

**Barley anthocyanin-less 1 (Ant1) encodes an R2R3 MYB transcription factor that is orthologous to the C1 gene of maize and rice**

Shin Taketa and Eiko Himi

Institute of Plant Science and Resources, Okayama University, 2-20-1,  
Chuo, Kurashiki, Okayama 710-0046, Japan

We recently isolated a strong candidate gene of barley anthocyanin-less 1 on the basis of an inference to wheat MYB genes that control coleoptile pigmentation (Himi and Taketa, in press). We have reinforced this hypothesis by molecular mapping using barley EST based-markers. Mapping was performed in 96 F2 plants derived from a cross between ant1.1 (induced in Bonus) and OUI026 (red pigmented leaf sheath). Public SSR (Varshney et al. 2007) and EST (Sato et al. 2007) markers and 3G12 marker (Taketa et al. 2008) were employed. F2 plants and their parents were grown in autumn 2013 to 2014 spring season in an unheated green house. Plant materials were fully exposed to winter coldness and sunlight. Extra care was taken to avoid mechanical damages to the plants, which could cause stress-induced erroneous red pigmentations. Phenotypes of anthocyanin-less 1 were surveyed several times from the tillering stage to the booting stage.

The ant1.1 phenotype completely cosegregated with the presence or absence polymorphism of an R2R3 MYB transcription factor, HvC1, named after its significantly high homology to the maize and rice C1 transcription factor. The HvC1 was flanked by markers k01269GR and Bmac0187 with the genetic distances of 2.1 and 1.1 cM, respectively, on the proximal region of the short arm of barley chromosome 7H (Fig. 1). With 2.1-cM further proximally, another EST marker k07683GR was mapped, thereby, the HvC1 candidate region (a 5.3-cM region) was covered by barley EST markers, which shows micro-collinearity to the 3.77 Mb-interval on the short arm of rice chromosome 6 (Fig.1). In the candidate HvC1 region, gene-based barley markers showed highly consistent order with rice homologues except for one minor inversion. This reinforces our conclusion that barley Ant1 encodes an R2R3 MYB transcription factor orthologous to the C1 gene.

So far, four induced mutants and one naturally occurring ant1 mutant that is represented by the ant1.b allele of cv. Bowman, have been documented (Franckowiak 2012). DNA sequencing of mutant HvC1 alleles relative to the wild-type one revealed that two alleles, namely ant1.1 and ant1.2 are complete deletions, probably reflecting their irradiation induced origins (ant1.1 by alpha-rays and ant1.2 by neutrons). In ant1.4 and ant1.56, no DNA sequence changes were detected from the start codon to the stop codon. Allelism tests were performed using the ant1.1 null mutant as a tester. As expected, F1 hybrids between ant1.1 x ant1.2 had colorless leaf sheath. Ambiguous results were obtained for ant1.4; this mutant shows a faint pink pigmentation, and F1 hybrids between ant1.1 and ant1.4 show a fainter pigmentation. The allelic status of ant1.4 needs to wait more detailed analysis of ant1.4. This is because mutation or epigenetic changes might be found in the neighboring regions including the promoter region. However, ant1.56 and its F1 hybrids with ant1.1 had dark red leaf sheath (Fig.2). Thus, ant1.56 is not an allele at the ant1 locus, but that the original true stock has likely been lost (personal communication from Dr. Udda Lundqvist).

The present identification of a key regulator for anthocyanin pigmentation, Ant1, in barley will accelerate future flavonoid biosynthesis pathway studies. It should also advance relevant research of barley grain traits, such as seed dormancy and brewery quality attributes.

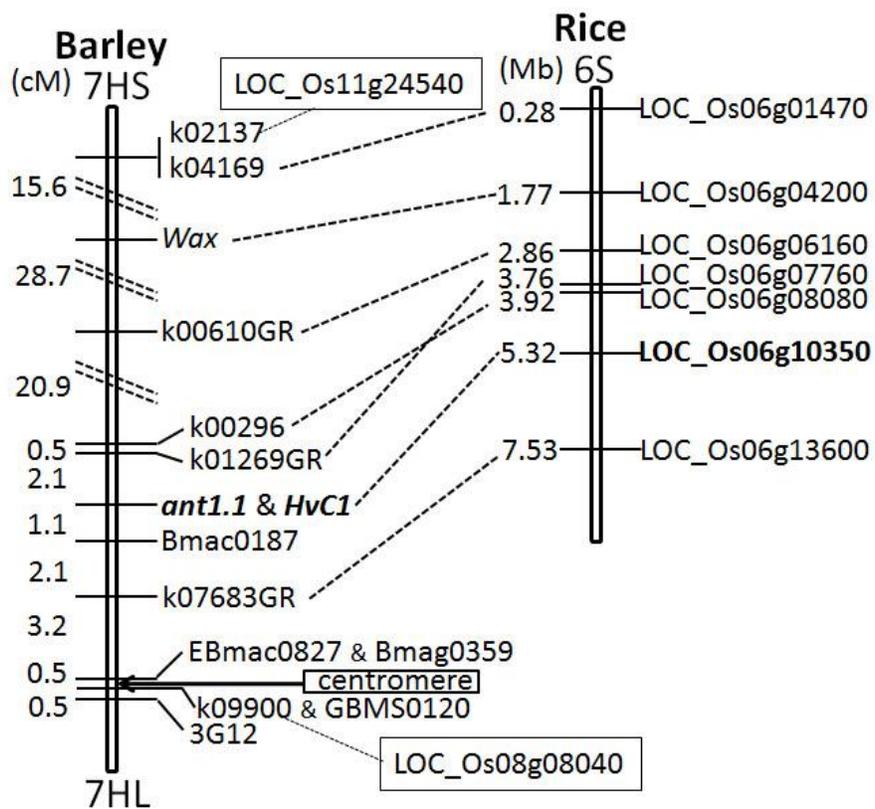


Figure 1. Molecular map of *ant1.1* in a 96 F<sub>2</sub> population derived from a cross with OUI026, and microcollearity of barley gene-based markers with rice chromosome 6 genes.

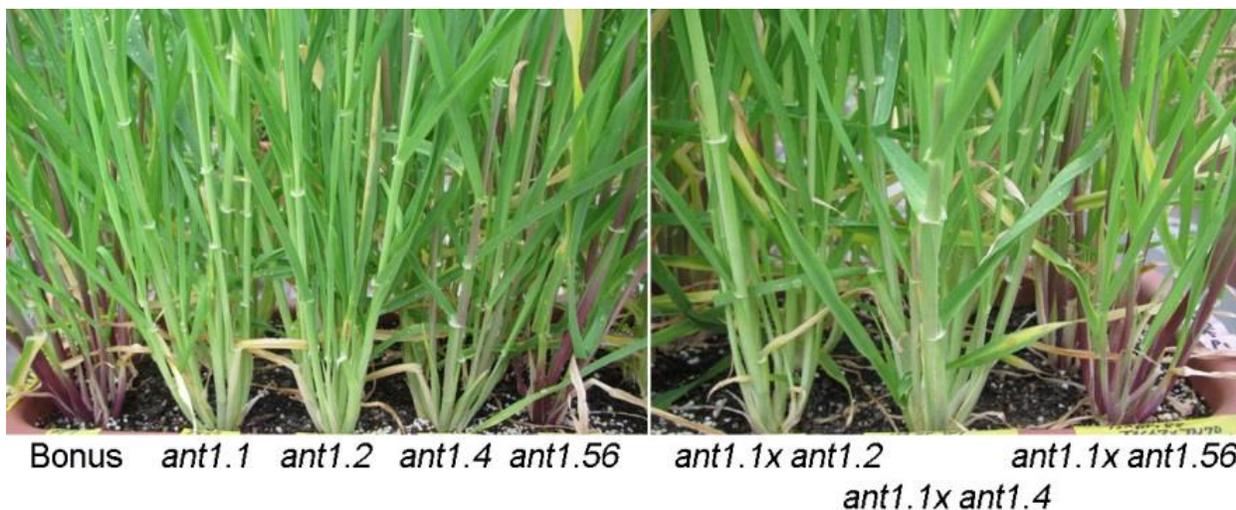


Figure 2. Leaf sheath base colors in the wild type Bonus and their four induced *ant1* mutants. Allelism tests in F<sub>1</sub> hybrids of *ant1.1* with the others.

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