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Fine Mapping of *C* (*Chromogen for Anthocyanin*) Gene in Rice

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Abstract: Seven residual heterozygous lines (RHLs) displaying different genotypic compositions in the genomic region covering probable locations of *C* (*Chromogen for anthocyanin*) gene on the short arm of rice chromosome 6 were selected from the progenies of the indica cross Zhenshan 97B/Milyang 46. Seeds were harvested from each of the seven plants, and the resultant $F_{2:3}$ populations were used for fine mapping of *C* gene. It was shown in the populations that the apiculus coloration matched to basal leaf sheath coloration in each plant. By relating the coloration performances of the populations with the genotypic compositions of the RHLs, the *C* locus was located between rice SSR markers RM314 and RM253. By using a total of 1279 $F_{2:3}$ individuals from two populations showing coloration segregation, the *C* locus was then located between RM111 and RM253, with genetic distances of 0.7 cM to RM111 and 0.4 cM to RM253. Twenty-two recombinants found in the two populations were assayed with seven more markers located between RM111 and RM253, including six SSR markers and one marker for the *C* gene candidate, *OsC1*. The *C* locus was delimited to a 59.3-kb region in which *OsC1* was located.

Key words: *C* locus; fine mapping; candidate gene; residual heterozygous line; rice

Rice cultivars have a usual green plant, but accumulation of anthocyanins is widely observed in tissues such as stigma, pericarp, sterile lemma, lemma, palea, apiculus, awn, leaf blade, basal leaf sheath and internode. The tissue-specific pigmentation, especially the apiculus coloration, has long been used as a morphological marker for the variety identifying and linkage analysis in rice.

Occurrence of the tissue-specific pigmentation is determined by the CAP control system, in which the *C* (chromogen) is the basic gene for the production of chromogen, *A* (activator) exerts its activation effect on *C* and turns the chromogen into anthocyanin, and *P* (distributor) is responsible for the distribution of color in specific tissues [1]. It was reported in 1928 [2] that a gene or a gene cluster for the coloration of apiculus, stigma and leaf sheath was loosely linked to a glutinous gene. With extensive studies on the anthocyanin pigmentation in rice plants, the CAP control system was revealed and the *C* gene was located on the Group I of the Japanese classical linkage map of rice [3]. After the publication of the first molecular linkage map of rice by the Tanksley's group of the Cornell University, USA [4], Japanese researchers constructed another molecular map and aligned it with the classical map [5]. *C* locus was mapped between XNpb165-1 (G165) and XNpb200 (G200) on rice chromosome 6. More studies have located *C* in the same region with a little variation [6-8]. According to the physical positions of the markers flanking *C* locus in these studies, *C* could be located in the interval spanning from 4.9 Mb to 6.5 Mb on the short arm of rice chromosome 6. Recently, the rice homologue *OsC1* of the maize *C1* anthocyanin regulatory gene was mapped between RFLP markers RZ588 and G200. Although the presence of other interacting genes for pigmentation made it unable to test the linkage between *C* and *OsC1*, *OsC1* was consider a candidate for *C* since they were located in the same region [9].

In this study, rice populations derived from residual heterozygous lines (RHLs) that displayed different genotypic compositions in the vicinity of *C* locus were used for gene fine mapping. The *C* locus was tagged to DNA markers on the short arm of rice chromosome 6 and delimited to a 59.3-kb region in

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which *OsC1* was located.

MATERIALS AND METHODS

Rice materials and field trial

A rice RHL was identified from a recombinant inbred line population of the indica rice cross Zhenshan 97B/Milyang 46. Tested with 208 polymorphic rice SSRs (simple sequence repeats), this RHL was shown to carry a 7.3-Mb heterozygous segment on the short arm of chromosome 6 in an inbred background. Such a plant was equivalent to an F_1 produced by crossing a pair of near isogenic lines (NILs), and the self progenies would compose a population that segregates a single region covering the target heterozygous segment.

A population derived from the RHL was grown in 2005 in the paddy field at the China National Rice Research Institute (CNRRI), Hangzhou, Zhejiang Province, China. Twenty-two polymorphic SSR markers located in the target interval were used to assay the population. Seven sub-RHLs, named as RHL1 to RHL7, were selected. They carried smaller heterozygous segments and contained all the three genotype classes in the presumable region covering the *C* locus (Fig. 1). The seven resultant F_2 populations with 202, 208, 506, 689, 395, 880 and 399 plants, respectively, were grown from November 2005 to April 2006 in Lingshui, Hainan Province, China. Coloration of apiculus and basal leaf sheath of each F_2 plant was recorded. F_3 families of the populations showing coloration segregation were grown at the CNRRI in 2006 to determine the F_2 genotype at the *C* locus.

SSR genotyping and data analysis

At 7–10 d after transplanting of the seven F_2 populations, a 3-cm long young leaf was collected from each F_2 plant for DNA mini-preparation following the method of Zheng et al [10]. SSR genotyping and linkage analysis were performed for the two populations that exhibited coloration segregation, including 880 plants from RHL6 and 399 plants from RHL7.

A three-step analysis was employed for mapping *C* locus in this study. Firstly, marker intervals harboring the *C* locus was determined by comparing the marker genotypes of the seven sub-RHLs and the coloration performance of the resultant F_2 populations. Secondly, the *C* locus was mapped using the populations derived from RHL6 and RHL7. Thirdly, recombinants between the flanking markers of the *C* locus were selected and assayed with more markers in this region. The region for *C* locus was narrowed down.

In addition to SSR markers that were selected from Gramene (www.gramene.org), a marker derived from portions of the *C* gene candidate *OsC1* was also used. Mikami et al [11] sequenced PCR products amplified with primers derived from *OsC1* and found that rice lines showing colorless apiculus have a 10-bp deletion in the third exon of *OsC1*. This deletion was later located in 795–804 bp of the *OsC1* gene [9]. Since the primer combinations reported previously did not generate expected products for the parental lines used in this study, a new primer was applied in combination with primer 3# that has the sequence of 5'-GATCG ATCGTGTATATATGTTGTCAGGT-3' [11] and the location of 713–740 bp in *OsC1*. The new primer, designed according to the *OsC1* sequence of Nipponbare (www.gramene.org), has a sequence of 5'-GCACGACGGAGCTGGACGAC-3' and the position of 898–917 bp in *OsC1*. This primer combination was called C3C8 in this paper.

PCR conditions were as follows: initial denaturing at 94˚C for 2 min followed by 30 cycles of denaturing at 94˚C for 45 s, annealing for 45 s, extension at 72˚C for 1 min, and a final extension at 72˚C for 8 min. The annealing temperature was 50˚C for RM19559, 59˚C for C3C8, and 55˚C for the others. PCR products were visualized on silver-stained non-denaturing polyacrylamide gels as described by Shi et al [12]. Linkage analysis was performed with MAPMARKER/EXP 3.0 [13], and map distances presented in centiMorgans (cM) were derived from the Kosambi function.

RESULTS

Primary mapping of the *C* locus

In the seven F_2 populations grown in Hainan Province from November 2005 to April 2006, the