

2. Development of the chromosome segment substitution lines (CSSLs) derived from a hybrid rice cross, Pai64S/93-11 with super high yield potential

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Development of chromosome segment substitution lines (CSSLs), in which each line carries a single or a few defined chromosome segment of donor genome and has a pure genetic background from a recurrent genotype, is a fundamental approach to conduct the QTL mapping in order to improve the mapping precision (Nadeau et al. 2000).

In the present study, a set of chromosome segment substitution lines carrying overlapping chromosome segments of Pai64S in a genetic background of elite cultivar 9311 has been constructed. Pai64S/9311 is an elite hybrid rice planted in the largest area in China, and so should be an ideal hybrid for analyzing heterosis (Zou et al. 2003). The typical *indica* restoring line, 93-11, had been sequenced in 2002 (Yu et al. 2002). Pai64S, whose whole genome has also being sequenced by Beijing Genomic Institute (personal communication), is a thermo-sensitive genic male sterile line with a broad spectrum of wild compatibility. In order to elucidate the genetic mechanism of yield heterosis of the hybrid rice, development of a set of chromosome segment substitution lines derived from the rice cross, Pai64S/93-11, was proposed. So far, the present study is the first report of development of the CSSLs population derived from the parents with sequenced whole genome.

To develop the CSSLs, total 775 primers were surveyed and 138 primers (17.8%) showed polymorphism between the parent varieties, Pai64S and 93-11. The linkage map, constructed with 123 SSR loci, spanned a total of 1,595.7 cM on all 12 chromosomes with an average interval of 12.97 cM between adjacent markers. The objective introgression lines were selected from the progenies for future study with the above SSR markers.

In year 2000, Pai64S, which was used as the female parent, was crossed by 93-11. The F₁ and its

progenies were backcrossed by 93-11 successively for three times. In year 2002, four hundred BC₃F₁ lines, the progenies of consecutive backcross to 93-11, were grown in the experimental station of Jiangsu Academy of Agricultural Sciences (Nanjing, China). The leaves of two hundred plants, each from one BC₃F₁ line, were collected and their DNA was extracted for genotype analysis. Forty-four candidate BC₃F₁ individuals were selected with SSR markers according to the following criteria: *a*) a high purity of recurrent parent background, and *b*) a wide coverage of the genome with donor introgressions. In subsequent generations, the candidate individuals were self-pollinated or backcrossed by 93-11. Presently, a set of chromosome segment substitution lines, which is composed of minimum 75 individuals selected from the generations of BC₃F₅, BC₃F₆, BC₄F₁, BC₄F₄, BC₄F₅, BC₄F₆, BC₅F₁, BC₅F₂, BC₆F₁ and BC₆F₂, respectively, were developed (Fig. 1).

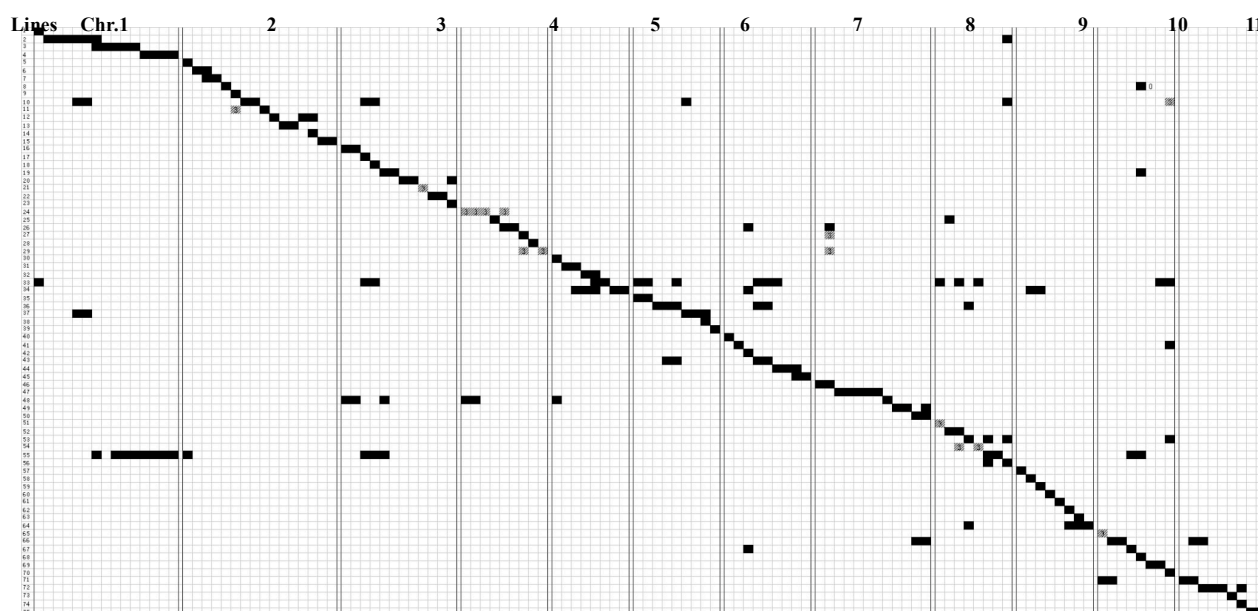


Fig.1. Graphic genotype of candidate individuals of chromosome segment substitution lines.

□ ■ ▨ denotes the genotype of 93-11, Pai64S and heterozygous type, respectively.

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/supinfo/nature03895.html>). The genome compositions analysis of the 75 CSSLs, which was estimated according to Young et al. (1989), was conducted with the GGT software (<http://www.dpw.wau.nl/pv/pub/ggt/>). The results showed that the average percentage of background genome segment was 97.61%, with a range from 96.6% for

chromosome 9 and 11 to 98.3% for chromosome 2. There are great differences in the number of substitution chromosome segments among the CSSLs, with a range from 1 to 10. There were 69 CSSLs containing introgression chromosome segments less than 3, among them 46 lines were singletons. The percentages of donor segment to whole genome varied from 0.1% to 14.1% in the CSSLs, with 91% of the total introgression lines lower than 5%. Further development of the CSSLs is underway by additional selection with denser genetic linkage map.

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