Plant Genomics Group, BioGreen 21 Program, RDA (Ramada Plaza Jeju, November 2-3, 2005)

과제 일련번호: 8

Construction of Genetic marker and BAC library related for cloning of the Clubroot resistance gene(CRb)

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Clubroot disease, caused by *Plasmodiophora brassicae* Wor., is a highly damaging disease of Chinese cabbage. For cloning of the Clubroot single dominant resistance gene (CRb) in the CR Shinki DH line of Chinese cabbage, we construct genetic marker and BAC library. An F2 population was analyzed using developed DNA markers, and a genetic map around *CRb* locus covering a total distance of 6.75 cM was constructed. Using three nearest surrounded markers (TCR01, TCR04, and TCR08), 18 recombinants were detected in a segregating F3 population (487 individuals). Among them, 11 were between TCR08, *CRb* and seven between *CRb* and TCR01, respectively. To construct a saturated map around *CRb*, AFLP technique was employed by using 256 (16 *Pst*I+GNNx16 *Mse*I+CNN) primer combinations. Though, we could not identify more closely linked markers in *Eco*R I/ *Mse* I, yet among 240 *Sno* I/ *Mse* I tested in a bulked segregant analysis, 30 candidate markers were identified. The confirmation of these candidate markers is in progress. For constructing BAC library high molecular weight (HMW) DNA was isolated from leaves of Chinese cabbage inbred line 'CR Shinki DH line' were partially digested with *Bam*H I enzyme and cloned into the pCUGIBAC1 vector. The *Bam*HI library consisted with 14,256 clones which covers 3.4-fold of the Chinese cabbage genome. The average insert size of the BAC clones were 132 kb. Three chloroplast gene, *rbc*L and *psb*A from rice and *ndh*A from tobacco, were employed for testing organelle DNA contamination. The result of screening indicates that 2.1% of BAC clones were containing organelle DNA.

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† 주관과제명 (과제책임자): 생명공학 기법을 이용한 배추 주요병해 저항성 품종개발 (농촌진흥청 원예연구소 윤무경)

‡ 총연구기간 (년차): 2002년 - 2006년 (4년차)