

A high throughput method of EcoTILLING for detecting haplotype diversity within the aluminium resistance gene (*TaALMT1*) of wheat

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TaALMT1 (Aluminium activated Malate Transporter of *Triticum aestivum* L), the gene controlling aluminium resistance via an Al-activated malate efflux mechanism, has recently been cloned, characterised and mapped on the long arm of chromosome 4D of wheat. The identification of novel alleles associated for Al resistance is required for further continual improvement. Besides, SNP and CEL 1 restriction enzyme based methods for EcoTILLING, gene specific markers based on INDELS and SSR could be utilised for preliminary genotyping and subsequently as a result of allele binning, unique haplotypes can be verified by sequencing. In order to validate this approach, we determined allelic diversity at the *TaALMT1* locus in a wide range of 1425 germplasm collections that included wheat cultivars, landraces, *T. aestivum* ssp. *spelta*, ssp. *macha*, ssp. *sphaerococcum*, ssp. *compactum* and ssp. *vavilovii*, and D genome containing accessions of *Aegilops tauschii*, *A. cylindrica* and tetraploid and hexaploid *A. crassa*, *A. ventricosa*, *A. vavilovii* and *A. juvenale*. *TaALMT1* gene specific markers targeting repetitive INDELS within intron 3 region and an upstream region were employed sequentially using pooling approach. The repetitive INDEL marker detected 9 alleles. A subset of germplasm with different alleles was genotyped with the long and short fragment markers targeting the upstream sequence of the *TaALMT1* gene. Alleles were binned and the data were confirmed by sequencing the unique haplotypes. The haplotype data were compared on a subset of genetically diverse wheat collections previously identified utilising DArT marker loci. The presence of *TaALMT1* alleles in *A. tauschii* and the absence of *TaALMT1* alleles within 'non-D' genome species reconfirmed that *A. tauschii* is a donor source of Al tolerance in modern wheats. Al-activated malate efflux measurements indicated that Al-resistance is indeed conditioned by the *TaALMT1* gene.