Fine mapping of an oil content quantitative trait locus in the linkage group 7 of *Brassica napus*

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ABSTRACT
Based on the QTL analysis of oil content in multiply environments with SG-DH population, a fine mapping study for the most significant QTL in the linkage group 7 was conducted using developed QTL-NILs of BC3F1/BC3F2, BC3F2/BC3F3 and BC4F1/BC4F2 progenies. The OilA7 was firstly validated in a population of BC3F1/BC3F2 and further fine mapped to 1240kb and 528kb genomic regions by BC3F2/BC3F3 and BC4F1/BC4F2 generations, respectively. The comparative analysis of oil content between homozygous BC4F2 sister sub-NILs carrying "Gaoyou" fragment (n=65, 46.4%) and NILs containing "Sollux" segment (n=61, 44.3%) in the 528kb target region showed significant difference of 2.1% (*p*=0.001) in oil content. For the next step, a large population of 4000-5000 BC4F3 and BC5F2 plants is being constructed to further fine mapping OilA7 mainly focus on the 528kb target region.

Key words: *Brassica napus.*L, oil content, QTL, fine mapping

INTRODUCTION

Oil content in seeds is definitely the critical objective for rapeseed breeding. However, accumulation of seed oil is a complicated metabolic and genetic regulatory process. It has been well documented in *Arabidopsis* that hundreds of genes were involved in Acyl Lipid Metabolism pathways (Beisson et al., 2003), which makes its genetic control system difficult to be understood. Up to date**,** a number of QTL for oil content in rapeseed have been identified (Ecke *et al.* 1995; Burns *et al.* 2003; Zhao *et al.* 2005; Qiu *et al.* 2006; Delourme *et al.* 2006; Yan *et al.* 2009; Chen *et al.* 2010), however fine mapping and map based gene cloning have not yet been reported. The current study was based on the previous mapping analysis of oil content in multiply environments and aim to fine mapping the most significant QTL for oil content in linkage group 7 (OilA7) and search for candidate gene/genes underlying this QTL.

MATERIALS AND METHODS

Brassica rapa BAC sequences (http://www.brassica.info/resource/sequencing/status.php, **http://brassicadb.org:8081/brad/**) were selected for locus specific marker design. Near-isogenic lines (NILs) containing introgressions from donor parent "Gaoyou" were developed using advanced backcross approach to get BC3F1/BC3F2, BC3F2/BC3F3 and BC4F1/BC4F2 plants/lines (Figure 1). Plants from BC3F1, BC3F2 and BC4F1 populations were genotyped individually at marker loci in the target region, while phenotypic data were obtained from respective selfing progenies BC3F2, BC3F3 and BC4F2 lines, which were tested in field with two replications and single row for each line. At seed maturity, around 20 g of seeds were bulk harvested from the terminal raceme of replicate plants in each plot. Seed oil content was determined by near-infrared reflectance spectroscopy (NIRS) for three times per line.

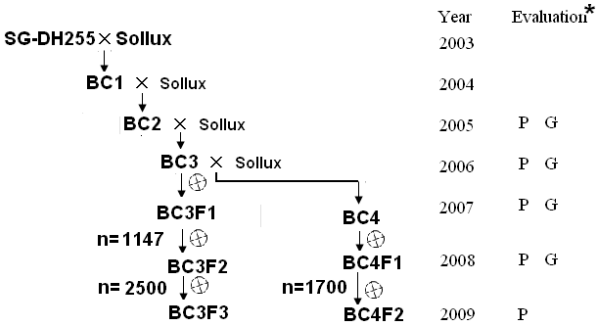

Figure 1. Development of fine mapping populations by marker assistant and phenotypic selection

RESULTS

In the initial QTL mapping for oil content over 11 environments using SG-DH population of 282 lines, OilA7 was detected significantly in all locations with LOD value of 12.54 and additive effect of 0.63% in average (Fig 2). This QTL explained 3.59% to 24.69% of the total phenotypic variation in SG-DH population and showed Chinese allele favored to oil content. Subsequently, OilA7 validation was performed using the genotypes of 1147 BC3F1 plants by 11 markers covering the whole QTL region of 21.1cM (Fig 2) and the correspondent phenotypes of BC3F2 lines obtained from field test with two replications. The OilA7 was then mapped in a 12.6 cM region between the markers ZAAS176 and EM4ME8b (Figure 3a). The further fine mapping was carried out applying 2500 BC3F2/BC3F3 and 1700 BC4F1/BC4F2 families and newly developed 23 markers between ZAAS176 and EM4ME8.

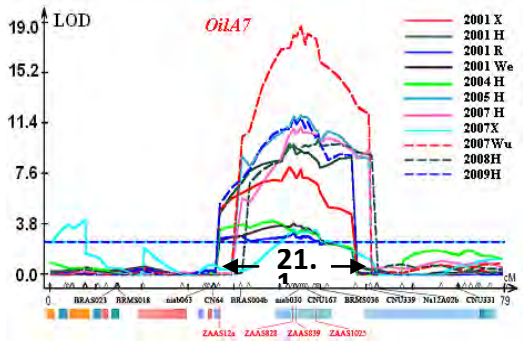

Figure 2. The initial QTL mapping for oil content in linkage group 7 with SG-DH population over 11 environments.

X: Xian of China

H: Hangzhou of China

OilA7 was then localized to a high-resolution linkage map by progeny testing of homozygous recombinant lines and narrowed the locus to 5.8cM (BC3F2/BC3F3) (Figure 3b) and 528kb (BC4F1/BC4F2) regions, respectively, after alignment between flanking markers of OilA7 and *Brassica rapa* scaffold sequences on A7 (Figure 3c). The comparative analysis of oil content between homozygous BC4F2 sister sub-NILs carrying "Gaoyou" fragment (n=65, 46.4%) and NILs containing "Sollux" segment (n=61, 44.3%) in the 528kb target region showed significant difference of 2.1% (p=0.001) in oil content (Fig. 4)

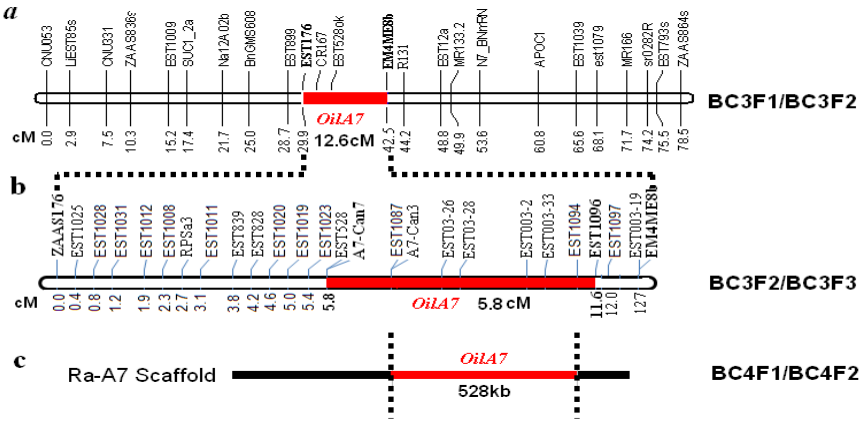

Figure 3. Genetic and physical maps of the OilA7. ***a*** Linkage map of A7 constructed using 282 SG-DH lines. The OilA7 locus was mapped between marker EST176 and EM4ME8b with genetic distance of 12.6 cM, using 1147 BC3F1/BC3F2 plants/lines. ***b*** Fine mapping of OilA7. The locus was mapped between marker A7-Can7 and EST1096 and the distance in between is 5.8cM. ***c*** Physical map of OilA7. The locus was narrowed down to a 528kb region in a *Brassica rapa* A7 scaffold (DNA fragment).

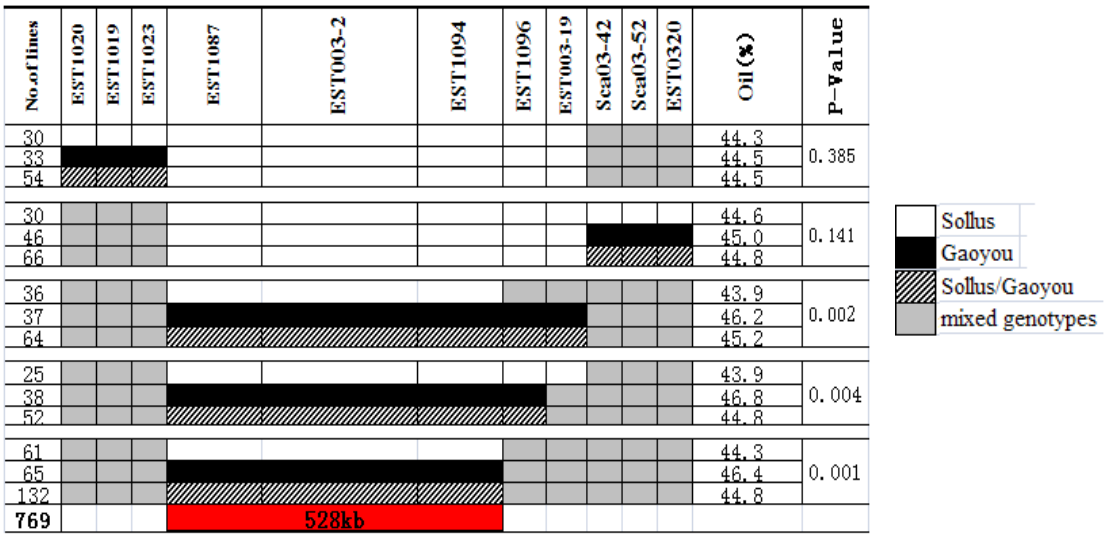

Figure 4. Progeny testing of fixed recombinant plants /lines of BC4F1/BC4F2, narrowed the OilA7 locus to the correspondent physical region of 528kb. Oil contents of BC4F2 lines carrying “Gaoyou” fragment were significantly higher than that containing “Sollux” segment in the target region.

In this 528kb genomic region across OilA7, we identified 108 predicted ORF by FGENESH and found that one of them was highly homologous with a candidate gene (E-value=0), which was involved in the fatty acid biosynthetic process in *Arabidopsis.* This gene might be considered as the first candidate for OilA7. In next step, a large population of 4000- 5000 BC4F3 and BC5F2 plants, focusing on the 528kb target region
(Figure 5) is currently being constructed and a more precise fine mapping for OilA7 will be conducted.

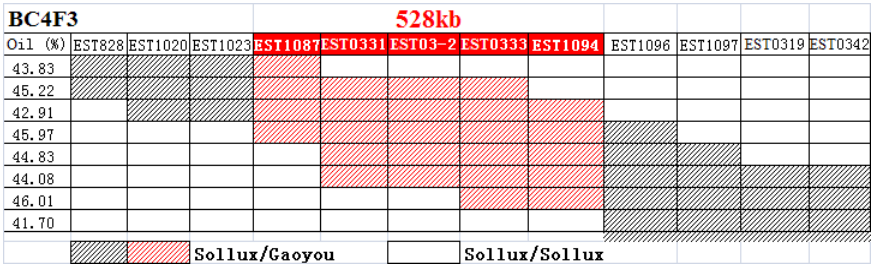

Figure 5. Around 4000-5000 plants derived from BC4F3 and BC5F2 generations mainly focus on 528kb target region (sowing on Oct. 2010).

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