

Towards Fine Mapping of *Cdu1*, a Major Gene Regulating Cadmium Accumulation in Durum Wheat Grain

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INTRODUCTION

Cadmium (Cd) is an environmental contaminant that can have negative effects on human health. The dominant source of human exposure to environmental Cd is through contaminated food. Among cereals, some durum wheat (*Triticum turgidum* L. var *durum*) cultivars accumulate Cd in grain to levels exceeding proposed international standards of 100 ppb. Genetic variation in grain Cd levels are known and genotypic differences are most closely associated with differences in Cd root-to-shoot translocation. A single gene for Cd concentration, designated as *Cdu1*, has been reported (Knox et al. 2009), but the genetic factor(s) conferring the low Cd phenotype are currently not known. Our ultimate goal is to achieve sufficient saturation of this region to allow positional cloning of *Cdu1* in durum wheat.

MATERIALS AND METHODS

Marker *ScOPc20* is linked to *Cdu1* and was first mapped in the Chinese Spring (CS) x CS DIC 5B mapping population because this population has been well saturated with expressed sequence tag (EST) markers (Lu and Faris, 2006). This allowed identification and development of additional EST-derived markers (ESMs, Expressed Sequence Markers) that were analyzed for polymorphisms in Kofa (high Cd) and W9262-260D3 (low Cd) using capillary electrophoresis and/or single strand conformational polymorphism (SSCP) assays. Polymorphic ESMs were mapped relative to *Cdu1* in a doubled haploid population of 155 lines from the cross Kofa x W9262-260D3. Grain Cd data from two environments (Knox et al. 2009) were used to localize a quantitative trait locus (QTL) using composite interval mapping. BLASTX of EST sequences was used to establish macro-colinearity between the *Cdu1* region and rice chromosome 3.

RESULTS AND DISCUSSION

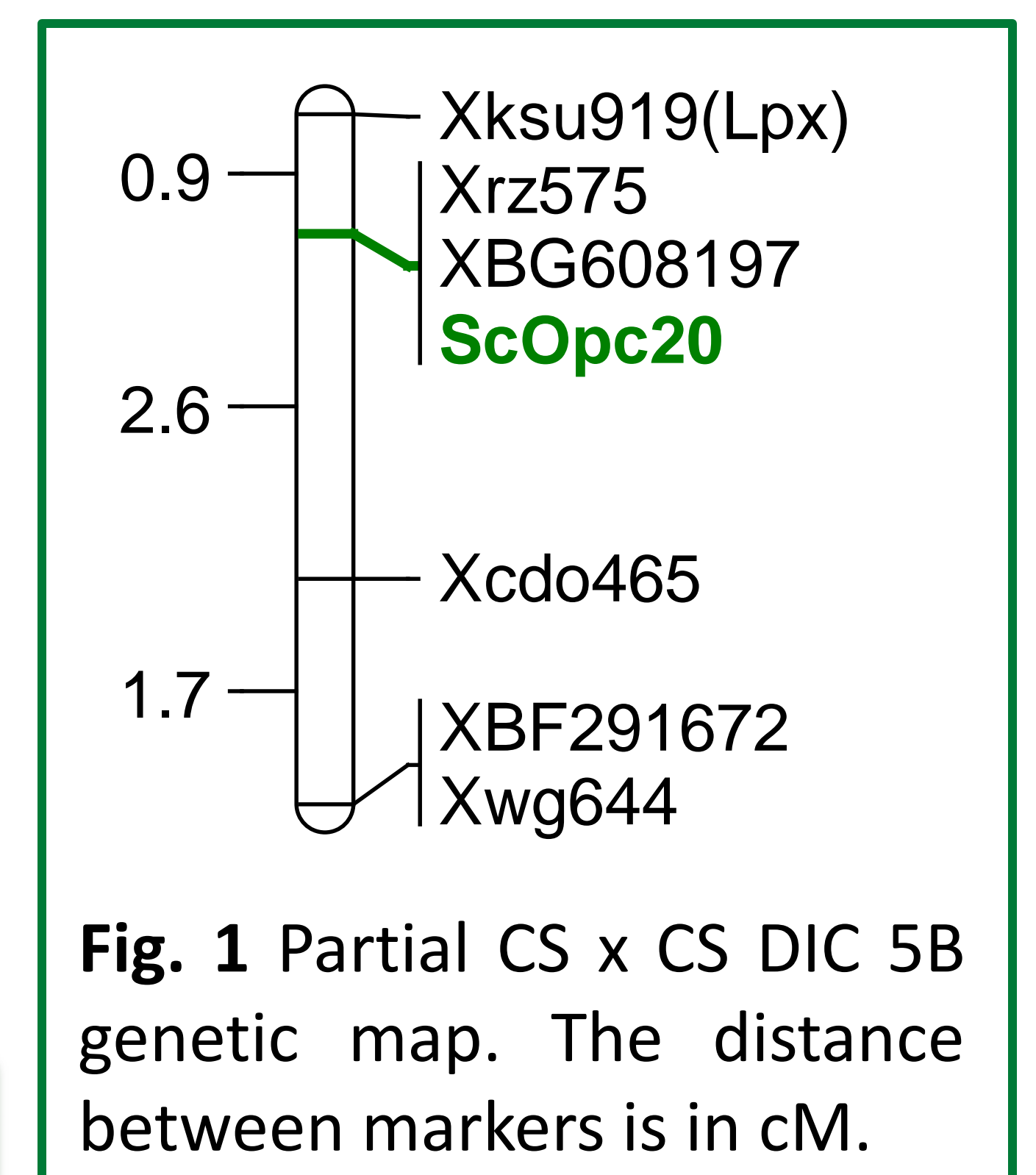


Fig. 1 Partial CS x CS DIC 5B genetic map. The distance between markers is in cM.

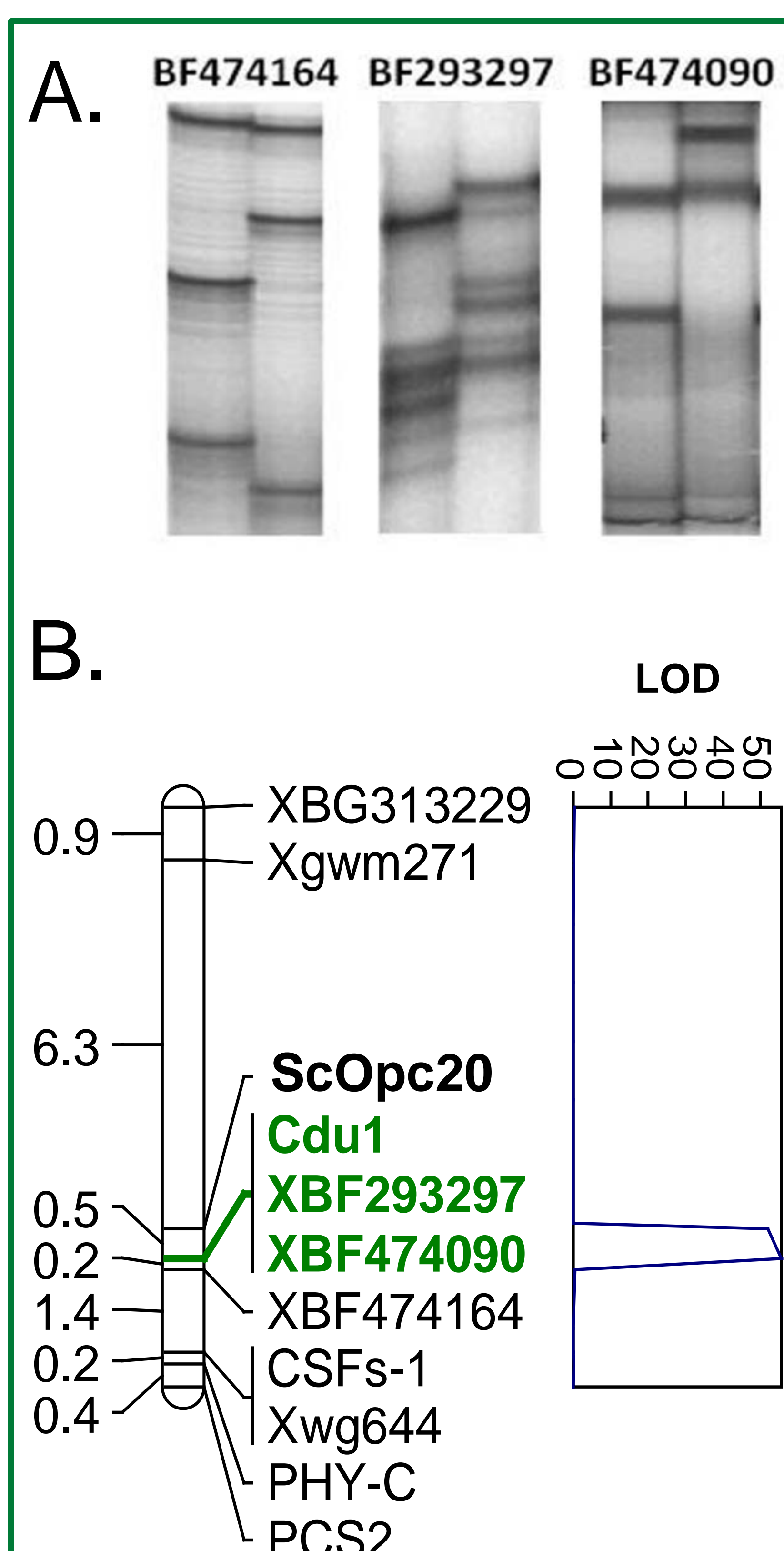


Fig. 2 (A) Polymorphic ESMs identified using SSCP. For each marker, lane 1 is Kofa and Lane 2 is W9262-260D3. **(B)** Composite interval map of *Cdu1*. Distance between markers is in cM.

ScOpc20 was polymorphic in the CS x CS DIC5B population and mapped 4 cM proximal to *Xwg644* and co-segregated with *XBG608197* (Figure 1). Both markers have been localized previously to BIN 5BL9 0.76-0.79 (Lu and Faris, 2006). As such, primers were designed for approx. 30 ESTs known to map to that BIN. EST-derived markers (ESMs) *BF474164*, *BG313229*, *BF293297* and *BF474090* were polymorphic between Kofa and W9262-260D3 (Figure 2A) and mapped to chromosome 5B (Figure 2B). In addition, four ESMs, including *Xwg644*, *Phy-C*, *Phytochelatin synthase 2 (PCS2)* and *CSFs-1* were also mapped (Figure 2B). Two of these markers were of interest as *Xwg644* is an ABC transporter which are associated with Cd accumulation in *Arabidopsis* (Bovet et al. 2005), and *PCS2* is a known chelator of Cd in plants (Cazale et al. 2001). In addition these two genes are linked to *Vrn-B1* (and thus vernalization response in wheat) and any associations with grain Cd concentrations could have consequences for durum wheat breeding. These markers however mapped >1.4 cM from *Cdu1* and could be ruled out as candidates (Figure 2). Two ESMs, *XBF293297* and *XBF474090*, co-segregated with *Cdu1* and were flanked by *ScOpc20* and *XBF474164*. This was confirmed with QTL analysis (Figure 2) where *XBF293297* and *XBF474090* explained approx. 80% of the phenotypic variation in grain Cd concentration. We have used these ESMs to anchor to the rice genome (Figure 3) and results suggest good macro-colinearity between the *Cdu1* region and a region on rice chromosome 3. This level of colinearity should allow identification of additional ESMs to further saturate this region for finer mapping. We are currently screening a large F₂ (>3000 gametes) population derived from crossing a pair of high and low Cd accumulating isogenic lines with *BF293297*, *BF474090* and *BF474164* for finer mapping of this region. We have already identified BACs containing these ESMs and these are currently being sequenced to provide an additional source of markers to aid in the localization of *Cdu1* in durum wheat.

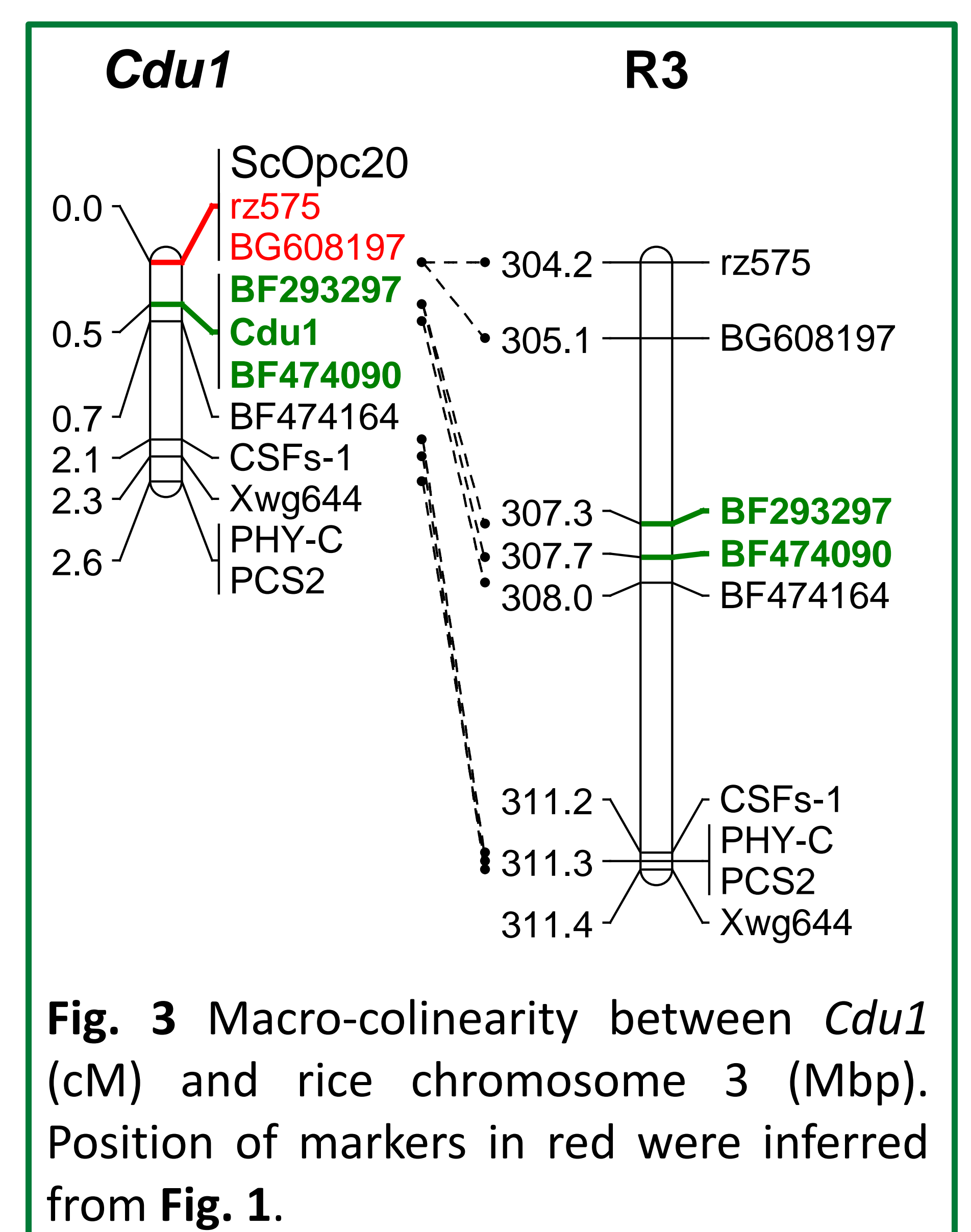


Fig. 3 Macro-colinearity between *Cdu1* (cM) and rice chromosome 3 (Mbp). Position of markers in red were inferred from Fig. 1.

References

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