

Construction of an ultra-high-density consensus genetic map and analysis of recombination rate variation in *Sorghum bicolor*

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Manuscript received: 31 October 2021. Revision accepted: 12 March 2022.

Abstract. Satrio RD, Nikmah IA, Fendiyanto MH, Pratami MP, Awwanah M, Sari NIP, Farah N, Nurhadiyanta. 2022. Construction of an ultra-high-density consensus genetic map and analysis of recombination rate variation in *Sorghum bicolor*. *Asian J Agric* 6: 47-54. Sorghum is one of the most widely grown cereal crops on a global scale. A consensus map is a method for combining genetic information from multiple populations, and it is an effective way to increase genome coverage and marker density. This study constructed a consensus map by combining publicly available marker data from four mapping populations. A total of 3,449 non-redundant polymorphic markers at the nucleotide level were used to construct a single consensus map on ten sorghum chromosomes. This study generated an ultra-high-density sorghum consensus map consisting of many markers spanning 1,571.68 cM and averaging one marker per 0.46 cM. Due to the markers' high density, only 0.06% of the markers had an interval greater than 5 cM. The local recombination rates were estimated using a set of all markers' genetic and physical positions along each of the ten chromosomes. The analysis of the recombination rate on ten sorghum chromosomes revealed that it decreased as the centromere position was getting closer. The consensus map generated in this study can be used to integrate information related to sorghum genetic resources and QTLs into the genome sequence, thereby accelerating the discovery of novel potential genes in sorghum.

Keywords: Genetic map, linkage, recombination rate, single nucleotide polymorphism, *Sorghum bicolor*

INTRODUCTION

Sorghum is a major cereal crop on a global scale, often placing fifth in yearly volume (FAOSTAT 2021). Due to crop resistance to a broad range of biotic and abiotic stressors, sorghum is widely planted in marginal cropping zones and water-scarce conditions in developed and developing countries (Leff et al. 2004). Sorghum is a staple food and a source of fodder in underdeveloped countries for the impoverished. In industrialized nations, it is mostly utilized as animal feed. Sorghum cultivars adapted in tropical regions have generated significant relations as a potential cellulosic biofuel-producing plant (Vermerris 2011). Sorghum genetic improvement projects worldwide are attempting to increase varieties quality, disease resistance, drought tolerance, and agronomic features (Bernardino et al. 2019, 2021). Molecular-assisted breeding techniques are broadly used to construct linkage maps and discover chromosomal regions associated with essential sorghum traits, like stay-green, disease resistance, abiotic stress tolerance, high yield productivity, and photoperiod insensitivity (Harris et al. 2007; Morris et al. 2013; Girma et al. 2019).

High-density genetic or linkage maps are necessary to investigate the inheritance of qualitative and quantitative traits, design markers for molecular breeding, perform map-based gene cloning, and conduct comparative

genomic investigations. Molecular breeding is more effective when a densely-marked molecular map (Hufnagel et al. 2018). In addition, the consensus genetic map increases the diversity and quality of markers and the frequency of polymorphic markers at important chromosomal intervals. In the early 1990s, the sorghum genome was genetically mapped using DNA markers, and multiple genetic linkage maps were published during the previous decade (Mace et al. 2009, 2019). Early maps were constructed using Restriction Fragment Length Polymorphism (RFLP) markers, but more recent maps have incorporated Amplified Fragment Length Polymorphism (AFLP), Simple Sequence Repeat (SSR), Diversity Array Technology (DArT), and, more recently, microarray and sequencing-based Single Nucleotide Polymorphism (SNP) markers (Pennisi 2017; Miftahudin et al. 2021b).

The development of innovative marker technologies enables rapid and sequence-independent whole-genome analyses of any plant species. Due to the massively parallel and automated nature of the high-throughput sequencing (HTS) technique, the cost per data point is lowered massively compared to earlier technologies (Elshire et al. 2011). Moreover, the HTS-based marker permits direct incorporation into the reference sequence for the sorghum genome (Bouchet et al. 2012). Therefore, integration of the constantly growing number of genetic linkage data generated by various novel marker technologies is

essential. Aside from that, the vast majority of published sorghum genetic linkage maps were constructed using wider crossovers than the number of crossings generated in sorghum breeding projects (Mester et al. 2015; Bouchet et al. 2017). However, genetic linkage maps derived from large crossings are typically insufficient for molecular breeding methods because they do not adequately represent the gene pool's genome architecture and function. In addition to providing an invaluable reference resource, constructing a consensus map by combining data from several mapping populations with various genetic constitutions also provides for mapping a greater number of loci than is possible in most single crosses. Thus, the number of potentially usable markers is increased across various genetic backgrounds and genome coverage levels while simultaneously offering chances to confirm marker order (Mace et al. 2009; Qu et al. 2021).

On a genetic map, the distance between markers is proportional to the frequency of recombination. Meiotic homologous recombination is crucial in plant breeding because it generates novel combinations of preexisting genetic diversity. Over the past decade, our knowledge of meiotic recombination and genomic diversity in plants has evolved dramatically (Lambing et al. 2017). DNA sequencing technology has allowed the discovery of high-resolution genetic information in plant genomes, boosting plant breeding methods via high-throughput genotyping, linkage analysis, and association mapping. The genetic distance was divided by the physical distance to determine the recombination rate using linkage disequilibrium mapping and molecular marker-based linkage mapping (Apuli et al. 2020). Understanding the genome distribution of recombination rates helps forecast the population's potential response to environmental change in quantity and breeding strategy (Shen et al. 2017).

The research aimed to integrate the linkage maps obtained from four distinct maps in sorghum and construct them into a single consensus genetic map. Additionally, the rate of local recombination of the chromosomes was evaluated. The consensus genetic map constructed in this study can be linked to a well-annotated reference physical map that may accelerate gene discovery in sorghum.

MATERIALS AND METHODS

Data collection

A total of four *Sorghum bicolor* (L.) Moench mapping populations were utilized to incorporate approximately 5000 individual loci (Table 1), predominantly SNP markers, into a single consensus map. The BTx623

was chosen as one of the population parents for the sorghum genome sequencing research by Kong et al. (2018). The usage of BTx623 streamlines the process of integrating the consensus genetic map generated in this work with the publicly accessible physical map sequence. Three additional mapping populations with a varied parental line were employed in this analysis, including the F₂ population used by Ji et al. (2017), the RIL population used by Lopez et al. (2017), and Phuong et al. (2019).

Consensus map integration

We constructed a consensus map for sorghum using four different genetic maps based on SNP, SLAF, DArT, and SSR markers, which combined to form a single map. Before integrating SLAF, DArT, and SSR markers into a single consensus map, they were converted to SNP markers. The nucleotide sequences of all primers from those markers were compared using an automated batch BLASTN search (Altschul et al. 1990), with $E < 1e-10$ against the BTx623 sequence as the reference genome (McCormick et al. 2018). The best hit for each marker was chosen to derive the map position by integrating the positions of adjacent markers. The average of each marker's start and end coordinates were used to determine its physical location throughout the genome. The actual marker locations relative to the reference genome were identified using a custom Perl script. Additionally, numerous markers were removed from the downstream analysis since their physical position could not be determined. Consensus maps for each sorghum chromosome were generated using the LPmerge package (Endelman and Plomion 2014) of R software. This tool extensively uses linear programming to reduce the mean absolute error associated with integrating many genetic or linkage maps.

Estimation of recombination rates

After finding the physical placements of the markers and integrating them genetically, the rates of local recombination along each of the ten chromosomes were assessed using MareyMap (Rezvoy et al. 2007). To demonstrate the relationship between genetic and physical positions, a scatter plot was used to compare the genetic (cM) and physical (Mb) locations of the markers (Shen et al. 2017). The Loess technique was used to create and show the recombination map for each marker through MareyMap. The centromere positions for each chromosome were determined using Evans et al. (2013) physical map. The correlation between the position of genetic markers and their physical map was evaluated using Spearman correlation (Sedgwick 2014).

Table 1. A summary of the mapping data used to construct the consensus map for sorghum

Study	Type of Population	Number of individuals	Parental of population	Type of marker	Number of makers	Length of a genetic map (cM)
Kong et al. (2018)	RIL	399	BTx623 × IS3620C	SNP	381	1408.8
Ji et al. (2017)	F ₂	130	Keter × J204	SLAF	2246	2158.1
Phuong et al. (2019)	RIL	140	HYP × DYP	DArT, SSR	184, 9	1212.0
Lopez et al. (2017)	RIL	135	Early Hegari-Sart × Bk7	SNP	2833	1559.9

RESULTS AND DISCUSSION

Consensus genetic map of *Sorghum bicolor*

Multiple mapping investigations of the sorghum genome using DNA markers have been conducted over the last decade, first using RFLP markers and, more recently, using AFLPs, SSRs, DArT, and, most recently, SNP markers (Nadeem et al. 2018). Integrating SNPs' constantly expanding set of linkage data with the numerous genetic linkage maps is important for sorghum gene identification. The objective of this article was to examine the collinearity of four independent sorghum component maps and integrate them into a single reference resource using many markers. The maps with four components were generated using the LPmerge package of R software. The lengths of the published individual maps varied from 1212 to 2158 cM (Table 1). A weighted technique based on population size implemented in LPmerge was used to construct the framework consensus map. The Sorghum consensus map comprised 3449 markers covering 1571.68 cM and an average of one marker per 0.46 cM (Figure 2).

The markers and their genetic and physical position on ten sorghum chromosomes were deposited in the Zenodo (DOI: 10.5281/zenodo.3474022). A total of 2204 markers were omitted from the newly developed consensus map or were determined redundant with those generated across studies in this meta-analysis. These markers were removed and not included in developing the consensus map process. The consensus map contains an average of 345 markers per chromosome. Non-random patterns were discovered in the distribution of DNA markers, with some locations visibly rich with markers and others being sparse (Figure 1). The typical distance between markers was quite short; most chromosomes were separated by less than 5 cM (Figure 2). On chromosome 7, however, there was still a 9.52 cM interval. The low recombination rate may account for the region's difficulty in mapping. Our result improves the consensus genetic linkage map previously constructed using combined RFLP, AFLP, SSR, and DArT markers (Mace et al. 2009). The number of markers increased from 2029 to 3449, and the mean markers density was narrowed from 0.79 to 0.46 markers per cM. The genetical chromosome size was relatively similar, i.e., 1603.5 and 1571.7 cM in previous and current studies.

The sorghum genome has 818 Gb of DNA and is made up of 10 chromosomes (Paterson et al. 2009). Sorghum chromosomes have vast pericentromeric regions encompassing around 50% of the genome and were characterized by low gene density and low recombination rates. Euchromatic DNA has a higher number of genes since it covers the outermost section of each chromosome arm. The current reference Tx623 genome assembly spans 720 Mb of DNA, consisting of 10 sorghum chromosomes (683.65 Mb) and several small large-contigs that were not integrated into the reference genome sequence (Cooper et al. 2019; Ruperao et al. 2021). The final consensus map enabled us to map more markers than any individual, get more comprehensive genome coverage, and complete several gaps in individual maps.

The final consensus map allowed us to map more markers than any individual map, acquire a more comprehensive coverage of the sorghum genome and complete multiple gaps in previously published maps (Ji et al. 2017; Lopez et al. 2017; Kong et al. 2018; Phuong et al. 2019). Apart from the fact that the sequence of markers was consistent across individual component maps, excellent agreement in the total distances between common marker pairs was discovered throughout the component maps utilized in this investigation using a different ratio approach (Zhang et al. 2018; Hu et al. 2021). The generated consensus genetic map may be used as a reference for genomic investigations in individuals with various genetic origins and a framework for genetic data transfer across various marker technologies and for combining SNP markers with other genomic resources. The SNP markers are a low-cost, high-throughput marker technology that is especially beneficial in genetic mapping, through QTL mapping or association study, and molecular breeding efforts for crops such as sorghum.

The ultra-high-density consensus genetic map constructed in this study could be used to facilitate QTL mapping to discover novel genes controlling valuable traits. The traits can be observed in agronomy (Satrio et al. 2021), morphology (Fendiyanto et al. 2019a; Miftahudin et al. 2021a), physiology (Fendiyanto et al. 2019b), metabolites (Fendiyanto et al. 2020, 2021b), or transcriptional level (Satrio et al. 2019; Fendiyanto et al. 2021a; Ratnadewi et al. 2021). In addition, published QTL maps from any previous study could be integrated with the consensus genetic linkage map constructed in this study as the meta-QTL analysis. QTLs detected from meta-QTL analysis generally have higher robustness than individual QTL studies, such as in the cases of wheat (Hu et al. 2021), rice (Khowaja and Price 2008; Trijatmiko et al. 2014), potato (Danan et al. 2011), and wheat (Qu et al. 2021).

Recombination rate estimates in the sorghum genome

The collinearity of the consensus genetic map and the physical map depicted in Figure 2 were studied on all sorghum chromosomes. The findings suggested that these markers were quite useful for building a genomic map. The bulk of chromosomal markers overlapped significantly between the genetic and physical maps. The Spearman correlation coefficient was typically consistent with the degree of genetic collinearity between each genetic and physical location. On all chromosomes except for chromosomes 7 and 10, the Spearman correlation value was more than 0.95. A high Spearman correlation coefficient suggested a significant association (Shen et al. 2017).

The topography of recombination rate variation throughout the genome is shown in Figure 2, which calculates the rate of recombination per physical distance (Mb) along each of the ten sorghum chromosomes. The consensus linkage map created in this research was used to calculate recombination estimates. The average genome-wide recombination rate did not follow a random distribution, and recombination occurred at a greater rate in distal chromosomal areas than in proximal ones. One

broad-scale and persistent trend within chromosomes was a lower recombination rate at centromeres. While this might be explained by selection against recombination in highly repeated areas, a repetitive sequence is not essential. Moreover, species lacking or with few centromeric repeats also demonstrate decreased centromere recombination (Stapley et al. 2017). Suppression is probably definitely a function of chromatin shape; double-strand DNA breaks

are less prevalent in condensed heterochromatin. The chromatin environment may affect the chance that a double-strand break is repaired without a crossover. A double-strand break and subsequent repair by a non-crossover may be widespread in centromeres. That might account for the accumulation of repetitive components and diversity of centromeres despite the apparent paucity of crossings (Talbert and Henikoff 2010).

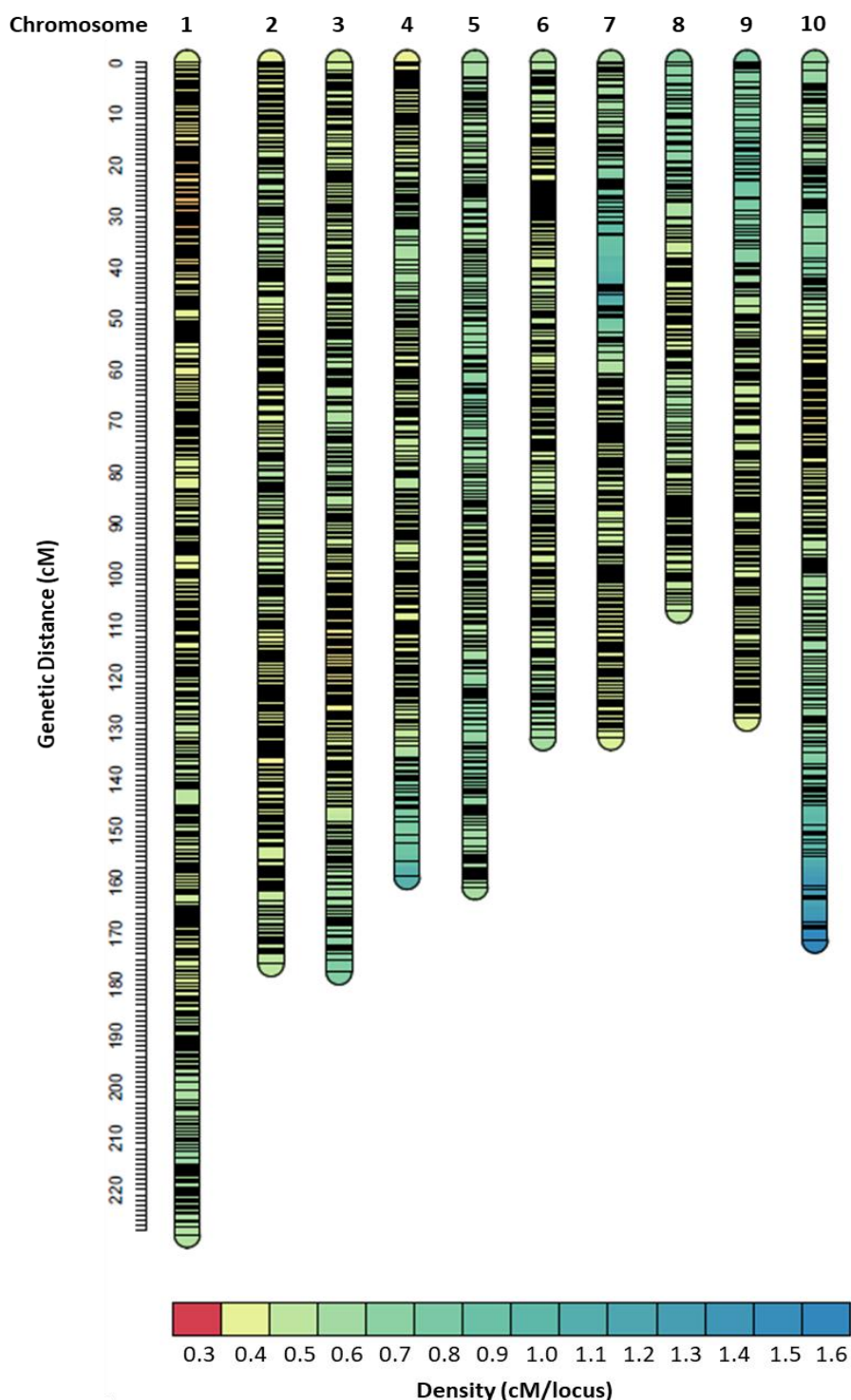


Figure 1. Ultra-high-density consensus genetic map of 10 sorghum chromosomes constructed from a large number of markers

Table 2. Characteristics of the ultra-high-density consensus genetic map constructed from a large number of markers in sorghum

Chr.	Physical chr. size (Mb)	Genetical chr. size (cM)	Number of markers	Average distance between markers (cM)	Maximum distance between markers (cM)	Gap < 5 cM (%)
1	80.88	228.84	591	0.39	3.14	100
2	77.74	175.87	430	0.41	2.54	100
3	74.39	177.46	414	0.43	2.89	100
4	68.66	158.92	350	0.46	3.77	100
5	71.85	160.92	288	0.56	2.65	100
6	61.28	131.81	308	0.43	1.86	100
7	65.51	131.70	266	0.49	9.52	99.62
8	62.69	106.88	222	0.48	3.09	100
9	59.42	128.01	267	0.48	3.11	100
10	61.23	171.27	313	0.55	5.67	99.68
Whole	683.65	1571.68	3449	0.46	9.52	99.94

Note: Gap < 5 cM: the average distance between neighboring markers that is less than 5 cM; Chr. = chromosome

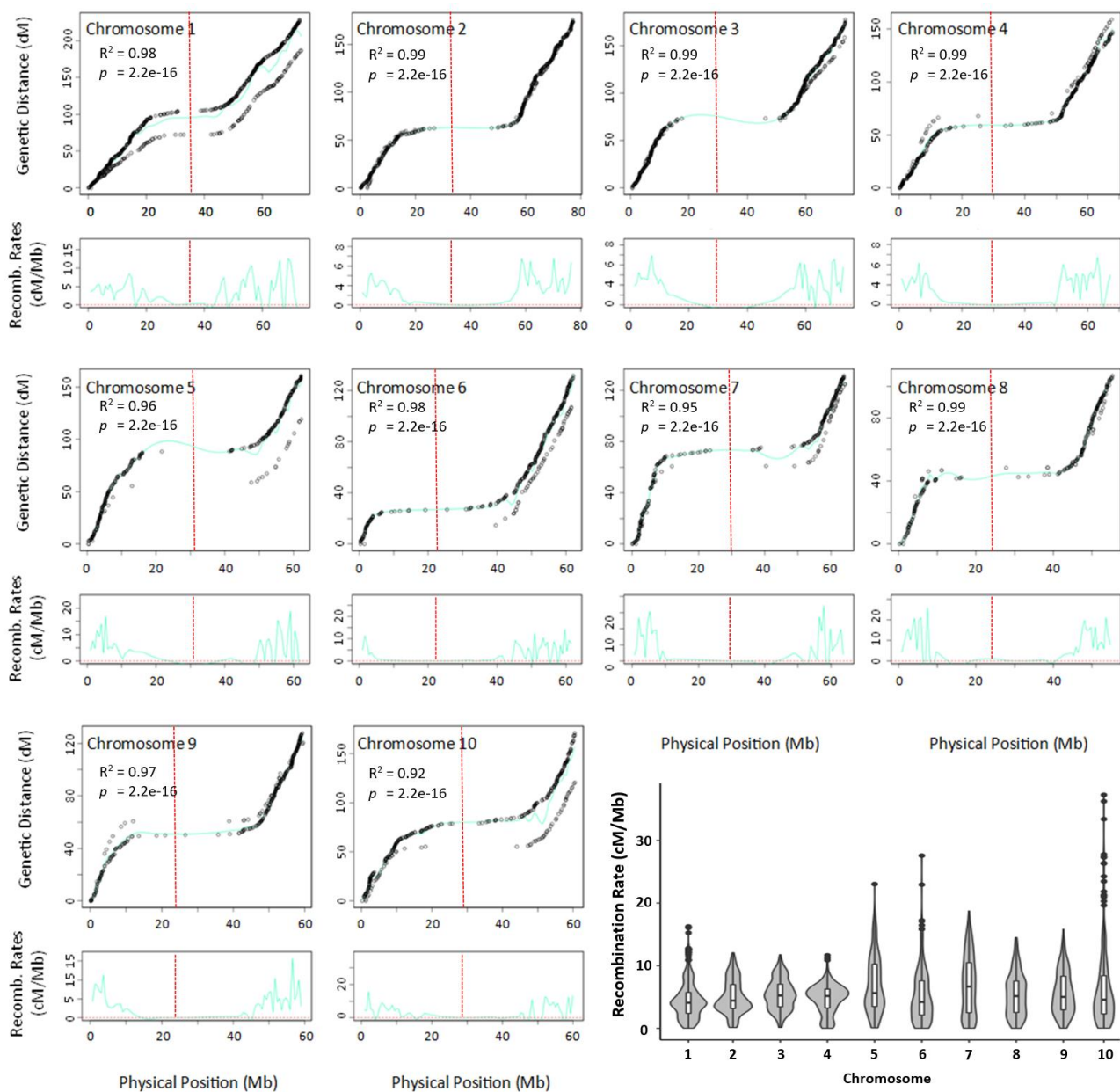


Figure 2. The relationship of genetic and physical maps and their estimated local recombination rates of the sorghum genome. The R^2 value represents the Spearman correlation coefficient between the genetic and physical maps. The light blue curves below the scatter plots represent the estimated local recombination rates. The dashed red line indicates the position of the centromere for each chromosome. The summary of the recombination rates in the whole genome of sorghum was represented as the violin and box plot

The rate of recombination differed across chromosomes (Figure 2). In summary, recombination rates varied on chromosomes 4, 6, and 8 from 0.01 cM/Mb to 37.28 cM/Mb. The calculated median rate of recombination was 4.83 cM/Mb. The estimated mean rate of recombination was 5.44 cM/Mb. Recombination rates computed using the consensus linkage map and polymorphism data revealed a large variance across all chromosomes on Mb scales (Figure 2). Most of our observations fell within the range of 0-37.28 cM/Mb for the consensus genetic map-based estimates, which is consistent with what was observed in other plants, such as *Arabidopsis thaliana* (L.) Heynh. (Giraut et al. 2011), *Populus trichocarpa* Torr. & A.Gray ex. Hook. (Slavov et al. 2012), and *Eucalyptus grandis* W.Hill (Silva-Junior and Grattapaglia 2015). Additionally, recombination of hot and cold spots have been observed in *Zea mays* L. and *Oryza sativa* L. (He and Dooner 2009). However, recombination hotspots or coldspots are typically reasonably modest in size, spanning just a few Kb (Choi and Henderson 2015). Sorghum's average recombination rate is several times that of many other plant and animal species (Tiley and Burleigh 2015), meaning that it possesses one of the greatest recombination rates ever reported in the plant kingdom.

Our high-density sorghum consensus genetic map was a useful resource for *S. bicolor* and comparative genomics investigations within the genus *Sorghum*. Recombination estimates rates generated from a consensus genetic map constructed from various linkage maps were generally consistent across studies. Our results imply that, since recombination estimates were based on population-scale variation, they may be especially helpful for discovering fine-scale recombination variation and identifying hot- or coldspots recombination in the genome. Thus, more study is necessary to ascertain the relative role of positive and negative selection in sculpting sorghum genome-wide diversity, and having access to the tools produced here would aid these investigations.

ACKNOWLEDGEMENTS

This research was funded by the "Ministry of Defense, Republic of Indonesia" on behalf of Dr. Rizky Dwi Satrio. In addition, the authors acknowledge the Bioinformatics Laboratory of the Universitas Pertahanan Indonesia for the computation facility.

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