plants, which is an intermediate step in recombination.

The maize genome contains 404,000 Capia superfamily retrotransposons, the integrase domains of these are very similar to the integrase core domain of BARE that underwent recombination in prbs. We speculate whether the homologous maize and barley integrase sequences may have interacted with each other, mediating the recombination. Expression analyses, in situ hybridization and microarray revealed that Vrs4 is actively expressed during inflorescence development, corresponding to the stage at which the barley inflorescence was soaked in maize genomic DNA to generate the prbs mutant. Due to its transcriptional activity, this region is likely to have an open chromatin conformation, which could provide an opportunity for maize DNA to interact with the BARE integrase domains and promote the recombination. The high concentration of conserved retrotransposon sequences would make binding and recombination in a retrotransposon sequence more likely than elsewhere. While recombinations between endogenous retroviruses (ERVs) are structurally similar to LTR retrotransposons—have caused genetic deletions through recombination, to our knowledge, there has not been an earlier demonstration of this in plants.

Horizontal gene transfer (HGT) is well documented in prokaryotic genome evolution. It is relatively clear that there are several HGT pathways, including transformation, conjugation, and transduction. In eukaryotes, direct DNA exchanges may occur during grafting, symbiosis, parasitism, pathogenesis, and epiphyte or entophyte. Some vectors, such as pollen, fungi, bacteria, viruses, plasmids, insects, and transposons, may also be involved in HGT. Transposable elements (TEs) have been recognized as important vectors for the horizontal movement of genes between eukaryotic genomes. Transposons, with their inherent ability to mobilize, can proliferate and integrate into genomic DNA and generate HGTs with ease. Transposons have also captured and transduced genomic DNA sequences in both Daphnia pulex and Drosophila species. The transfer of Mu-like transposons between Setaria and rice has been documented. LTR retrotransposons can produce virus-like particles, which may work as more frequent vectors for HGT. Such cases have been demonstrated in LTR-retrotransposon RIRE1 within the genus Oryza and the LTR-retrotransposon Route66 in Poaaceae. With the increasing availability of eukaryotic genome sequences, more evidence will be available that plants are also likely to undergo HGT. However, the results have been based on incongruences in molecular phylogenetic trees. On the other hand, there are numerous reports in the literature that have directly introduced foreign DNA by injecting exogenous DNA or directly DNA soaking or pollen tube pathway into rice, barley, wheat, sorghum, maize, cotton, oats, rye, cucumber, pumpkin, kidney bean and soybean to create new genetic diversity. This is especially true for LTR-retrotransposons as the LTR is sufficient in itself to activate TE transcription in response to stress. It is possible that soaking the barley spike in the maize DNA solution created stress conditions for the developing spikelets, which activated LTR-TE mediated recombination. In this scenario, the maize genomic DNA may be not essential for the mutation. Further research is required to test this assumption by soaking the developing barley spikes in water or salt solution to provide similar stresses for identification of new mutants.

**Materials and Methods**

**Plant materials.** A poly-row branched spike (prbs) barley mutant was obtained by soaking a two-rowed barley inflorescence (cv. Pudamai-2) in maize genomic DNA solution. The method followed that described earlier for wheat. Flowering barley spikes were soaked in total maize DNA at 1.6 ug/ul in 0.1 x SSC for 24 hours. After soaking, the head was moved from the solution and air-dried under ambient conditions. Plants were self-pollinated and seeds harvested. The mutant was identified at flowering of the next generation plants.

**Genetic mapping** was conducted in two populations: one recombinant inbred line (RIL, F2) population consisting of 207 plants derived from a cross between the prbs mutant and a six-rowed barley cultivar Kunlun 12, and an F2 population consisting of 285 spike mutant plants derived from a cross between the prbs mutant and a six-rowed barley cultivar Zangqing 320. The prbs mutant, RIL 11R258-95 with a branched spike phenotype, Pudamai-2, and var. Morex were used for DNA sequence analysis.

**Genomic DNA extraction and genotyping analysis.** Genomic DNA was extracted from leaves of individual plants and their parents using a modified CTAB method. DNA samples were quantified using a Unican UV300 UV/Vis spectrometer (Thermo Electron Corporation, Cambridge, UK), and then adjusted to 25 ng/ul. Because a DQ327702 marker associated with the mutant is closely linked with the Vrs4 gene, new molecular markers Cbic43 and Cbic44 were designed around the Vrs4 gene using the barley genome sequence from the IPK Barley BLAST server (http://webblast.ipk-gatersleben.de/barley/viroblast.php). Primer pairs specific to the
Vrs4 gene (AS12, AS34, and AS56) were designed for Vrs4 haplotype analysis. Primers were synthesized by Shanghai Sunny Biotechnology (Shanghai, China). PCR reactions were performed in 10 μL volumes containing approximately 25 ng genomic DNA, 0.2 μM of each primer, and 5 μL 2 × Taq Master Mix (Gene Solution, Shanghai, China) using the following program: 94 °C for 3 min, 32 cycles of 94 °C for 30 sec, 55 °C for 45 sec, 72 °C for 1 min, and 72 °C for 5 min. PCR products were separated on 8% polyacrylamide gels.

Cloning of the deletion in mutant prbs. BAC sequences were identified by blasting the Vrs4 against the International Barley Genome Sequencing Consortium database (unpublished data). PCR primer pairs were designed at 2 kb intervals in the region near the Vrs4 locus using the Primer-Blast tool (http://www.ncbi.nlm.nih.gov/tools/primer-blast/). PCR reactions were described as above. Annealing temperatures were optimized for each primer pair (Supplemental Table 1). PCR products were examined by electrophoresis on 1% agarose gels.

Quantitative RT-PCR. RNA was extracted from immature spikes at lemma primordium stage of the prbs mutant and wild parent Pudamai-2 using Spin Column Plant total RNA Purification Kit (Sanggon Biotech (Shanghai) Co., Ltd.). cDNA was prepared from 1 μg RNA using AMV First Strand cDNA Synthesis Kit (Sanggon Biotech (Shanghai) Co., Ltd.). qPCR reactions were performed using SYBR Green (SG Fast qPCR Master Mix (HighRox), BBI) and the Applied Biosystems Stepone plus Real-time PCR System. The Real-time PCR assays were performed in triplicate for each cDNA sample. Vrs4, Vrs1 and HvActin primer sequences used for quantitative RT-PCR. The HvActin gene was used as reference gene for normalization.

Sequence analysis. Alignments of mutant prbs and barley genomic sequences were constructed using MEGA 6.014 and BLASTN 2.3.0. The prediction of transposable elements was identified through LTR Finder 1.05 (http://life.fudan.edu.cn/ltr_finder/) and BLAST66 against the Triticeae Repetitive Element (TREP) database (http://wheat.pw.usda.gov/GG2/blast.shtml). Searches for sequence homology to maize was conducted with MaizeGDB against the sequence database B73 RefGen_v3 (MGSC), using the BLAST program BLASTN with an E-value cutoff < 1e-50.

References

1. Doust, A. N. & Kellogg, E. A. Inflorescence diversification in the panicle "bristle grass" clade (Paniceae, Poaceae): evidence from molecular phylogenies and developmental morphology. *Am. J. Bot.* 89, 1203–1222 (2002).
2. Sreenivasulu, N. & Schnurrausbusch, T. A genetic playground for enhancing grain number in cereals. *Trends Plant Sci.* 17, 91–101 (2012).
3. Forster, B. P. et al. The barley phytochrome. *Ann. Bot.* 100, 725–733 (2007).
4. Komatsuda, T. et al. Six-rowed barley originated from a mutation in a homeodomain-leucine zipper I-class homeobox gene. *Proc. Natl. Acad. Sci. USA* 104, 1424–1429 (2007).
5. Ramsay, L. et al. INTERMEDIATE-C, a modifier of lateral spikelet fertility in barley, is an ortholog of the maize domestication gene TOSONTE BRANCHED 1. *Nat. Genet.* 43, 169–172 (2011).
6. Koppolu, R. et al. Six-rowed spike4 (Vrs4) controls spikelet determinacy and row-type in barley. *Proc. Natl. Acad. Sci. USA* 110, 13198–13203 (2013).
7. Lundqvist, U. & Lundqvist, A. Induced intermediate mutants in barley: origin, morphology and inheritance. *Hereditas* 108, 13–26 (1988).
8. Lundqvist, U., Franckowiak, J. D. & Konishi, T. New and revised descriptions of barley genes. *Barley Genet. News.* 26, 22–516 (1997).
9. Liu, S. et al. Studies on inheritance and spike characters of poly-row- and branched spike mutant in barley. *Fujian Science and Technology of Rice and Wheat.* 18, 37–39 (2000).
10. Ji, H., Chen, Q. & Lin, X. Study on poly-row barley with multi-branches of spikelets emerged by directly of DNAs. *Journal of Fujian Agriculture University* 24, 9–13 (1995).
11. Huang, B., Wu, W., Liu, S. & Huang, Z. Genetic Analysis on Poly-row- and Branched Spike Mutant in Barley. *Hereditas (Beijing)* 26, 903–906 (2004).
12. Shang, Y. et al. Characterization and mapping of a Prbs gene controlling spike development in Hordeum vulgare L. *Genes Genomics* 36, 275–282 (2014).
13. Suoniemi, A., Tanskanen, J., Pentikainen, O., Johnson, M. S. & Schulman, A. H. The core domain of retrotransposon integrase in Hordeum: predicted structure and evolution. *Mol. Biol. Evol.* 15, 1135–1144 (1998).
14. Tamura, K., Stecher, G., Peterson, D., Filipski, A. & Kumar, S. MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. *Mol. Biol. Evol.* 30, 2725–2729 (2013).
15. Krischnan, L. & Engelman, A. Retroviral integrase proteins and HIV-1 DNA integration. *J. Biol. Chem.* 287, 40858–40866 (2012).
16. Vitte, C. & Panaud, O. Formation of solo-LTRs through unequal homologous recombination counterbalances amplifications of LTR retrotransposons in rice Oryza sativa L. *Mol. Biol. Evol.* 20, 528–540 (2003).
17. Estep, M. C., DeBarry, J. D. & Bennetzen, J. L. The dynamics of LTR retrotransposon accumulation across 25 million years of panicle grass evolution. *Hereditas (Edinb).* 110, 194–204 (2013).
18. Bortiri, E. et al. Ramosa2 Encodes a LATERAL ORGAN BOUNDARY Domain Protein That Determines the Fate of Stem Cells in Branch Meristems of Maize. *Plant Cell* 20, 574–585 (2006).
19. Shirasu, K., Schulman, A. H., Lahaye, T. & Schulte-Lefert, P. A contiguous 66-kb barley DNA sequence provides evidence for reversible genome expansion. *Genome Res.* 10, 908–915 (2000).
20. Baidouri, M. E. & Panaud, O. Comparative Genomic Paleontology Across Plant Kingdom Reveals The Dynamics Of TE-driven Genome Evolution. *Genome Biol. Evol.* 5, 954–965 (2013).
21. Schulman, A. H. Retrotransposon replication in plants. *Curr. Opin. Gen.* 3, 604–614 (2013).
22. San Miguel, P. & Panaud, O. & Takahonov, A., Nakaizumi, Y. & Bennetzen, J. L. The paleontology of intergene retrotransposons of maize. *Nat. Genet.* 20, 43–45 (1998).
23. Vogel, J. P. et al. Genome sequencing and analysis of the model grass Brachypodium distachyon. *Nature* 463, 763–768 (2010).
24. Mayer, K. F. et al. A physical, genetic and functional sequence assembly of the barley genome. *Nature* 491, 711–716 (2012).
25. Liu, R. et al. A GeneTrek analysis of the maize genome. Proc. Natl. Acad. Sci. USA 104, 11844–11849 (2007).
26. Zhou, G. Y. et al. Introduction of exogenous DNA into cotton embryos. Meth. Enzymol. 101, 433–481 (1983).
27. Peña, A. D. L., Lörz, H. & Schell, J. Transgenic rye plants obtained by injecting DNA into young floral tillers. Nature. 325, 274–276 (1987).
28. Barber, G. N. STING-dependent cytosolic DNA sensing pathways. Trends Immunol. 35, 88–93 (2014).
29. Ma, Z. et al. Modulation of the eGAS-STING DNA sensing pathway by gammaherpesviruses. Proc. Natl. Acad. Sci. USA 112, 4306–4315 (2015).
30. Abe, T. et al. STING Recognition of Cytoplasmic DNA Instigates Cellular Defense. Mol. Cell. 50, 5–15 (2013).
31. Kawai, T. & Akira, S. Toll-like Receptors and Their Crosstalk with Other Innate Receptors in Infection and Immunity. Immunity 34, 637–650 (2011).
32. Takaoka, A. et al. DAI (DLM-1/ZBP1) is a cytosolic DNA sensor and an activator of innate immune response. Nature 448, 501–505 (2007).
33. Kondo, T. et al. DNA damage sensor MRE11 recognizes cytosolic double-stranded DNA and induces type I interferon by regulating STING trafficking. Proc. Natl. Acad. Sci. USA 110, 2969–2974 (2013).
34. Samanic, I., Cvitanic, R., Simunic, J. & Puizina, J. Arabidopsis thaliana MRE11 is essential for activation of the cell cycle arrest, transcriptional regulation and the DNA repair upon the induction of double-stranded DNA breaks. Plant Biol (Stuttg). 18, 681–694 (2016).
35. Tenaillon, M. I., Hufford, M. B., Gaut, B. S. & Rosselbarra, J. Genome size and transposable element content as determined by high-throughput sequencing in maize and Zea luxurians. Genome Biol Evol. 3, 219–229 (2011).
36. Schnable, P. S. et al. The B73 maize genome: complexity, diversity, and dynamics. Science. 326, 1112–1115 (2009).
37. Shuvarikov, A. et al. Recurrent HERV-H-mediated 3q13.2-q13.31 deletions cause a syndrome of hypotonia and motor, language, and cognitive delays. Hum. Mutat. 34, 1415–1423 (2013).
38. Stegemann, S. & Bock, R. Exchange of genetic material between cells in plant tissue grafts. Science 324, 649–651 (2009).
39. Finan, T. M. Evolving insights: symbiosis islands and horizontal gene transfer. J. Bacteriol. 184, 2855–2856 (2002).
40. Burger, G. & Lang, B. F. Parallels in genome evolution in mitochondria and bacterial symbionts. IUBMB Life 55, 205–212 (2003).
41. Yoshiida, S., Maruyama, S., Nozaki, H. & Shirasu, K. Horizontal gene transfer by the parasitic plant Striga hermonthica. Science 328, 1128–1128 (2010).
42. Sharp, A. J. et al. Discovery of previously unidentified genomic disorders from the duplication architecture of the human genome. Nat. Genet. 38, 1038–1042 (2006).
43. Bock, R. The give-and-take of DNA: horizontal gene transfer in plants. Trends Plant Sci. 15, 11–22 (2010).
44. Richards, T. A. et al. Phylogenomic analysis demonstrates a pattern of rare and ancient horizontal gene transfer between plants and fungi. Plant Cell 21, 1897–1911 (2009).
45. Broothaerts, W. et al. Gene transfer to plants by diverse species of bacteria. Nature 433, 629–633 (2005).
46. Hull, R., Harper, G. & Lockhart, B. Viral sequences integrated into plant genomes. Plant Biol. 110, 2969–2974 (2013).
47. Dieterich, C. et al. The Pristionchus pacificus genome provides a unique perspective on nematode lifestyle and parasitism. Nat. Genet. 40, 1193–1198 (2008).
48. Diao, Y. et al. Next-generation sequencing reveals recent horizontal transfer of a DNA transposon between divergent mosquitoes. PloS One 6, e16743 (2011).
49. Novick, P., Smith, J., Ray, D. & Boissinot, S. Independent and parallel lateral transfer of DNA transposons in tetrapod genomes. Genet. 449, 85–94 (2010).
50. Roulin, A. et al. Whole genome surveys of rice, maize and sorghum reveal multiple horizontal transfers of the LTR-retrotransposon Route66 in Poaceae. BMC Evol. Biol. 9, 58 (2009).
51. Sormacheva, I. Vertical evolution and horizontal transfer of CR1 non-LTR retrotransposons and Tc1/mariner DNA transposons in Lepidoptera species. Gene 345–54 (2008).
52. Abe, T. Recurrent HERV-H-mediated 3q13.2-q13.31 deletions cause a syndrome of hypotonia and motor, language, and cognitive delays. Hum. Mutat. 34, 1415–1423 (2013).
53. Liu, P. & Kang Z. Oat (Avena sativa L.) exogenous DNA introduction into common wheat and PAPD analysis. Agricultural Research in the Arid Areas. 24, 100–103 (2006).
54. Wang, S. et al. Molecular verification of DNA flow from wild rice (O. minuta) to cultivated rice. Molecular Genetics and Genomics. 278, 501–505 (2012).
55. Sorensen, A. et al. Vertical evolution and horizontal transfer of CR1 non-LTR retrotransposons and Tc1/mariner DNA transposons in Lepidoptera species. Mol. Biol. Evol. 9, 3685–3702 (2012).
56. Loreto, E. L. S., Carareto, C. M. A. & Capy, P. Revisiting horizontal transfer of transposable elements in Drosophila. Heredity. 100, 545–554 (2008).
57. Schäck, S., Choi, E., Lynch, M. & Pritham, E. J. DNA transposons and the role of transposition in mutation accumulation in Daphnia pulex. Genome Biol. 11, R6 (2010).
58. Diao, X., FREELING, M. & Lisch, D. Horizontal transfer of a plant transposon. PLoS Biol. 4, 119 (2006).
59. Silva, J. C., Loreto, E. L. & CLark, J. B. Factors that affect the horizontal transfer of transposable elements. Curr. Issues Mol. Biol. 6, 57–71 (2004).
60. Roulin, A., Piegu, B., WANG, R. A. & Panaud, O. Evidence of multiple horizontal transfers of the long terminal repeat retrotransposon RIRE1 within the genus Oryza. Plant J. 53, 950–959 (2008).
61. Luo, H., Zhong, B., Yang, Z., Li, Y. & He, G. The SSR molecular evidence of rice transformation via pollen tube pathway. Mol. Plant Breed. 2, 501–505 (2004).
62. Liu, P. & Fang Z. Oat (Avena sativa L.) exogenous DNA introduction into common wheat and PAPD analysis. Agricultural Research in the Arid Areas. 24, 100–103 (2006).
63. Wang, S. et al. Molecular verification of DNA flow from wild rice (O. minuta) to cultivated rice. Scientia Agricultura Sinica. 39, 2170–2177 (2006).
64. Casacuberta, E. & Gonzalez, J. The impact of transposable elements in environmental adaptation. Molecular Ecology 22, 1503–1517 (2013).
65. Huang, B. & Wu, W. Mapping of Mutant Gene prbs Controlling Poly-Row- and Branched Spike in Barley (Hordeum vulgare L.). Agric. Sci. China 10, 1501–1505 (2011).
66. Bai, F. et al. A new Wheat Strain of Early Maturity and Another One of Good Dwarf Quality by Introducing Exogenous Maize Nuclear DNA. Acta Agronomica Sinica. 25, 260–264 (1999).
67. Liu, T. & Zheng, K. A simple method for isolation of rice DNA. Chinese Journal of Rice Science 6, 47–48 (1992).
68. Zhang, Z., Schwartz, S., Wagner, L. & Miller, W. A greedy algorithm for aligning DNA sequences. J. Comput. Biol. 7, 203–214 (2000).
69. Xu, Z. & Wang, H. LTR_FINDER: an efficient tool for the prediction of full-length LTR retrotransposons. Nucleic Acids Res. 35, 265–268 (2007).
70. Altschul, S. F. et al. BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res. 25, 3389–3402 (1997).

Acknowledgements

We are grateful to Professor Jing Zhang, Institute of Crop Germplasm Resources, Chinese Academy of Agricultural Science, for providing the mutant prbs, and to Dr Nils Stein Leibniz Institute of Plant Genetics and Crop Plant Research (IPK) for accessing the unpublished barley BAC sequence. This work was partially supported by the Natural Science Foundation of Zhejiang Province (LY15C130004), China Agriculture Research System (CARS-05), the National Nature Science Foundation of China (31501309) and the Academy of Finland, Project 266430.
Author Contributions
C.L. and J.Y. conceived, designed and supervised the study; Y.S. and F.Y. are principal investigators; A.H.S., F.Y.W.Z. and Y.J. data analysis; J.Z., J.W., W.H. phenotyping; N.S., X.Z. and Q.J. DNA sequencing; Y.S., F.Y. A.S. and C.L. wrote the paper.

Additional Information
Supplementary information accompanies this paper at http://www.nature.com/srep

Competing Interests: The authors declare no competing financial interests.

How to cite this article: Shang, Y. et al. Gene Deletion in Barley Mediated by LTR-retrotransposon BARE. Sci. Rep. 7, 43766; doi: 10.1038/srep43766 (2017).

Publisher's note: Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

This work is licensed under a Creative Commons Attribution 4.0 International License. The images or other third party material in this article are included in the article’s Creative Commons license, unless indicated otherwise in the credit line; if the material is not included under the Creative Commons license, users will need to obtain permission from the license holder to reproduce the material. To view a copy of this license, visit http://creativecommons.org/licenses/by/4.0/

© The Author(s) 2017