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Short Communication

Application of SV markers developed from Dongxiang common wild rice in analysis of cultivated rice

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Abstract

Dongxiang common wild rice (*Oryza rufipogon* Griff., DXWR) is an important genetic resource for the improvement of cultivated rice. For the past three decades, great achievements have been made in the field of molecular marker development. Although structural variations (SVs) had been studied between DXWR and Nipponbare (*Oryza sativa* L. ssp. *japonica*), the development and application of SV markers in DXWR has not been reported. In this study, based on the genome-wide SV loci, we developed and synthesized a total of 195 SV markers that were evenly distributed across the 12 rice chromosomes. Then, these markers were tested for their stabilities and polymorphisms. Of these 195 markers, 147 (75.4%) were successfully amplified and displayed abundant polymorphisms between DXWR and Nipponbare. Meanwhile, through the genotyping of 20 rice varieties from 13 countries and areas, we concluded that these SV markers have a wide application prospect in the analysis of cultivated rice. Therefore, these molecular markers greatly enrich the number of markers available for DXWR, which will facilitate genomic research and molecular breeding for this important and endangered germplasm resource.

Keywords: genetic resource, germplasm utilization, molecular marker, rice wild relatives, structural variation

Introduction

Dongxiang wild rice (*Oryza rufipogon* Griff., DXWR) is a common wild rice, with its northernmost habitat located at 28°14′N latitude in Dongxiang county, Jiangxi province, China (Mao *et al.*, 2015). DXWR has abundant genetic diversity and many valuable agronomic traits (Zhang *et al.*, 2006). However, the introgression of favourable genes from DXWR to cultivated rice through traditional breeding could be associated with complications due to the substantial amount of linkage drag from DXWR.

Molecular marker assisted selection (MAS) can greatly improve the efficiency of rice breeding through precise transfer of genomic regions of interest (Kaur *et al.*, 2015). The most frequently used molecular markers include simple sequence repeat (SSR), insertion-deletion (InDel), and single nucleotide polymorphism (SNP) markers. These markers reflect specific sites in the genome with polymorphisms between individuals from one to hundreds of nucleotide bases (Hayashi *et al.*, 2004; Yadav *et al.*, 2017). Additionally, with the rapid development of high-throughput sequencing, structural variation (SV) is receiving increasing attention for molecular marker development (Davey *et al.*, 2011). SV markers are genomic structural variation markers that represent deletions, insertions, duplications, inversions, and translocations of DNA segments

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in the genome (Xu *et al.*, 2011). SV markers can not only detect structural variations of up to several thousand nucleotide bases, but also have the advantages of co-dominance, repeatability, stability, and simplicity of use (Wang *et al.*, 2015). Although SVs had been studied in DXWR (Zhang *et al.*, 2016), the development of SV markers in this valuable genetic resource has not been reported. Therefore, the main objectives of this study were to: (i) develop a set of genome-wide SV markers for DXWR; and (ii) test their stabilities and polymorphisms in DXWR and cultivated rice.

Experimental

Whole-genome sequencing, reads filtering, and clean reads mapping to the reference genome were performed as previously (Zhang *et al.*, 2015). Based on the paired-end sequence method, one read should be mapped to the forward strand, and the other to the reverse strand. The distance between the two locations should be in accordance with insert size (Eren *et al.*, 2013). Therefore, the alignment of the two paired reads to the genome is regarded to be of normal direction and appropriate distance. If the direction or distance is abnormal, then the region might has SVs. Abnormal pair-end alignments were further analysed by clustering and compared with previously defined SVs (Wang *et al.*, 2015). The SVs were detected using the SOAPsv program as described by Li *et al.* (2011), with support from at least three abnormal paired-end reads.

Primer pairs were designed using Primer3 (v. 0.4.0) software based on the region including flanking sequences on both sides of SV loci (Untergasser *et al.*, 2012). Genomic DNA was extracted from fresh leaves using the CTAB method (Abdel-Latif and Osman, 2017). Polymerase chain reaction (PCR) amplification reactions were performed in a mixture, including 1 µl DNA (200 ng/µl), 0.2 µl dNTP (10 mmol/l), 0.2 µl primers (10 µmol/µl), 1.5 µl 10 × buffer (with Mg^{2+}), 0.2 µl Taq DNA polymerase (5 U/µl), and 11.9 µl ddH_2O. The reaction parameters were set as follows: initial denaturation at 95°C for 5 min, followed by 35 cycles at 95°C for 30 s, 56°C for 30 s, 72°C for 2 min, and a 10 min final extension step at 72°C. PCR products were separated on agarose gels and stained with GelRed nucleic acid staining dye. A total of 21 accessions of rice germplasm, including DXWR and 20 rice cultivars (online Supplementary Table S1), were collected in our laboratory.

Discussion

In our previous study, SVs between DXWR and Nipponbare (*Oryza sativa* L. ssp. *japonica*) had been detected to investigate lost/acquired genes during rice domestication (Zhang *et al.*, 2016). However, the development of SV markers in DXWR has not been reported. To enrich the number of molecular markers available for DXWR, we detected SVs at a genome-wide level based on the results from the comparative genomics analysis. Totally, 17,893 SVs were detected between DXWR and Nipponbare using the SOAPsv program (Table 1). The genome-wide average SV density was one SV per 19.97 kb, whereas the density of SVs was varied among the 12 rice chromosomes (Table 1). The most abundant chromosomes for SVs were chromosomes 1, 12, 10, and 6, with one SV per 18.24, 18.67, 18.81, and 18.99 kb, respectively (Table 1). Of these, chromosomes 1, 6, and 10 also have a high density of InDels and SNPs (Zhang *et al.*, 2015, 2017), implying that these chromosomes had more abundant genetic variations than the other chromosomes.

Table 1. Densities of SVs on 12 chromosomes detected between Dongxiang common wild rice (DXWR) and Nipponbare genomes

Chr. ID	Nipponbare length (bp)	DXWR aligned length (bp)	Number of SVs	Average density of SVs[a]
1	43,270,923	41,887,727	2296	18.24
2	35,937,250	34,943,035	1538	22.72
3	36,413,819	35,125,571	1746	20.12
4	35,502,694	33,825,642	1712	19.76
5	29,958,434	29,110,085	1471	19.79
6	31,248,787	29,591,680	1558	18.99
7	29,697,621	27,397,024	1434	19.11
8	28,443,022	27,778,151	1127	24.65
9	23,012,720	22,166,476	1104	20.08
10	23,207,287	22,252,558	1183	18.81
11	29,021,106	27,100,318	1319	20.55
12	27,531,856	26,228,926	1405	18.67
Total	373,245,519	357,407,193	17,893	19.97

[a]Refers to a SV density in DXWR, and equals to aligned length (bp)/number of SVs/1000.

With the rapid development of high-throughput sequencing, SVs are receiving increasing attention for molecular marker development (Davey *et al.*, 2011). For example, Ren *et al.* (2012) constructed a high-resolution genetic map for watermelon with a total of 953 molecular markers, including 36 SV markers. Wang *et al.* (2015) developed 104 SV markers and constructed a linkage map for the basidiomycete *Volvariella volvacea*. In this study, we developed 195 SV markers for DXWR. These molecular markers were mostly derived from the type of insertion and evenly distributed across the 12 chromosomes (online Supplementary Table S2). To validate their stabilities and polymorphisms, we synthesized these molecular markers for PCR amplification. The results of agarose gel

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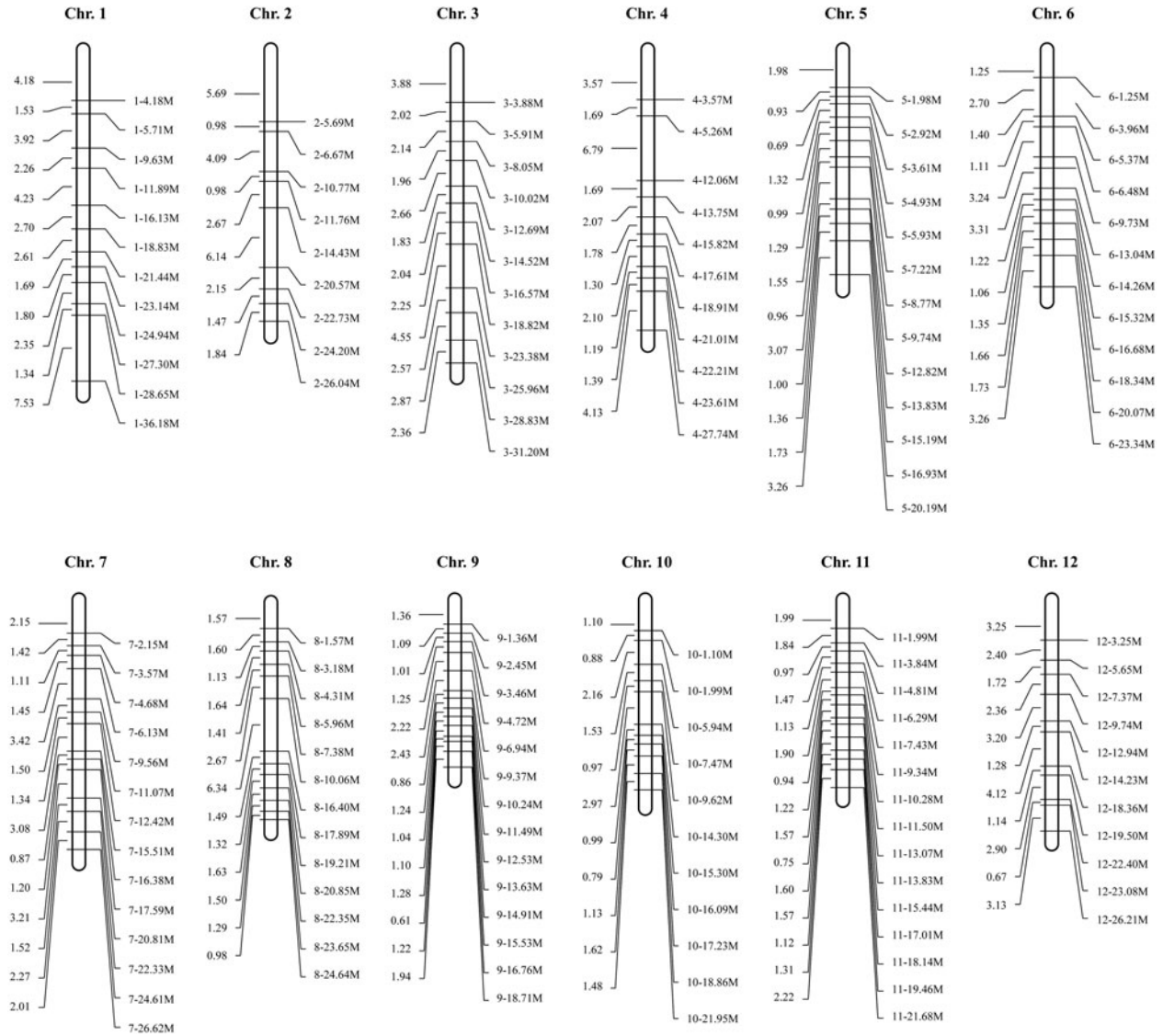

Fig. 1. Distribution of polymorphic SV markers on 12 chromosomes. The numbers to the left of each chromosome indicate the physical locations in Mb.

electrophoresis showed that 147 (75.4%) of the 195 markers can be successfully amplified and showed abundant polymorphisms between DXWR and Nipponbare (Fig. 1 and online Supplementary Table S3). Furthermore, to test these SV markers if they can be applied in the analysis of cultivated rice, we amplified the genome DNA fragments from 20 rice cultivars using the polymorphic SV markers. As a result, abundant polymorphisms were detected among these rice varieties (online Supplementary Fig. S1), which suggested that these polymorphic SV markers have a wide application prospect.

In conclusion, these SV markers greatly enrich the number of markers available for DXWR, which can serve as additional resources for assessing genetic diversity, constructing genetic maps, and identifying valuable genes from this important germplasm resource in the future.

Supplementary material

The supplementary material for this article can be found at https://doi.org/10.1017/S1479262119000145

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