**Background**

Single nucleotide polymorphisms (SNPs), generally defined as only a single base change in a DNA sequence among individuals [1], quickly became one of the most popular molecular markers in plant molecular genetics with the advantage of being abundant, ubiquitous in most organisms and highly amenable to high- and ultra-high-throughput automation [2, 3]. It also provided a powerful tool for marker-assisted breeding and quantitative trait locus (QTL) analysis and genome association analysis [3, 4]. The application of next-generation sequencing (NGS) technology has facilitated the identification of massive number of SNPs in various organisms through resequencing, including human [5], rice [6, 7, 8], maize [9, 10], soybean [11], Arabidopsis [12], etc. Genome-wide analysis revealed that SNPs were distributed unevenly on different genomic regions and fewer SNPs fell in some regions of higher conservation which are functional regions like coding sequences (CDSs) and regulatory elements [13, 14]. SNPs found in plant genomes may cause serious phenotype changes or apparent trait variations. For example, while studying the loss of seed-shattering habit in rice domestication, an SNP was found in *qSH1* gene responsible for this important phenotype change event.

Recent years have witnessed numerous studies identifying and analyzing SNPs through resequencing with NGS, and in addition to great effort put on studying SNPs in protein-coding genes, several investigations of miRNA-related SNPs were performed in Arabidopsis [15] as well as rice [16, 17], in which researchers focused on the changes SNPs may bring to the miRNA structure stability and target alteration as well as miRNA evolution.

MiRNAs are small endogenous non-coding RNAs originated from endogenous loci. The miRNA gene loci are transcribed into self-complementary primary RNAs (pri-miRNAs) that can form hairpin structures [18] in nucleus and then pri-miRNAs are excised to liberate precursor miRNAs (pre-miRNAs) by DCL1 [19]. Next, a duplex of about 21nt RNAs with 3’ overhangs is further produced from hairpin precursor, and after methylation on both 3’ nucleotides, the duplex would then be cleaved and loaded into AGO protein to form RNA-induced silencing complex (RISC) with a miRNA\* strand degraded [20]. The remaining strand is the so-called mature miRNA and is thought to guide RISC to target RNAs through complementarity to the miRNA binding site. MiRNAs are key regulators in process of plant growth and development and often target genes that are themselves regulators such as transcription factors. Studies have reported SNPs involved in the miRNA-mediated gene silencing caused distinct changes to agronomic traits. In rice, it was reported that one point mutation in the osa-miR156 binding site of OsSPL14 perturbed the outcome of osa-miR156-mediated silencing, thus resulted in an ideal plant with reduced tiller number, increasing lodging resistance and enhanced grain yield [21]. While in barley, SNPs perturbed the interaction between miR172 and its target gene HvAP2 and brought variations to the spike density of barley inflorescence [22].

In plants, miRNAs repress the mRNAs of their targets with high complementarity mainly through transcript cleavage [23], and this high complementarity requirement formed the basis of many bioinformatic softwares for miRNA target prediction, one of which is the web-based Plant Small RNA Target Analysis Server (psRNATarget) [24]. Besides in silico method, recent years, there are several methods developed to verify the true miRNA:target relationship, such as overexpression of miRNA or miRNA-resistant target, RNA ligase-mediated 5’-RACE, degradome sequencing, and etc. [25] Owing to the complexity of plant miRNA target recognition, bioinformatic methods may product miRNA targets that are not subjected to functionally relevant miRNA regulation[25], so how to filter the false-positives remain a headache.

Recent 3,000 rice genome project sequenced more than 3,000 rice cultivars and millions of genomic reads have been produced [26]. Huge number of SNPs were identified by aligning the sequence reads [27], including some rare tri- and tetra-allelic SNPs which represent the precious genotypes of the minority population of cultivars. The abundance of SNPs provides good opportunity for genome-wide identification and analysis into SNPs in miRNA-mediated silencing processes. Since SNPs can reflect the variations of genomes of different rice cultivars, and in turn help us uncover the variations in miRNA-mediated regulation between rice cultivars and possible effects of SNPs involved in this process on the phenotypes of cultivars. Based on the SNP data derived from 3,000 rice genome project, we studied the SNP distribution on both rice miRNAs deposited in miRBase [28] as well as identified miRNA targets and the effects SNPs brought to the miRNA:target interactions in rice. The relationship between variations of miRNA:target interactions and that of phenotypes was further analyzed.

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