Introduction

Bread wheat (Triticum aestivum) has large (17 Gb) and complex allohexaploid genome with high proportion (80%) of repetitive sequences. The complex nature prevents assembly of reference genome sequence from shot-gun sequence reads. To facilitate this work, genome complexity was decreased by construction of chromosome-specific physical maps. Subsequent steps involve orientation and anchoring of physical maps along the chromosomes using suitable markers. However, classical recombination maps provide insufficient resolution, including massive marker clustering due to uneven crossover distribution along chromosomes. In contrast, genome maps developed using chromosome specific radiation hybrid (RH) panels do not rely on natural recombination and offer high and uniform resolution (Kalavacharla et al., 2009). Two approaches are used to construct radiation panels. The Seed RH panel was constructed using pollen of plants from irradiated seeds. The Endosperm RH panes takes advantage of using irradiated pollen which allows use of high dosage of irradiation and create RH panels with higher density of deletions = higher resolution. To saturate the panel with markers the Infinium 90K SNP assay was used (Wang et al., 2014). Markers could be ordered using two approaches. The fist include classical approach based on recombination frequency. The second include network-topology approach previously used for physical map construction using LTC software (Frenkel et al., 2010).

Results

4A RH panel genotyping and construction

➤ 1069 4A specific Endosperm RH panel was constructed using crossing of Chinese Spring irradiated pollen with NT44A and NT44A plants (Fig. 1).
➤ DNA of 400 endosporms were extracted and characterized with 10 molecular markers per chromosomal arm. The markers were selected according to 4A GenomeZipper (IWGSC 2014) to evenly cover whole chromosome.
➤ 60 and 59 lines derived from NT44A and NT44A respectively showed high level of deletions, but were not missing entire chromosomal arms and were genotyped using Infinium 90K SNP assay. MDA amplified 4AS and 4AL DNA, DNA of four 4AS and six 4AL deletion lines derived by gametocidal approach (Endo and Gill, 1996), genomic DNA of cv Chinese Spring and water were used as controls.
➤ 2683, 2698, and 2699 reliable 4A specific markers were identified for bin, NT4A4B, and NT4A4D derived maps, respectively.
➤ 2698 were identical between NT4A4D, and NT4A4D derived maps.
➤ The bin map was developed using MS Excel and 18 bins were identified. Two the most distal bins of 4AS were not separated but two new were identified compared to described previously (Fig. 2, labeled with “+”). The first new bin represents very centromeric bin corresponding to overlap of 4AS and 4AL telosomes chromosomes. The second newly identified bin represents the most distal bin of 4AL chromosome.
➤ NT4A4D, and NT4A4D derived maps were assembled using two algorithms derived from LTC (network-topology) and MultiPoint (recombination frequencies) map building approaches and combined to final consensus map containing 2420 markers which passed the network-topology test (Fig. 3)

Conclusions

➤ 4A specific Endosperm RH panel was created
➤ 129 RH lines and lines of 4A deletion stock were selected for high throughput genotyping
➤ 90 K SNP Infinium assay was used to genotype selected RH lines and data were manually analyzed.
➤ 2699 4A specific SNP markers were identified.
➤ The deletion map was enhanced by two new bins and centromere of the 4A chromosome was more precisely delimited.
➤ After the network-topology test consensus RH map contains 2420 SNP markers, from which 757 can be considered as high quality skeleton markers. The skeleton map is 238,4 cM long.
➤ The 4A RH map will be used to anchor and orient contigs of the 4A physical map and 4A reference sequence scaffolds.

REFERENCES


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