Analysis of the genomic structure of a wheat NIL population segregating for resistance to glume blotch with a 90k ILLUMINA SNP chip

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Introduction and objectives

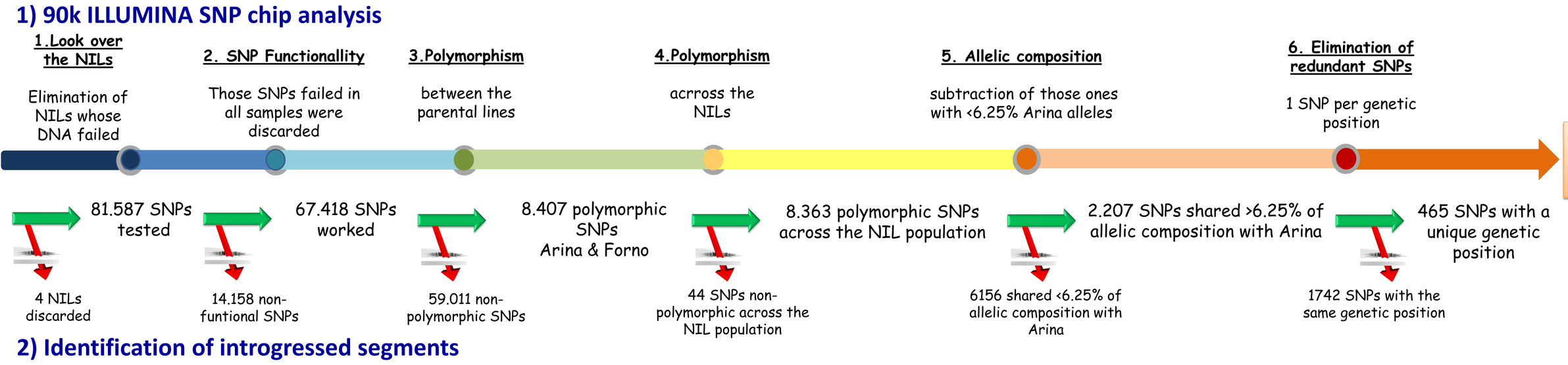
Single nucleotide polymorphism (SNP) markers have recently become highly relevant for genetic analysis in wheat because of new SNP genotyping technologies like the Illumina Golden-Gate Assay allowing a high-throughput and cost-effective genotyping, even in the polyploid wheat genome.
Stagonospora nodorum glume blotch (SNG) is a necrotrophic fungal disease affecting spikes in bread wheat (*Triticum aestivum* L.) and can result in devastating disease and yield losses of up to 30-40%. The high-resolution analysis of a major QTL against SNG placed on Chr3B with a fine mapping Near Isogenic Lines population (NIL), revealed presence of two distinct resistance loci in the target interval but it suggested that other genomic regions outside of the target region could contribute to resistance also [1]. To verify that, using the reliable and high throughput genotyping platform of the 90k ILLUMINA SNP chip, we wanted to unravel the genetic structure of our NIL population and to determine the extent and size of genomic fragments derived from the donor line in the NILs in order to see if indeed there would heterogeneities outside 3B region that could influence on the resistance.

Necotrophic lesions in Wheat spikes caused by SNG

Materials and Methods

Among an original population of 1320 NILs, derived from the introgression of that specific genomic region on Chr3B from Arina (resistant) into Forno (susceptible), two winter Swiss wheat varieties used in wheat breeding programs, we selected 89 homozygous NILs (28 BC3F8 and 61 BC3F7) showing recombination between the flanking markers of the target region. Each sample consisted of two pooled plants. For SNP-analysis a novel 90k ILLUMINA SNP chip [2] was used for all NILs.

1) 90k ILLUMINA SNP chip analysis



2) Identification of introgressed segments

The identification of introgressed segments was done based on the selected 465 SNPs distributed on the chromosomes displayed in the table below. To avoid false-positives, an introgressed segment was scored based on the presence of at least three consecutive markers with the Arina genotype, with a maximal inter-SNP distance of 5cM. Introgression boundaries were then defined by two consecutive markers with the alternative genotype Forno. As this is a NIL population selected originally for Chr3B, this chromosome was excluded to avoid an overrepresentation of donor line. Considering we worked with a BC3 population, the 6.25% of the donor line genome was expected to be present in each NIL. Having the SNP wheat map [2], the length of each chromosome was known. On the other hand, the total length of the introgressed segments was calculated for each chromosome. If that was higher that the 6.25% of chromosome length, these segments were treated as an overrepresentation of the donor line genome, if not, we excluded them in the further analysis.

Results

Chromosome analysis

The table on the right summarises the number, size and localization of the introgressions across the chromosomes. Most of the introgressed segments are concentrated on subgenomes A and B. As the introgressed segments from the donor line in Chr1D, 2A, 7A and 7D didn't exceed the 6.25% of their chromosome length, we considered them as introgressions with the expected donor line genome size, and, therefore, discarded for further analysis. For the rest of the chromosomes, the average number of introgressions per chromosome was 69.25, across the 85 NILs. The average number of NILs with introgressed genomic regions was 34.4 per chromosome and ranged from 15 (Chr 4A) to 59 (Chr 7B). The average introgression size was 14.7cM, with Chr5B having the lowest (7.3) and Chr5A the highest (22.2) mean length, respectively. These same chromosomes showed the lowest (3.9) and highest (18.8) introgression size per NIL, respectively.

Chr	Genomic size mapped (cM)	Total size of donor expected (cM)	Nº Intrg segments	N° NILs with a higher donor background expected	Introgression size (cM)	
					average	per NIL
1B	174.1	10.9	49	36	19.9	9.1
1D	209.1	13.1	39	0		
2A	185.5	11.6	21	0		
3A	207.3	12.6	34	32	10.9	4.4
4A	166.7	10.4	53	15	8.4	4.5
5A	148.5	9.3	131	56	22.2	18.8
5B	219.8	13.7	76	22	7.3	3.9
6A	183.4	11.5	30	22	13.6	4.8
6B	127.5	8.0	57	33	19.2	9.7
7A	244.2	15.3	38	0		
7B	188.6	11.8	124	59	16.1	13.3
7D	241.3	15.1	111	0		
Mean			69.25	34.36	14.7	8.56

The graphical genotype of NILs (Fig 2) provides an overview of the genetic map based on the SNPs selected. The percentages of "Arina" and "Forno" alleles for each chromosome is displayed in the top part. As a whole, there is an overrepresentation of "Arina" allele, being particularly high in Chr6B and Chr7B. Another interesting observation was that the introgressed segments did not show a randomized position across the chromosomes, but also "fixed" positions (Fig. 2).

NIL analysis

Fig 3 shows the n° of Nils with "x" introgressions coming from the donor line. The average number of introgressions per NIL was 3.24 and only three NILs didn't show any introgressions.

In only 9 NILs the total length of the introgressions represented less than 6.25% of the chromosome length, meanwhile in the rest of the NILs was higher (Blue bars Fig 4). However, when the total size of these introgressions is referred to whole genome size (Red bars Fig 4), in 35 NILs they represented between the 6.25% and 12.5% of the donor line genome –which is acceptable-, meanwhile in the other 50 NILs represented less that 6.25 % of the donor line genome.

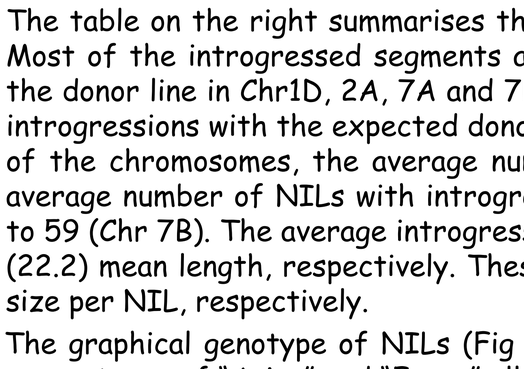


Fig 3. Distribution of introgression number based on SNP map.

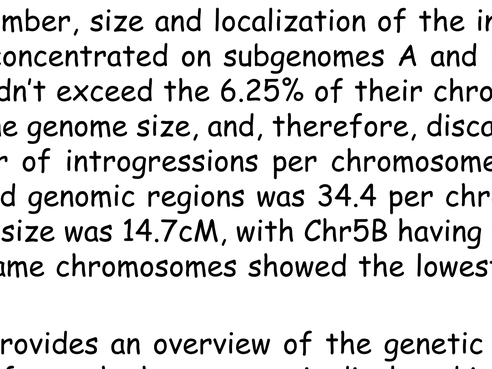


Fig 4. Distribution of the donor parent genome introgressed

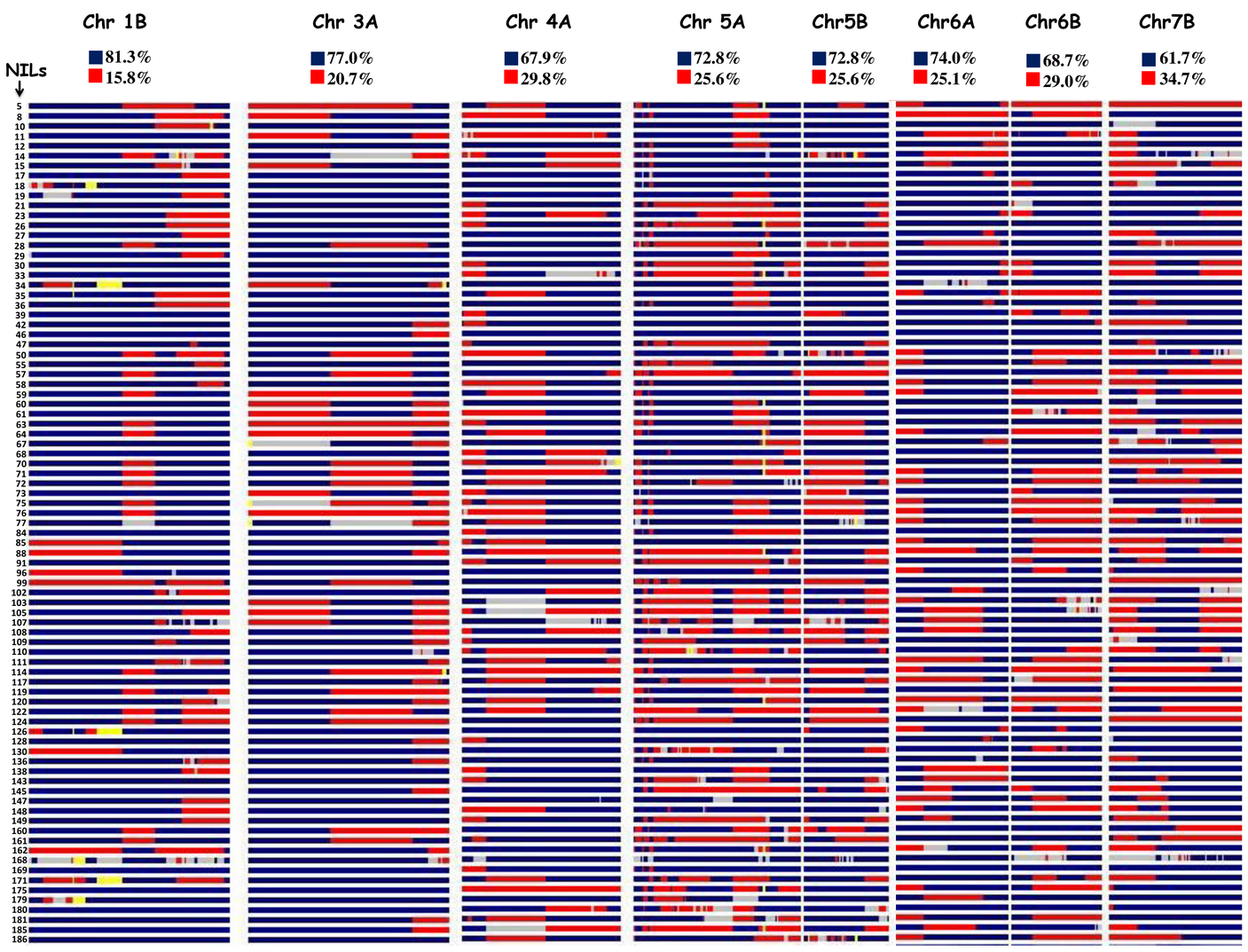


Fig 2. Graphical genotypes of NILs representing recurrent parent (Forno ■), donor parent (Arina ■), and heterozygous ■ alleles. Missing data ■

The SNP analysis revealed that the NIL population as a whole was properly selected with a donor line genomic background in an acceptable range. However, the presence of introgressed segments that represent an overrepresentation of the donor line and occupying fixed positions should be taken into account to study if the genes placed on those segments have an influence on SNG resistance in further analysis.

REFERENCES
[1] Shatalina et al (2013) TAG 127:573-586
[2] Wang et al (2014) Plant Biotechnol. J. doi: 10.1111/pbi.12183