Supplementary Materials:

Frequency and type of inheritable mutations induced by γ rays in rice as revealed by whole genome sequencing

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Table S1 Verification of single base substitution (SBS) mutations in P_2M_2 and P_3M_3 plants

SBSs	Mutated gene	Mutation	Genotype[1]	Forward (F) and reverse (R) primer (5'→3')	Verification in	
					M_2	M_3
SBS1	Os09g0359800	G→A	het	F:TGGCAGATATGGCAAAGG R:TGCAGTAAGATGCAAAGAAGC	F[2]	—[4]
SBS2	Os10g0158400	G→A	het	F:TCATTCATAGACGCATCGC R:GCTCGCTTTTGGTTAGACC	T[3]	—
SBS3	Os12g0424300	C→G	het	F:TTCCATACTAGCACATTCCCATA R:TCTTGATGTTCCCACTGATACCT	T	—
SBS4	Os08g0155700	A→G	het	F:TGATGCTGGAAGTAGTGCCC R:GGAATTTCTTACCGTACCATCATAT	T	—
SBS5	Os07g0572600	C→T	het	F:GCATTGGAAGGAAGGTTGTA R:CCTCAGCATCCGTGGTAAA	F	—
SBS6	Os05g0552550	T→C	het	F:CAGTCAGCGGCGAAAAGA R:GCCCATCACCAAGGAGGA	T	—
SBS7	Os04g0487400	C→G	het	F:ACGAACTAGCCGAACACTCCC R:AAGACAAAGACCGAAACCCAGA	F	—
SBS8	Os02g0528900	G→C	het	F:CCAGTAGTGCCTTCATCGTT R:CGTCGTTGAGAATGGGGAT	T	—
SBS9	Os03g0753500	G→C	hom	F:CCCAGCATTGGAGGAAGT R:AGCAAGCATCAAGCACGT	F	—
SBS10	Os01g0656400	A→G	hom	F:GTTGTTGGCGTGTTTTGATTAGTTT R:GCGTCGGCTGATGCTTTTG	T	—
SBS11	Os10g0380100	G→C	het	F:GGCGTTCCCGAGGATGAGA R:CACGACGGCACGAAGATGA	T	—
SBS12	Os08g0155700	A→G	het	F:CAAGGCTCGTGATGATGC R:CACAAGTCGTCGCTGGAT	T	—
SBS13	Os07g0598700	C→T	het	F:ATTTGTTAGCACTCCGATGG R:CAGCACTGAGAATCAGACCG	T	—
SBS14	Os05g0552600	G→T	het	F:CCCATCACCAAGGAGGACT R:TGACACGGTTACATGCTGAAC	F	—
SBS15	Os06g0175500	C→G	het	F:TCTGAACGAACCGAACCC R:GCCGCGTCTATCTGGAAT	T	—
SBS16	Os04g0115650	C→T	het	F:CGATGTCGCTATGTTTGC R:CCAGAAGAATGATGCCTTT	T	—
SBS17	Os02g0596100	C→T	hom	F:ACTGCTTGTGCCCTTCAT R:CAACAGCCATTGCTAACC	T	T
SBS18	Os02g0610500	G→T	hom	F:GGCTGATGCGGTACAGGG R:GGTTAGGCAGAAGAGGAGTAGTAGG	T	T
SBS19	Os03g0753500	C→T	het	F:CTTTGCCAAGAACTATCCCTAC R:TGGTGAGATCAGCCCAGAA	T	—
SBS20	Os01g0495200	G→C	het	F:GCAAAGTGCGGGCTATCA R:AGGAGGACGAGCAAGACG	T	—

[1]het for heterozygous and hom for homozygous mutations; [2]The mutation was proved to be false (F) by Sanger sequencing;
[3]The mutation was proved to be true (T) by Sanger sequencing or high resolution melting curve analysis; [4]Not tested (—)

Table S2 Verification of insertion and deletion (Indel) mutations in the P_2M_2 and P_3M_3 plants

Indels	Mutated gene	Mutation	Genotype[1]	Forward (F) and reverse (R) primer (5'→3')	Verification in	
					M_2	M_3
Indel1	Os03g0351400	GGCCCAAGAG deletion	het	F:GACGCCACTTCATTGACT R:GCATACCTTCTGTGAGCC	T[2]	—[4]
Indel2	Os05g0585500	AT deletion	het	F:TTATTATGTTATGGCTAGGTCG R:TTATGGAATCGGTTTTATCG	F[3]	—
Indel3	Os02g0218700	TGATGGT insertion	het	F:GGATGCCGCTGGTGAAGT R:CGTGCGTGCTATCCCGAC	F	—
Indel4	Os10g0142100	TATGTATGTA deletion	het	F:TGATGTTATGCGGTAAGTGG R:TTTCAAAAGCGTGATGCC	T	—
Indel5	Os04g0218600	GGTAGCGGAGGAGGAGGC insertion	het	F:GAGGGAGGCATCGTCGGA R:CGTAGCGGCAAGGGGTGT	T	—
Indel6	Os04g0298600	G deletion	hom	F:GTTGATGGAAGATGGACCCTT R:TTGCTCGTCGGAGATGGC	T	—
Indel7	Os04g0534100	A deletion	hom	F:CCAGACACTTATGCCTTATCC R:AACGAGCAGACGAGACCA	T	—
Indel8	Os04g0588200	T deletion	hom	F:CCGTATCGAACCGCCTAGC R:CCAAGTCACAACCTCCCCAC	T	—
Indel9	Os04g0643200	C insertion	hom	F:GATTTACAGCTACTTTTGGGTTT R:ATTCGGTTGGCATTTTCC	T	—
Indel10	Os04g0389600	T deletion	hom	F:TCCAGGTAAAAGTAAACGAGCAT R:CGGTGGTTGGTGGTGTATGT	T	—
Indel11	Os01g0715201	TCT insertion	het	F:CCTGCCAGTGCGACGGTAT R:GGCGCTGTCCTCTTATGGTAAA	T	—
Indel12	Os12g0246700	AGTAGGGTA insertion	het	F:CCGAATAGAAAGAAGAATG R:TAGTGGTGGTGAGTCCTG	F	—
Indel13	Os02g0528900	TACTATA insertion	het	F:ATGGGACTTGACATTTGC R:TTCCAAGATGATGTTTCG	F	—
Indel14	Os03g0755100	TCTGT insertion	het	F:CCATATTACATAGCAGCCAATC R:TCCTCCACTCCCTCCATT	F	—
Indel15	Os08g0155700	ACACAGCGTT insertion	het	F:TGGACTTTCTTATCCCTCAAT R:GTGGGTCTGGTACGGTCA	F	—
Indel16	Os01g0964133	AG deletion	het	F:TTAGGGTGATGTGGGTTAA R:CATGGGTGCCTGTTAGTG	T	—
Indel17	Os04g0282400	C insertion	hom	F:ACATACCTCGAACTCTTCCC R:GACGACGTACCAGCTAACAC	T	—
Indel18	Os04g0298600	G deletion	hom	F:GTTGATGGAAGATGGACCCTT R:TTGCTCGTCGGAGATGGC	T	—
Indel19	Os04g0534100	C deletion	hom	F:CCAGACACTTATGCCTTATCC R:AACGAGCAGACGAGACCA	T	—
Indel20	Os07g0569166	T insertion	het	F:ATGGAGGCGATGGGTAAT R:CGATGTAGAAACGTGCTAGTAAAA	T	—
Indel21	Os10g0492400	G deletion	hom	F:TTCAGGATAGCGAGGTGGAGCG R:GCGAGCGAAGTGGAGTGGGTTT	T	—
Indel22	Os12g0110700	TC deletion	het	F:GCATCCTTCTAATCGTGCCC R:GGTGGTTTTGACCCCAGTTTT	F	—
Indel23	Os01g0244400	GA deletion	het	F:ACAAAACGTGAGCACCAAACTGA R:CCTGGCTGGCGCATAGGA	T	—
Indel24	Os07g0620600	AG deletion	het	F:AACGTGAGGGAGGAGGACT R:CCCCAACCAAAACCAAAT	F	—
Indel25	Os06g0269151	AAG deletion	het	F:ACAGGTGATGTGCAAGCG R:CCAATTCAGGTTCTCCGTC	F	—
Indel26	Os05g0202500	A insertion	het	F:TCGGTCGTGCTAAGATAA R:CAGGGAGGTGTAGGTAGG	T	—
Indel27	Os09g0375100	T insertion	het	F:GGAGGGCAGGAGAATCAT R:GGTTCCAACGCTAAAGGT	T	—
Indel28	Os08g0355400	T insertion	het	F:GTTGGACACGGGAACTCA R:CGGAGGATTATGGTGCTG	F	—
Indel29	Os09g0471500	A insertion	hom	F:GCTGCTACGTTCCTCTGC R:GATTGGATTGACTGGCTTAC	F	—
Indel30	Os02g0528550	T insertion	het	F:CAAGGTTACAAGGAGCGAAAG	F	—

				R:CGCACGCATGACAATAGC		
Indel31	Os06g0286351	TGTTTCTTTGCCTGCAC deletion	hom	F:ATAGATGAAAGCGAGGAT R:GTACCACCACTTTAAGAAC	T[2]	T
Indel32	Os12g0208900	A deletion	hom	F:TCCTCAAGGTCTGGATGC R:ATGAACAGTGGGGAGTGAA	T	—
Indel33	Os05g0147133	GAGCAT deletion	het	F:TGGTGCCACTTTGGGTAG R:AAAGCACGGAGCATACCT	T	—
Indel34	Os12g0611300	A deletion	hom	F:GGACCAGGTGATGCTCTGC R:AGTCGAAGCTGCCGCTCT	T	—
Indel35	Os04g0385700	GC insertion	hom	F:TATTTCCCTTTCCAATGTTC R:GAATCGTCGTTCGACTGC	T	—
Indel36	Os04g0385700	T deletion	hom	F:GAGGACCTCCATGTGCTTG R:CCACTAGACTTTGTCACCGACT	T	T
Indel37	Os04g0387300	C insertion	hom	F:GTAGGAAGCCAATCGGAACT R:TTCAAGGGGAAAGAGCGTA	T	T
Indel38	Os04g0599100	G deletion	hom	F:GAAGCGGGCTGAGTTGAA R:AAGCGATTGTGAAGGGACA	T	—
Indel39	Os04g0687150	C deletion	hom	F:AAGCAAGAGGCAATTAGAGCG R:TGCAGGTGAGGGAGTGAGC	T	T
Indel40	Os04g0349600	T deletion	hom	F:CTGTTCGACGAATCCCTACTG R:CACCATTTCCTTTGTCTTGC	T	T
Indel41	Os04g0321800	C insertion	hom	F:TTTTGCCTCTGTTCCTCTTC R:CTGGTTGACGCCCTTGTT	T	—
Indel42	Os02g0207900	AG insertion	het	F:GGAGGGAAGAAGGTTTAG R:CCAAGTGGCACTCTAATC	T	—
Indel43	Os02g0528550	G insertion	het	F:TCTAGGGCAAATGAAGGC R:TCAGACCGAACTGGCAAC	F	—
Indel44	Os04g0218400	CTA insertion	het	F:CAAGGTGGGTACTCAACA R:CTGCTCTTCGTATGGTGC	F	—
Indel45	Os08g0313600	A insertion	het	F:CTGCTTCACTTGCCTTGT R:TTTGGCTAATGTGGTTTCT	T	—
Indel46	Os04g0317800	CGCGATACACGCCGGTCT AACCG deletion	het	F:ATTGGCAAAGTTAATCGC R:GAGGCATCAAGTCACCTACA	T	—
Indel47	Os01g0104800	AA deletion	het	F:AGTGGGATTATGGGAGTG R:GGTAGTGATGGTTGGGTG	T	—
Indel48	Os04g0312500	C insertion	hom	F:CTTGCTTCAACCAGACCCTT R:CCCACAGCCGAATACCAG	T	—
Indel49	Os04g0301700	G insertion	hom	F:ATGGGACGAATAGAGTAAATGCC R:CGTCGAGGTGGAGCAGGTAG	T	—
Indel50	Os04g0352700	G insertion	hom	F:TTACTCATCCTCGCTCTGGC R:GGGCTGGTTGTCGCTCAT	T	—
Indel51	Os09g0369500	TC deletion	het	F:AAGAAGCTGGGCAACACGG R:CTGAAGAGTTAGGCACCAAACATAC	F	—
Indel52	Os02g0132500	GCG deletion	het	F:ATGCTGTCGAACAGGGAGT R:GGGGAGGATCTGAAGAGGT	F	—
Indel53	Os07g0201800	TCT deletion	het	F:CAGGCTGCTGCTGATGCT R:TGGAGACGAAGGTGACGG	T	—
Indel54	Os04g0298600	G deletion	hom	F:ATCCGTCCTTCTACAGTTCC R:ACAGAGCGTGACCGACTT	T	—
Indel55	Os04g0675101	CT deletion	het	F:ATCGGCAAAGGAGAAGTC R:AGCAGATGATGCAGCACA	T	—
Indel56	Os10g0211900	G insertion	het	F:GAATGGACGGAACCCTAA R:GAAAGCGACACCCACTAAC	T	—
Indel57	Os05g0549700	CCC insertion	het	F:GACTACGAATCCGAGGCT R:GAAGGGTTTGGTTGAGGG	F	—
Indel58	Os08g0355400	TT insertion	het	F:AGGACAAGAGCCTAACGA R:CTCACTCCATCCCATTTT	F	—
Indel59	Os04g0312500	C insertion	hom	F:CTTGCTTCAACCAGACCCTT R:CCCACAGCCGAATACCAG	F	—
Indel60	Os04g0321800	C insertion	hom	F:TTTTGCCTCTGTTCCTCTTC R:CTGGTTGACGCCCTTGTT	T	—

[1]het for heterozygous and hom for homozygous mutations; [2]The mutation was proved to be false (F) by Sanger sequencing; [3] The mutation was proved to be true (T) by Sanger sequencing or high resolution melting curve analysis; [4]Not tested (—)

Table S3 Verification of structural variation (SV) mutations in P_2M_2 plants

SVs	Chromosome	Location	Mutations	Forward (F) and reverse (R) primer (5'→3')	Size (WT/MT, bp)[1]	Verification
SV1	6	28795143-28795784	184 bp deletion	F:ATGTGAGGGAACTGAAAGA R:GAGTGCATCGACCATAACT	846/662	F[2]
SV2	10	14899128-14899888	229 bp deletion	F:CCGAGGTGTTCGACGTAAT R:CCTGCCTGCCTTGTAGAGT	1247/1018	F
SV3	9	3858255-3858530	230 bp deletion	F:CAGAGCAGTGAGGCATTT R:TTGGCATAGCCCTACAGA	528/298	F
SV4	9	6249647-6249776	201 bp insertion	F:CAAATCAAAGGCTAATCCA R:ACTTCACAACAAGCACCAC	603/804	F
SV5	12	24668763-24669908	767 bp inversion	F:AAGTTTGTACCCTGAAGC R:GAAAGGCAAGAGTTGGAAT	1455/1455	F
SV6	9	3868727-3868907	218 bp deletion	F:CAGAAGCGTCTCAGCATA R:CGAAGTGTCGGAGGATTT	870/652	F
SV7	8	1718198-1718606	238 bp deletion	F:ATTCTTCCCTCATTCTGC R:CAATTCACTCGACAACCC	720/482	T[3]
SV8	2	28699544-28700165	195 bp insertion	F:TTACTTTGTCGGATTTGCC R:GATGGGTTGCTGTCTTCTG	1355/1550	F
SV9	9	6374464-6375052	194 bp insertion	F:GCACGACATCGTTCACTG R:GTCATCAACACGGCACAT	863/1057	F
SV10	6	1192705-1192933	154 bp inversion	F:TTGGTCCCGTGATGGCGAACT R:CCACCGGCGTGAGCGAAAA	547/547	F

[1]The expected fragment size for the wild type (WT) and mutant (MT) plant; [2]The mutation was proved to be false (F); [3] The mutation was proved to be true (T)

Table S4 Verification of copy number variation (CNV) mutations in P_2M_2 plants

CNVs	Chromosome	Location	Mutations	Forward (F) and reverse (R) primer (5'→3')	Verification
CNV1	12	21206601-21207300	700 bp deletion	F:ACCTCCCGAGAAGCGAAGA R:CGAAATCGAAACAACTTGAGAA	T[1]
CNV2	11	19082501-19083300	800 bp deletion	F:CAGACCTGTGGGTTTGGC R:TGCTCTTCCTCCTACCTTCC	F[2]
CNV3	7	5271901-5272700	800 bp deletion	F:GTCGTGAGGGTTACAAGC R:CAAGTAGGAACGGGTGGT	T
CNV4	6	21377101-21377700	600 bp deletion	F:CAAGTCAGAAAGACGAGCAG R:TGGGCAAAGGATACATTACA	F
CNV5	4	28745901-28749500	3600 bp duplication	F:TTCCCTGACTTCTGAGCC R:CAAAGCCTTTATGCCAAC	F
CNV6	2	15740801-15743500	2700 bp duplication	F:TTTCAGAATAAATGGGAGATAGGG R:CCGTGCTGGTAAAGTGGC	F
CNV7	1	24753201-24754100	900 bp deletion	F:GTGCTCGACGCTGGACAGT R:GCCCGAAGAAAGAAGTAACAAA	F

[1]The mutation was proved to be true (T); [2]The mutation was proved to be false (F)

Table S5 Frequency of single base substitution (SBS) and Indel mutations in individual chromosomes of the six γ rays-mutagenized P_2M_2 plants (mutation numbers per million base pairs)

Chromosome	165-MS		165-NP		246-MS		246-NP		389-MS		389-NP	
	SBS	Indel	SBS	Indel	SBS	Indel	SBS	Indel	SBS	Indel	SBS	Indel
1	5.55	2.10	9.43	2.59	5.87	2.73	8.16	2.54	5.04	2.15	4.83	1.99
2	6.73	2.25	7.99	2.28	8.10	2.45	7.76	2.20	4.73	2.20	5.04	2.64
3	6.73	2.17	8.60	2.53	7.99	2.00	6.43	2.77	5.36	2.66	4.83	2.97
4	14.17	2.37	18.28	2.51	14.17	2.87	17.58	2.23	12.76	3.01	14.34	2.93
5	9.18	1.94	10.25	1.57	9.45	2.04	9.11	2.14	5.27	1.77	6.38	1.77
6	10.43	1.66	9.25	1.79	10.78	1.89	9.89	2.02	6.27	1.50	6.08	1.73
7	6.87	2.02	6.84	1.85	6.36	1.72	6.33	1.95	4.51	2.02	3.13	2.09
8	8.82	1.44	9.84	2.21	7.98	1.86	9.84	1.65	5.87	1.97	6.01	1.79
9	8.04	2.04	9.56	1.39	7.43	1.65	11.95	1.83	3.82	2.17	6.34	2.39
10	10.56	2.28	14.61	2.50	12.75	1.77	13.40	1.81	9.61	1.98	9.18	1.94
11	8.20	1.62	8.20	1.03	8.65	1.34	9.20	1.45	5.41	1.55	7.03	1.69
12	11.01	2.14	13.87	2.43	12.46	1.78	18.31	2.40	6.90	1.93	7.59	2.62
Average	8.86	2.00	10.56	2.06	9.33	2.01	10.44	2.08	6.30	2.08	6.73	2.21

'Nipponbare' (RGAP 7) as reference genome

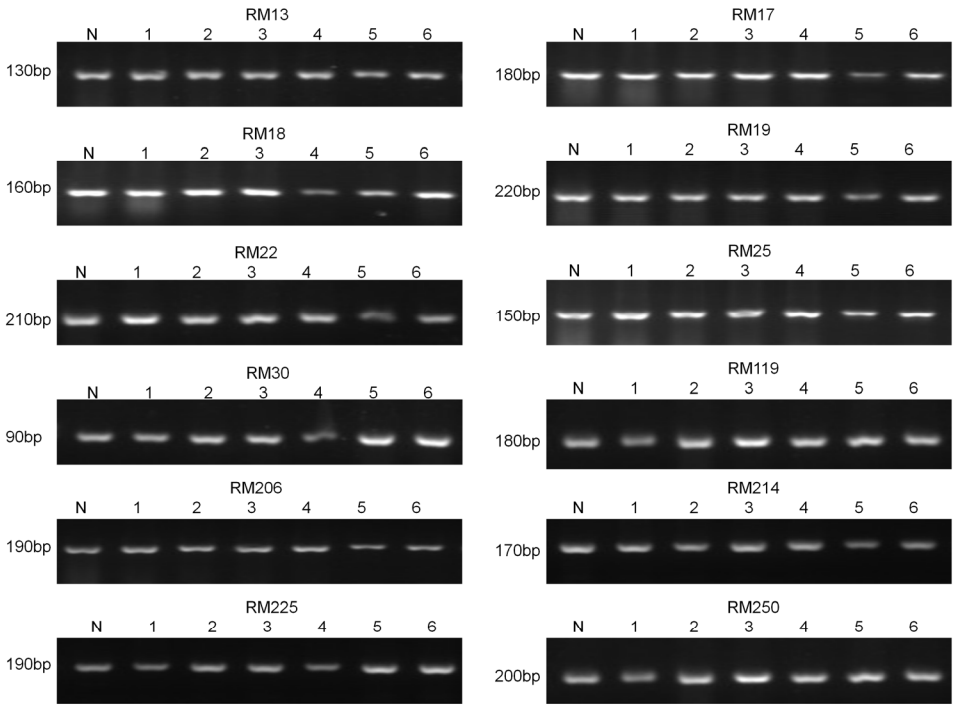

Fig. S1 Genotyping of the selected six P_2M_2 plants and Nipponbare with 12 representative SSR markers
N stands for Nipponbare; Lanes 1–6 represent 165-MS, 165-NP, 246-MS, 246-NP, 389-MS and 389-NP plants, respectively