1. Development of a series of chromosome segment substitution lines in a Chinese elite rice background

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Chromosome segment substitution lines (CSSLs) are useful tools for the evaluation of gene effects or interactions affecting importance traits (Nadeau et al., 2000). In addition, CSSLs are also widely used for the study of heterosis and, in some cases, direct breeding practices (Zachary et al. 2006).

The typical *indica* restorer line, 9311, has been sequenced in 2002 (Yu et al. 2002). "C418" is developed from the cross between *japonica* variety Wanlun 422 and *indica* variety Miyang 23, so it carries both *japonica* and *indica* segments. Since C418 have features of wide compatibility, high yield, good quality and lodging resistance (Zhao et al. 2000), thus it is widely cultivated in the north of China. In order to elucidate the genetic base for these superior characters of C418, developing of a set of chromosome segment substitution lines derived from the rice cross, C418 and 9311, was proposed.

A total of 1000 SSR markers and 40 Indel makers distributed throughout the 12 chromosomes of rice were used to detect polymorphism between 9311 and C418. It was found that 158 markers (15.19%) showed polymorphic between these two parents. The polymorphism between 9311 and C418 was low because "C418" carried *indica* segments simultaneously. Here, at last 133 polymorphic loci were used for CSSL analysis. The average distance between two flanking markers was 11.6 cM.

In year 2005, 2274 BC_3F_4 plants of 9311/C418 combination were developed using 9311 as recurrent parent (Fig. 1) and their DNA was extracted for genotype analysis with 8 SSR makers, which were uniformly distributed in chromosome 1, 4, 8 and 12. The criteria for selection were as following: if the line is harbored three or four C418 segments substituting in these selected makers areas, it was thought there existed too many substituted segments in the line, and was discarded for our construction of CSSL. Only the lines that have no or few introgressed segments were kept for next round screening. According to the criteria, 1000 candidate BC_3F_4 individuals harboring 0, 1 or 2 substituted segments were selected and grown in the experimental station of San'ya (Hainan, China). At harvest, the seeds of 1000 lines were obtained in bulk. In 2006, the 1000 BC_3F_6 plants were planted and the leaves of the second line per row was selected and mixed to construct 97 gene pools. Every gene pool was composed of about 9 to 11 individual plants that derived from the same BC_3F_1 parent. Then we genotype the 97 gene pools using 96 polymorphic molecular markers that evenly distributed 12 chromosomes for the selection of CSSLs. The criteria for selection were as follow: a single, relatively large chromosome segment of C418 was introgressed into the 9311 chromosome, whereas a high level of homozygosis of 9311 alleles remained in non-target chromosomal regions. In order to cover 12 rice chromosomes, the target chromosome segments should be partially overlapped in the selected lines. As a result, appropriate 51 gene pools were obtained.

In these 51 gene pools, we used all the 133 polymorphic molecular makers to genotype the 450 BC_3F_4 plants containing in these 51 gene pools and 47 candidate plants was obtained. 4 of these candidate plants were crossed with 9311 to produce BC_4F_6. Then 47 candidate plants were self-pollinated and produced 106 BC_4F_7 plants via marker assistant selection. On summer of 2007, the progeny of 106 BC_3F_7 and 4 BC_4F_6 plants were genotyped by the same set of markers described above to construct the CSSLs. Lastly, the CSSLs including 108 lines were obtained.

The marker position on the linkage map was estimated according to Temnykh et al. (2002), McCouch et al. (2002) and the International Rice Genome Sequencing Project (http://www.nature.com/nature/journal/v436/n7052/suppinfo/nature03895.html). The genome compositions of the 108 CSSLs, which was estimated according to Young et al. (1989), were conducted with the GGT software (http://www.dpw.wau.nl/pv/pub/ggt/). The average distance between two flanking markers was 11.6 cM. The substituted chromosomes in the CSSLs covered most of the 12 chromosomes, except for small region at the distal end of the long arm of chromosome 8 (defined by the SSR maker RM6948) (Fig. 2). The CSSLs carried 98.3% of the genome of C418. In each CSSL, only one single chromosome segment or a few chromosome segments was substituted in the genetic background of 9311. The CSSLs will be helpful for new gene identification and breeding practices.

Acknowledgments

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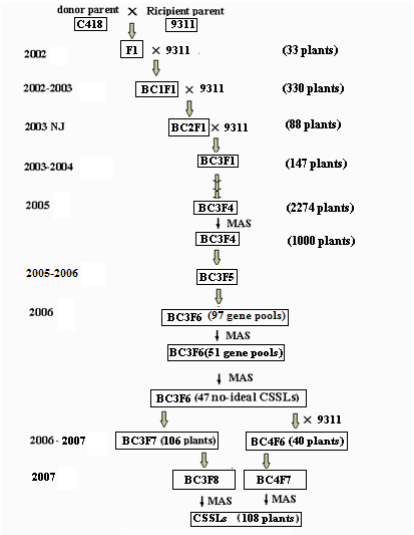

Fig. 1 Strategy for the development of the CSSLs in the present study

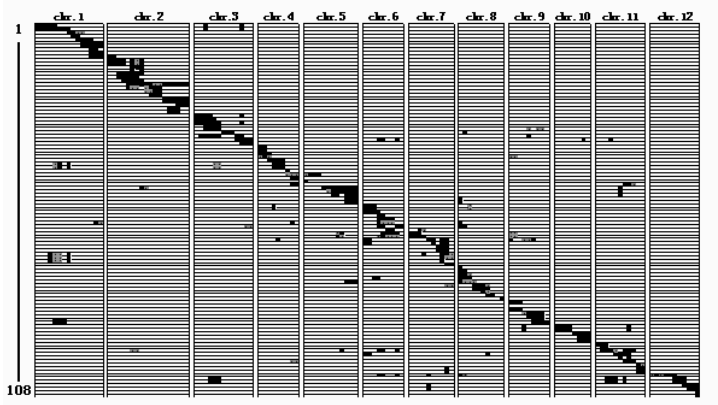

Fig. 2 Graphical representation of the introgression lines developed in this study. Each row represented a candidate introgression line. The black regions indicated the regions homozygous