Development and verification of SSR markers from drought stress-responsive miRNAs in common wild rice

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PREMISE: Dongxiang wild rice (*Oryza rufipogon* Griff., DXWR) is the northernmost common wild rice found in the world, which possesses abundant elite genetic resources. We developed a set of drought stress-responsive microRNA (miRNA)-based single sequence repeat (SSR) markers for DXWR, which will help breed drought stress-resistant rice varieties.

METHODS AND RESULTS: Ninety-nine SSR markers were developed from the drought stress-responsive miRNAs of DXWR. The SSR loci were distributed in all 12 rice chromosomes and most were in chromosomes 2 and 6, with di- and trinucleotides being the most abundant repeat motifs. Nine out of ten synthesized SSR markers were displayed high levels of genetic diversity in the genomes of DXWR and 41 modern rice varieties worldwide. The number of alleles per locus ranged from 2 to 6, and the observed and expected heterozygosity ranged from 0.000 to 0.024 and 0.461 to 0.738, respectively.

CONCLUSIONS: These SSR markers developed from drought stress-responsive miRNAs in DXWR could be additional tools for elite genes mapping and useful for drought stress-resistant rice breeding.

KEY WORDS common wild rice; drought stress-responsive microRNA; molecular marker; single sequence repeat (SSR).

Rice (*Oryza sativa* L.) is the staple food for half of the world's population and it has shaped billions of people's diets, economies, and cultures (Zhu et al., 2017). As the world population grows constantly, so does the demand for rice (Feng et al., 2021). However, securing rice production has become a great challenge because it is usually affected by various biotic and abiotic stresses (Liu et al., 2019). It has been estimated that rice production may decline by 3.8% in the Asian region in 21 century due to various stresses (Matthews et al., 1997). Among them, drought stress is one of the biggest factors seriously affecting rice growth and yield, because rice is a semi-aquatic plant (Berahim et al., 2021). Rice plants need more water during their whole life than other cereal crops, such as maize and wheat. Normally, early drought stress results in delayed transplantation, affecting seed germination and seedling growth, finally ending with harvest failure. During the rice reproductive stage, drought stress causes grain sterility and poor grain filling. Therefore, identification of drought stress-resistant gene resources and the subsequent development of drought stress-resistant rice varieties are of great importance for rice production.

Common wild rice (*Oryza rufipogon* Griff.) is the ancestor of modern rice varieties and possesses many valuable gene resources that have been lost during the process of domestication (Shishido et al., 2019). Dongxiang wild rice (DXWR) is the northernmost (28°14' N) common wild rice in the world and has strong drought stress resistance (Zhang et al., 2016). Therefore, DXWR is an elite germplasm resource for the development and breeding of new drought stress-resistant rice varieties. MicroRNAs (miRNAs) are a class of non-coding RNAs (approximately 21 bp long)

that can regulate gene expression at the post-transcriptional level in plants (Zhang et al., 2014). A considerable number of studies have shown that miRNAs play vital roles in drought stress resistance in many plant species, including sugarcane (Gentile et al., 2015), soybean (Li et al., 2016), wheat (Akdogan et al., 2016), rice (Nadarajah and Kumar, 2019), *Arabidopsis* (Niu et al., 2019), and tomato (Zhou et al., 2020). In our previous study, we found that miRNAs also play crucial roles in response to drought stress in DXWR, and 33 conserved miRNAs and 67 novel miRNAs were detected to be significantly affected by drought stress (Zhang et al., 2016; Zhang et al., 2018).

Developing drought stress-resistant plants with the aid of gene marker-assisted breeding has provided great benefits for conventional breeding (Luo et al., 2019). Simple sequence repeats (SSRs), also called microsatellites, are DNA fragments with 1-6 nucleotide motifs repeated a variable number of times. SSRs are ideal genetic markers because they have many advantages, such as genetic co-dominance, abundance, rich polymorphism, high reliability, and dense genome-wide coverage (Li et al., 2019). Consequently, SSR markers are considered to be powerful tools for evaluation of genetic diversity, germplasm resources identification, and genetic mapping. To date, some studies have developed SSR markers based on miRNAs in plants. For example, Mondal and Ganie (2014) developed a set of SSR markers from 130 members of salt stress-responsive miRNAs in rice. Sihag et al. (2021) developed 70 miRNA-based SSR markers using a set of 20 terminal heat stress-tolerant and heat stress-susceptible wheat genotypes. We recently developed the lncRNA-derived SSR markers. However, to our knowledge, there are few studies on the development of

miRNA-based SSR markers in common wild rice, which has strong resistance to various abiotic stresses. Therefore, the present study was aimed at the development of a set of additional promising SSR markers from drought stress-responsive miRNAs for the elite genes mapping and molecular breeding programs of DXWR.

METHODS AND RESULTS

Previously, we identified 100 drought stress-responsive miRNAs (33 conserved and 67 novel) in DXWR and obtained 99 corresponding pre-miRNA sequences through high-throughput sequencing approaches, because two conserved miRNAs (miR2871a-3p and miR2871a-5p) shared the same pre-miRNA sequences. In this study, the 99 pre-miRNA sequences were blasted with the Nipponbare MSU v7 reference genome (<http://rice.uga.edu/>) to obtain their flanking sequences, which included 500 bp upstream and 500 bp downstream regions of the pre-miRNA sequences. Finally, we obtained 99 sequences that contain 100 drought stress-responsive miRNAs and their corresponding flanking sequences. Then, the 99 sequences were screened by the SSRHunter software to find SSR loci with a 4 bp motif maximum and three repeat minimum (Li and Wan, 2005). A total of 673 miRNA-based SSRs were obtained in all sequences. Then, primer pairs of each of the SSR-containing sequences were designed using the program Primer3 Input (version 0.4.0) (<https://bioinfo.ut.ee/primer3-0.4.0/>). The parameters were set using the following criteria: (1) optimum primer length of 18-23 bp, (2) GC content of 40%-60%, (3) annealing temperature between 50°C and 60°C (55°C optimum), and (4) expected amplicon size of 100-300 bp; the rest of the parameters were set as defaults.

Finally, we developed 99 primer pairs for the 100 drought stress-responsive miRNAs of DXWR. These markers were distributed in all of the 12 chromosomes and were most abundant in chromosomes 2 and 6 (11), and chromosomes 1, 4 and 8 (10) (Table 1). Among them, dinucleotide (56, 56.6%) and trinucleotide (36, 36.4%) repeat motifs were the most abundant (Table 1). Meanwhile, the AT was the most abundant (21.4%) type of dinucleotide repeats, followed by AG (14.3%) and GA (12.5%) types (Table 1).

To investigate whether these miRNA-based SSR markers developed for DXWR could be applied to the modern rice varieties, we synthesized 10 pairs of primers and then amplified fragments using the genomic DNA of DXWR and 41 modern rice varieties worldwide. Among these rice varieties, 18 belonged to *indica*, and the remaining 23 belonged to *japonica* (Appendix 1). Fresh leaf tissues were used for total genomic DNA extraction using a Rapid Plant Genomic DNA Isolation Kit (Sangon Biotech, Shanghai, China). PCR amplifications were carried out in a 10 μ L reaction system containing 1 μ L (200 ng/ μ L) genomic DNA, 1 μ L (0.01 nmol/ μ L) primers, 3 μ L ddH₂O, and 5 μ L 2 $\times$ Fast Taq Premix (Tolo Biotech Co., Ltd, China). The PCR reaction conditions were as follows: initial denaturation at 95°C for 5 min; 35 cycles of denaturation at 95°C for 30 s, annealing at 56°C for 30 s, extension at 72°C for 30 s, and 5 min at 72°C for the final extension. The PCR products were separated on 6% denaturing polyacrylamide gels in 0.5 $\times$ TBE buffer and the bands were visualized via silver nitrate staining. All of the 10 primer pairs were amplified successfully for DXWR and the modern rice varieties. The electrophoresis results

suggested that nine out of the 10 primer pairs were polymorphic among DXWR and the modern rice accessions. The nine polymorphic SSR markers were analyzed using GenAlEx 6.5 software for the number of alleles per locus, observed heterozygosity, and expected heterozygosity (Peakall and Smouse, 2012). Software Arlequin ver 3.5 (Excoffier and Heidi, 2010) was used to determine Hardy-Weinberg equilibrium (HWE). Among the nine polymorphic loci, the number of alleles per locus ranged from two to six with a mean of 4.444 (Table 2). The levels of observed and expected heterozygosity were 0.000-0.024 and 0.461-0.738, with averages of 0.003 and 0.625, respectively (Table 2). Among the nine polymorphic primer pairs, seven pairs were able to detect levels of expected heterozygosity above 0.5, indicating a high level of polymorphism among these rice accessions (Table 2).

CONCLUSIONS

In this study, we developed a set of SSR markers for the drought stress-responsive miRNAs in DXWR and verified the validity and applicability of these valuable markers in rice varieties grown worldwide. This is the first report on the development and verification of miRNA-based markers for abiotic stress resistance in DXWR, which lays the foundation for future basic and applied research to make full use of elite miRNA genes from this valuable wild rice germplasm resources.

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AUTHOR CONTRIBUTIONS

F.-T.Z., and J.-K.X. conceived and designed the experiments. W.-L.Y., and G.-M.D. collected the samples. Y.C., and W.-L.Y., designed the primers. Y.C., and Y.-H.C. performed the molecular laboratory work. Y.C. analyzed the data. Y.C., Y.-W.F., and F.-T.Z. drafted the manuscript.

DATA AVAILABILITY STATEMENT

All of the sequences of microsatellite loci had been deposited into the National Center for Biotechnology Information (NCBI)'s GenBank database with the accession number of OK378244 - OK378342.

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APPENDIX 1. Worldwide rice cultivars used for verification of the developed SSR markers in this study.

	Name	Origin	Subspecies
1	Xieqingzao B	China	<i>indica</i>
2	Ai-Chiao-Hong	China	<i>indica</i>
3	Ming Hui	China	<i>indica</i>
4	ZHE 733	China	<i>indica</i>
5	Zhenshan 2	China	<i>indica</i>
6	Aijiaonante	China	<i>indica</i>
7	Hon Chim	Hongkong, China	<i>indica</i>
8	Binulawan	Philippines	<i>indica</i>
9	IR64	Philippines	<i>indica</i>
10	Byakkoku Y 5006 Seln	Australia	<i>indica</i>
11	Champa Tong 54	Thailand	<i>indica</i>
12	Chau	Vietnam	<i>indica</i>
13	Darmali	Nepal	<i>indica</i>
14	Oryzica Llanos 5	Colombia	<i>indica</i>
15	Criollo La Fria	Venezuela	<i>indica</i>
16	Radin Ebos 33	Malaysia	<i>indica</i>
17	Tchibanga	Gabon	<i>indica</i>
18	SL 22-613	Sierra Leone	<i>indica</i>
19	Zhonghua 11	China	<i>japonica</i>
20	Shangyu 394	China	<i>japonica</i>
21	Sung Liao 2	China	<i>japonica</i>
22	Caawa/Fortuna 6-103-15	Taiwan, China	<i>japonica</i>
23	Aichi Asahi	Japan	<i>japonica</i>
24	Jouiku 393G	Japan	<i>japonica</i>
25	Kibi	Japan	<i>japonica</i>
26	Nipponbare	Japan	<i>japonica</i>
27	Hiderisirazu	Japan	<i>japonica</i>
28	Hatsunishiki	Japan	<i>japonica</i>
29	Edomen Scented	Japan	<i>japonica</i>
30	Rikuto Norin 21	Japan	<i>japonica</i>
31	Shirogane	Japan	<i>japonica</i>
32	Kiuki No. 46	Japan	<i>japonica</i>
33	Arias	Indonesia	<i>japonica</i>
34	Geumobyeo	Korea, South	<i>japonica</i>
35	Lemont	United States	<i>japonica</i>
36	Moroberekan	Guinea	<i>japonica</i>
37	Ostiglia	Italy	<i>japonica</i>
38	Bombon	Spain	<i>japonica</i>
39	Coppocina	Bulgaria	<i>japonica</i>
40	WC 4443	Bolivia	<i>japonica</i>
41	Tropical Rice	Ecuador	<i>japonica</i>

TABLE 1. Characteristics of SSR markers derived from the drought stress-responsive miRNAs of DXWR.

Locus	miRNA	Motif repeat	Primer-F (5'-3')	Primer-R (5'-3')	Expected amplicon size (bp)	Chr.	GenBank accession no.
SSR1	miR1846d	(CGC) ₅	ATCTCGCGGTAGGTTTCGATC	CAGCTGGAGTAGGGCGAG	159	1	OK378249
SSR2	miR319b	(AG) ₁₃	CCTGAAAGCCACATCACAAAGT	GTACTTTCCTTAGCCCGGGG	170	1	OK378260
SSR3	miR408	(CT) ₁₂	TGGTGTTGTTGTTGCTCCCT	GCAACAGCCCTTGAAGTGTC	205	1	OK378261
SSR4	miR5076	(AT) ₄	GAAGCTCATGTTGTTGTTTGCA	TGGAACAGGATCAGCTCTACC	191	1	OK378266
SSR5	miR7692	(CG) ₄	CTACTCGCAACCAATCCAGC	CTCTCCCAGAAGAAGTCCGG	227	1	OK378273
SSR6	miR818a	(AAT) ₃	AAATGACAAGTTGTGCCTAAAGT	GCTGTTTGTATGGGTCCTTCG	203	1	OK378275
SSR7	novel-miR100	(CA) ₅	AAAACCCTCCCTCCACTAGC	GCAGATCAGGACGAGAAGGA	187	1	OK378277
SSR8	novel-miR24	(CA) ₃	GAAGCATGACCTATTGACAGCA	AAGGGCATAGTTGAGGGACC	243	1	OK378297
SSR9	novel-miR26	(GGA) ₅	CTGCACCGTTGTACCGATG	TCTTCCCTACCGCAGTGAG	159	1	OK378298
SSR10	novel-miR99	(TGCC) ₃	GTTGATCGTAGTGGCTCTGG	CGGCGACACATATAGAACCC	165	1	OK378342
SSR11	miR166h	(AC) ₅	AGCCAGACAACAAACCCCTT	CTGGCTCGAGGTATCCACAT	169	2	OK378246
SSR12	miR172d	(AG) ₆	GAACCACATACGCCAAACAGA	TCTTCCTCCTCCACCTCCTC	197	2	OK378248
SSR13	miR1861c	(AT) ₄	GACCGAGCCTGTGAATATGC	CAAAGATTGTGTACGCGGCT	246	2	OK378251
SSR14	miR2863b	(CAC) ₃	CGTCAGGCCCAATTCATGAG	ATCAACTCCACCTCCACCTC	217	2	OK378256
SSR15	miR444d	(TG) ₄	ACACTCTGATGCACTTGTGC	GCCTCCCTCTGCCAAATCTA	214	2	OK378263
SSR16	novel-miR103	(GGC) ₅	ACCCTAACCCCTAACCCCTCCC	CGCGGTAGGGCAGGTATG	161	2	OK378278
SSR17	novel-miR105	(TA) ₄	ACTTCCATCCAAACCAGGGG	AGCCTCAAATTCAACCCGTA	229	2	OK378279
SSR18	novel-miR29	(CGG) ₆	CCCTCTCTCTCCCCGT	TTTTCGGTCGTACATCTAA	221	2	OK378299
SSR19	novel-miR33	(AG) ₃	AAAGCTCTGCCCTTCCATCT	CTCTACGCCTAGGACACCAG	159	2	OK378301
SSR20	novel-miR34	(ATA) ₃	TCAGAAGAAAAGAACACCACCT	TCTATATGTATGTGGGCAATGCT	110	2	OK378302
SSR21	novel-miR36	(GCA) ₃	GAAGTGATGGTAAGAAGGTACGA	TGCTCAAATTGTGCGAGGTGG	197	2	OK378303

SSR22	novel-miR108	(AT) ₄	CCTGATACTTGGTAGAAATCGCG	TCAGAAACCAGTATAAGGACCTG	100	3	OK378280
SSR23	novel-miR110	(AAT) ₇	TTTGAAATGGAGGGAGTACTTGA	ACCACGAAATTTTCTGTACAA	158	3	OK378281
SSR24	novel-miR38	(CA) ₆	TCTTGCTGCCGAGTCAATTC	ATTGTCTCGCTTCTCTGGGT	162	3	OK378304
SSR25	novel-miR40	(AC) ₄	TTTGACCCTGCAACCGGAAT	CGTTATGCTGATGGTGAGTCC	220	3	OK378306
SSR26	novel-miR41	(GT) ₅	CACTACTCCCTTCTGCTGCT	TATCCCCATGTGTTGCCAAC	185	3	OK378307
SSR27	novel-miR42	(GA) ₅	CCAGCTACAGTGTTTTGCCA	GCTCTCTATCTCCACCACGG	172	3	OK378308
SSR28	miR1861d	(CT) ₃	ACTCGTGCGGTAAAATTGC	AGCGCAACGGAATCTTCAAA	204	4	OK378252
SSR29	miR2871b	(TCTT) ₃	GTGCAACGATATCCATGGCT	TTTTTCGACTAATGGAGGACA	179	4	OK378259
SSR30	novel-miR111	(CGG) ₅	GACGTGGGAGAGGATAAAGTCT	CGTGCAATTGATGGGTCTGG	230	4	OK378282
SSR31	novel-miR114	(AT) ₃	TGGATGTTCTATGCCAGGTG	AATCGGAAGTGTCAGGAGGC	176	4	OK378283
SSR32	novel-miR115	(GA) ₆	TTCCCGAAGCAGTGAAGAGT	CGGTGGTTTTTGGTCTACGTG	158	4	OK378284
SSR33	novel-miR3	(AG) ₃	ACTAGGGTTCAATGAATGGAGAA	TGCTTTTCGCTATCAACCAGA	181	4	OK378300
SSR34	novel-miR44	(TTGG) ₃	GATGACTCGCGATTGGTTGG	ATCACACAAGGCATGCTCAC	206	4	OK378309
SSR35	novel-miR45	(CG) ₃	GTCATGATGGCCAGCTCCT	CGCAACGTGGAGTAGATGGA	165	4	OK378310
SSR36	novel-miR46	(GGC) ₄	CAACAAAGGGATCTGGCGTC	TGAAGAGGACGATGACAGGG	158	4	OK378311
SSR37	novel-miR48	(CA) ₂₂	CACGGACTCCCACCATTTTG	TGGAGAAGAAGGCCATGGAC	201	4	OK378312
SSR38	miR164c	(CT) ₁₁	TGCAAACCCACTCCAACACT	CGAGTGTGCATGCAATATGGC	189	5	OK378245
SSR39	miR2871a	(AGGG) ₃	CGTAGTGGAGCGCTCTTAGA	TCGATGGATTTGAGCCTTTGAC	238	5	OK378258
SSR40	novel-miR116	(GA) ₇	CGTAGTAGTGGTGCCCGTAC	AAACACAAACCACCACCACC	207	5	OK378285
SSR41	novel-miR50	(ATAA) ₃	AGTGCCGAAAGAATGCCTC	TGTTAACCGAAGAGATTGAACCT	234	5	OK378314
SSR42	novel-miR51	(CGC) ₄	CCTCCCTCTCTTTCAGGTCG	GAGGAGGAGTCACTGCCAG	172	5	OK378315
SSR43	novel-miR52	(TTG) ₄	AGCGAGAACAAGTGCGTTTC	AGTGGAGCTTCTTAACCCATCT	212	5	OK378316
SSR44	miR1861h	(AT) ₄	CAAAGATTGTGTACGCGGCT	CGAGCCCGTGAATATGCAAA	243	6	OK378253
SSR45	miR5072	(CCG) ₃	ATTTAAGGGACAAGGCCGGT	AGGATAACTACCCGTCTCGTG	211	6	OK378265
SSR46	miR5828	(TTA) ₃	CCCAATTGCTTGATTACCG	GCTGATTTTGACAATCCAGCG	184	6	OK378269
SSR47	miR6250	(CGC) ₄	CTAACCCACTGTCCCCACTC	TGGTGATATATTGGTGCCCC	203	6	OK378270

SSR48	novel-miR122	(GCA) ₃	CAGTAGGTTCCATTTCGCTGC	TGTGTGGCAGTGTGGGTATT	161	6	OK378287
SSR49	novel-miR125	(GT) ₃	GCTGACAAGATGGTTCTCGC	CGGGATGTAATGTGCATGGT	224	6	OK378288
SSR50	novel-miR54	(CGG) ₃	GAACCCACTCCTCCTCCTC	GGAGGAGGGGAAAGGCTG	178	6	OK378317
SSR51	novel-miR55	(AG) ₉	TCCACTCTAGCTATTCTCGGA	AGACTCCTTTTCTTCCCTAGGG	229	6	OK378318
SSR52	novel-miR57	(AAG) ₃	AGTAGTAACCCGTTTCATCCAAA	TGGCTACTTCTCCACCTACC	183	6	OK378319
SSR53	novel-miR60	(AC) ₄	CCTCGCAATGCATCTCAGAG	ATGCCTAACCTTTTCTCCGT	155	6	OK378321
SSR54	novel-miR61	(TCC) ₃	AAATTCTCCTCTCCTGCCCCG	CTAAGTGCCAACGCCCTC	166	6	OK378322
SSR55	miR6253	(AC) ₃	AAGCTTAACCCAACCTGCCCA	CCGTTGTCATGTTACCCTGC	121	7	OK378271
SSR56	miR6255	(GA) ₃	ACCAATCACTCATCTGCAACG	TCGTCTTCCCGTGCATTAGT	209	7	OK378272
SSR57	novel-miR129	(TG) ₃	GGATCAGTGAATGTGCATTGC	CATGCATGAACTCCCCACAC	203	7	OK378289
SSR58	novel-miR130	(TA) ₄	CTAGCAGGGAGTAGTGACG	ATAAGCACATAGGGCAGGCA	203	7	OK378290
SSR59	novel-miR66	(AAT) ₃	ACATTGCTGCTCTGTAGGGT	TGCAACAATTGATCGCGTTTG	152	7	OK378323
SSR60	novel-miR68	(AGG) ₃	GGATTAGGATGCTCACCGGT	TGACCCATACATAGGCAGGC	194	7	OK378324
SSR61	novel-miR69	(CGG) ₆	TGGTTAGTTGGAGCCTTGGA	GGAGAGGGAGGAGAGAGAGT	153	7	OK378325
SSR62	novel-miR70	(GGT) ₃	GAGTTGGAGGGGAGGATGAA	GGAGCGTGGAGTTGGAAGA	150	7	OK378327
SSR63	miR160f	(ACG) ₃	GATGGGATTAACGCTGCCTG	AAGTGCACCATACCTGAGCT	168	8	OK378244
SSR64	miR1861j	(AT) ₁₁	ATAGCGTGGTCCGTCTGA	GTAGCCGCATACACAATCTT	199	8	OK378254
SSR65	miR444a	(AG) ₃	CTGAAGACACGACCGAAACC	CCCAATTCTTGTGCCCCTC	196	8	OK378262
SSR66	miR444e	(ATT) ₃	ACATAAGCTCAGCCATTTCGC	ACTTGCGGCAAAATCATTGG	207	8	OK378264
SSR67	novel-miR133	(TG) ₃	CTTCAGTGTCTGCTACGGTTG	AAAACCATACAGCTGCACGG	201	8	OK378291
SSR68	novel-miR134	(AT) ₄	GCTCTCTTCACAGCCTCTTC	ACAATGGAGCCTATCATCATGC	246	8	OK378292
SSR69	novel-miR135	(AAT) ₃	TGCTACCTCCGTCCCAAATT	TTTTGACGGACGATGGAAGC	221	8	OK378293
SSR70	novel-miR71	(CCG) ₅	CGCGGTAGGGCAGGTATG	TCAAAACCCTAACCCCTCCCC	156	8	OK378328
SSR71	novel-miR73	(AGAA) ₄	ACACGAGCACTAGAGACTGG	AGGTATCCCATCCCATCCCA	155	8	OK378329
SSR72	novel-miR74	(AG) ₃	CCTGTAGTGATTTGATCCATGGA	AATGCCATGTCAGGAAACGT	244	8	OK378330
SSR73	miR1846e	(GA) ₄	GCGGGAAGAAGAGAATTGGG	GTACCGCGCTCTGAAACTA	186	9	OK378250

SSR74	novel-miR137	(CT) ₆	GTCATCGTCGTCTCCCATCT	CCGTTGCAGTAGTGAAGTGA	172	9	OK378294
SSR75	novel-miR79	(TA) ₃	TCGTAACCCAAGAAGCACCC	AGGGACTCGGGTTAAATTCCA	172	9	OK378331
SSR76	novel-miR82	(CCA) ₄	CTATCTCTGGTCGTCGCCC	GTGGTCACCGAGGAGAAGAC	250	9	OK378332
SSR77	novel-miR84	(GA) ₁₂	AGCAACAGATGGGTGCAAAA	AGGTTGAGATCTGGCAATGTG	175	9	OK378333
SSR78	novel-miR85	(AT) ₅	TTTGAGCAATGCCTTACCGT	CCTGCAGGCAAATTTTGAATATC	190	9	OK378334
SSR79	novel-miR2	(TC) ₄	TGTACGCACTAGACTGGACG	CAACGAGGTGGAGGTCAGAC	154	10	OK378296
SSR80	novel-miR4	(GA) ₅	GGGAGAAGGCTGAGGAGTAG	CTGCGCACAAAACGAGAAAC	150	10	OK378305
SSR81	novel-miR5	(TA) ₈	AGCCACTCCTAGAATTGCAGT	TCTTCCTCCATCGCTTCAGG	250	10	OK378313
SSR82	novel-miR6	(TA) ₄	TCACATGCAAAACACCTCCA	CGCTCTCGGTCTCTTTGTCT	190	10	OK378320
SSR83	novel-miR7	(AT) ₃	TCCGGATGCCACCTTATCTC	CGGTTTCGGGTTGTTTCGTT	230	10	OK378326
SSR84	miR810a	(TA) ₄	CGCATGCTTAACAAACTGTGG	CGCATGCTTAAATAACTAACGGC	233	11	OK378274
SSR85	novel-miR10	(CA) ₃	CAGACTTTTGGTGCTGTGGA	TGGTTGACAACATGTGCCAT	203	11	OK378276
SSR86	novel-miR12	(AT) ₄	GCAGCTCCCTCACCTGATAG	GTTCAAAGGTGTGCTCTGCT	236	11	OK378286
SSR87	novel-miR88	(GGC) ₆	CATGCGGTGACATCAGAGTG	TTATAGATCAGCGCGCTTGG	166	11	OK378335
SSR88	novel-miR89	(GTT) ₃	CCCATTGCCATCTCAACAGG	ATTAGTCCCCTGGTGCTGAG	183	11	OK378336
SSR89	novel-miR93	(AGG) ₃	CACCACCAACCTCGTCCTAG	TGACGAAACCCTCATCACCA	171	11	OK378337
SSR90	novel-miR94	(CT) ₃	AGGTGCTAGATTGCTGGTAGT	GAATGGAAGAGCGGCACTG	159	11	OK378338
SSR91	miR167h	(TC) ₆	ACAAGGCGAGATGAGATGACA	TCATGGAGGAGAGGAGGAAGA	151	12	OK378247
SSR92	miR1861n	(AG) ₄	AAAAGATTGTGTACGCGGCT	TGCTCCCGAATGATCTCTCT	215	12	OK378255
SSR93	miR2864	(AT) ₄	GGGAGGGCTGCAGATAATCT	TTCAGTTTCAGCAGCGTACC	231	12	OK378257
SSR94	miR5505	(AT) ₄	GATAGATGGATGGGGCCTGG	GGCTACTGTCCCATGCAAAG	157	12	OK378267
SSR95	miR5805	(GCC) ₄	CCCCAAACGCTAGCTATCCT	TCAAGTCCATGTCAGCCCAT	221	12	OK378268
SSR96	novel-miR17	(GTA) ₃	TGTCCTCCCTAATGCTGGTCA	TAGTGGGAGGGACATGGAGA	152	12	OK378295
SSR97	novel-miR96	(GGC) ₃	GCCAATCTTCAAGCCAGAGG	TACGAGTCATACCCAGCGG	206	12	OK378339
SSR98	novel-miR97	(ACC) ₃	CCTACAGGCCTAGTGTGCAC	TGTGATTGTAGCATGCATTGG	176	12	OK378340
SSR99	novel-miR98	(ATTT) ₃	CTGATTACTGCTGCCTTCACA	TCAAAACCACGAACCATGCC	239	12	OK378341

TABLE 2. Genetic properties of the 9 polymorphic SSR markers among DXWR and 41 modern rice varieties.

Locus	miRNA	A	H_o	H_e^a
SSR2	miR319b	2.000	0.000	0.672**
SSR3	miR408	5.000	0.000	0.461**
SSR23	novel-miR110	5.000	0.000	0.738**
SSR37	novel-miR48	6.000	0.024	0.683**
SSR38	miR164c	4.000	0.000	0.673**
SSR51	novel-miR55	4.000	0.000	0.622**
SSR64	miR1861j	5.000	0.000	0.498**
SSR77	novel-miR84	6.000	0.000	0.622**
SSR81	novel-miR5	3.000	0.000	0.656**
Mean	-	4.444	0.003	0.625

Note: A = number of alleles per locus; H_o = observed heterozygosity; H_e = expected heterozygosity.

^a Deviations from Hardy-Weinberg equilibrium: ** $P < 0.01$.