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HIGH RESOLUTION MAP OF THE ALUMINUM TOLERANCE GENE (*ALT3*) REGION IN RYE (*SECALE CEREALE* L.)

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ABSTRACT

Cloning an aluminum (Al) tolerance gene in rye (*Secale cereale* L.) is very difficult because of the lack of a heterologous probe, a transposon tagging system and a powerful strategy for map-based cloning of the gene. In addition, rye has a large and complex genome that makes cloning the gene through map-based cloning almost impossible. The goal of this research was to construct a high resolution map of the Al tolerance gene (*Alt3*) region in rye by utilizing the rye-rice (*Oryza sativa* L.) syntenic relationship. A rice bacterial artificial chromosome (BAC) sequence was used to develop new molecular markers to saturate the *Alt3* region in rye. Restriction fragment length polymorphism (RFLP), amplified fragment length polymorphism (AFLP), and polymerase chain reaction (PCR) techniques were all used to achieve the goal.

Aluminum tolerance in a rye F_6 RIL population derived from Al-tolerant (M39A-1-6) and Al-sensitive (M77A-1) parents was determined to be controlled by a single dominant gene (*Alt3*) located on the long arm of chromosome 4. Five RFLP markers, five AFLP markers and two PCR-based co-dominant markers were linked and/or co-segregating with the *Alt3* gene. The high resolution map of the *Alt3* region showed that *Alt3* was located 0.05 cM between markers BCD1230 and B6. The *Alt3* spanning region between the two flanking markers corresponds to a 10 kb region of the rice BAC that contains four rice candidate orthologue *Alt3* genes. Two RFLP markers, B11 and B26,

developed from two of the rice candidate orthologue *Alt3* genes, co-segregated with the *Alt3* gene in rye. The other two rice candidate orthologue *Alt3* genes could not be mapped to the *Alt3* region in rye due to a lack of polymorphisms.

A syntenic relationship between the *Alt3* region in rye and the rice BAC was established, and six genes in the rice BAC sequence were mapped to the *Alt3* region in rye in the same order as the genes located in the rice BAC sequence. The microco-linearity between the two species at the kilobase level has made imminent the isolation of the *Alt3* gene through utilization of the map-based cloning technique and the rye-rice syntenic relationship.