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Locating the *Dw6* Dwarfing Locus using Near Isogenic Lines

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Introduction

In crosses involving multiple height modifying genes, deducing the genotype of intermediate height plants can be a challenge. Molecular markers linked to each locus could be used diagnostically in such situations.

Milach et al (1977) determined that the *Dw6* dwarfing locus was closely linked to the RFLP locus umn145 (3.3 +/- 1.3 cM) in their study population (OT207 x Kanota) but were unable to assign them to a linkage group. They were also unable to map the *Dw6* locus on the Kanota x Ogle (KO) reference map since neither *Dw6* nor the linked crown rust resistance gene *Pc 91* is segregating in KO and since the probe umn145 was monomorphic. However, subsequent aneuploid analysis suggested that the *Dw6* dwarfing locus is on KO 33 (Fox et al 2001). Recently a SNP-REMAP marker and a SNP-RAPD marker were developed which are located 5.2 and 12.6 cM from *Dw6* in the F2 mapping population Aslak x Kontant (Tanhuanpaa et al 2006) but the map location of these three loci was not yet determined.

We have utilized a set of seven pairs of Near Isogenic Lines (NILs) (Kibite 2001) to locate the *Dw6* locus and associate with it multiple molecular markers.

Materials and methods

Dr. Kibite used OT257 as the *Dw6* donor to develop seven pairs of NILs. The first three were from the cross Jasper/OT257; the fourth from the reciprocal cross; the fifth and sixth from OT256/OT257; the seventh from OT257/N326-7 (Kibite 2001). Each NIL pair consists of two F8:10 families, selected from a single heterozygous F8 individual, derived by Single Seed Descent from a single F2 individual.

Molecular marker analysis used standard Random Amplified Polymorphic DNA (RAPD), Amplified Fragment Length Polymorphism (AFLP) and Restriction Fragment Length Polymorphism (RFLP) protocols (Wight et al 2003) but a fourth selective nucleotide was used to simplify the AFLP patterns. Markers were selected for this study based on the Kanota x Ogle (Wight et al 2003) and Terra x Marion (De Koeyer et al 2004) recombination maps. The Sequence Characterized Amplified Region (SCAR) marker ubc333ks was developed by Orr and Molnar (unpublished).

Results and discussion

We found that the probe umn145 was polymorphic in the TM mapping population and were successful at mapping a umn145 locus to TM 25 (De Koeyer et al 2004). However, the umn145 allele sizes in TM differ from those reported by Milach et al (1997) in OT207 x Kanota, suggesting that the umn145 locus on TM 25 may be either homologous or homoeologous to the region carrying the *Dw6* locus. Comparative mapping between KO 33 and TM 25 revealed that KO 33 has a cdo 1321b locus and TM 25 has a cdo 1321a locus but that since allele sizes differ, these are likely homoeologous linkage groups (data not shown). The putative homoeology of KO 33 and TM 25 is further supported by the fact that KO 33 in fact shares more marker loci with TM 1 (figure), and TM 25 with KO 36, which are therefore their putative homologs respectively.

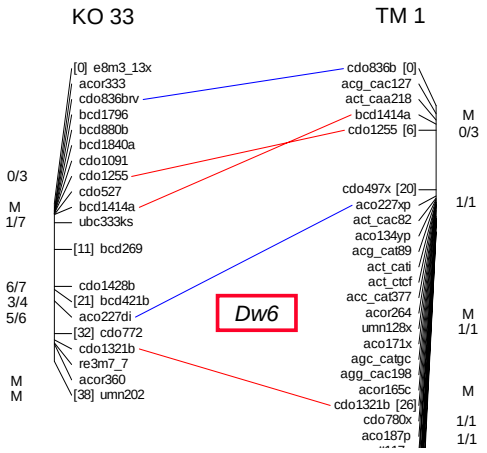

Figure: Location of Dw6 on KO33 deduced from the fraction of polymorphic NILs whose marker genotypes correlated with dwarf/tall for markers from KO33 and TM1. M=monomorphic

To more precisely locate the *Dw6* locus, all available RFLP and PCR-based markers selected from KO 33, and its homolog TM 1 (figure), were used to genotype the *Dw6* NILs to test for association. The seven pairs of NILs were developed from three parental combinations and only polymorphic parental combinations provided any useful information.

A perfect correlation was not found for any marker. However, excellent correlations were found for neighbouring KO markers cdo1428b (6 of 7 NILs), bcd 421b (3 of 4), and aco227di (5 of 6) indicating that *Dw6* is closely linked to this chromosomal region. Flanking marker ubc333ks (1 of 7) defines one boundary for the *Dw6* region, and this conclusion is supported by the TM marker cdo1255 (0 of 3). The other boundary is not well defined due to low frequencies of polymorphism in both KO and TM.

AFLP analysis using eight primer pairs identified an additional two potential markers for *Dw6*, however these have not yet been mapped on KO. Based on approximately 100 polymorphisms found between the parents of each family of NILs, we conclude that on average there remains approximately 5% polymorphism between the members of each NIL pair. This is much higher than the 0.4% expected and indicates extensive residual heterozygousity remaining in regions of the genome unlinked to *Dw6*.

Conclusions

We utilized a set of seven pairs of NILs to locate the *Dw6* locus to a small chromosomal region on KO 33 near RFLP loci cdo1428b, bcd421b and aco227di. This identifies many markers linked to *Dw6* which complement the two reported by Tanhuanpaa et al (2006). Such markers have potential for marker assisted breeding for *Dw6* as well as for the linked *Pc91* crown rust resistance locus. Higher than expected residual heterozygousity within the NILs addresses the development and genetic structure of NILs.

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