Development of EST and AFLP markers linked to a gene for resistance to ryegrass blast (*Pyricularia* sp.) in Italian ryegrass (*Lolium multiflorum* Lam.)

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Introduction Ryegrass blast, caused by the fungus *Pyricularia* sp. is one of the most serious diseases of Italian ryegrass (*Lolium multiflorum* Lam.). However, in Italian ryegrass, except for a new cultivar 'Sachiaoba', no resistant cultivars were found in Japan (Mizuno *et al*., 2003). For these reasons, improving the disease resistance of Italian ryegrass is one of the most important goals in the breeding programs. The aim of this study was to identify genes for resistance to ryegrass blast and to develop EST and AFLP markers linked tightly to the resistance genes.

Materials and methods To develop genetic mapping populations, we generated a segregating F_1 population from a cross between two heterozygous individuals: a resistant individual of cv. 'Sachiaoba' and a susceptible individual of cv. 'Minamiaoba'. Three-week-old F_1 plants were inoculated with a suspension of spores at a concentration of 5×10^4 spores/ml. The location of the resistance gene was determined from the marker order in the linkage group and the phenotypic data of the F_1 population by interval mapping in MapQTL. The position of the gene was estimated at the maximum LOD score with a 1-LOD support interval.

Results The phenotypes of the F_1 population consisting of 161 individuals showed 81 resistant individuals and 80 susceptible individuals. From the set of 512 AFLP primer combinations, we screened 25 combinations with polymorphic bands. All of the screened AFLP markers segregated in a 1:1 (present:absent) ratio in the F_1 population (Figure 1a). Linkage analysis revealed that all 25 markers formed one linkage group with one resistance gene (*LmPi1*) from the resistant parent (Figure 2). Of 30 EST-CAPS markers mapped on a reference population for Italian ryegrass, one linked EST marker, p56, was found in the segregating F_1 population and the parents. The restriction patterns of p56 amplification with *Hha*I in the F_1 population showed two short fragments (Figure 1b). One fragment of 289 bp, derived from the resistant parent, segregated in a 1:1 (present:absent) ratio in the F_1 population and the fragment was corresponding to the resistant allele at the *LmPi1* locus. In our previous study, p56 was mapped on linkage group 5 (lg5) of the reference population. Therefore, the *LmPi1* locus is also located on lg5 in Italian ryegrass.

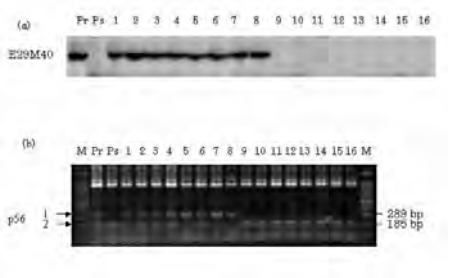

Figure 1 Segregation pattern of markers linked tightly to the resistance gene *LmPi1* in the F_1 population. The first two lanes are the resistant (Pr) and susceptible (Ps) parents, followed by eight resistant (1 to 8) and eight susceptible (9 to 16) individuals of the F_1 population.

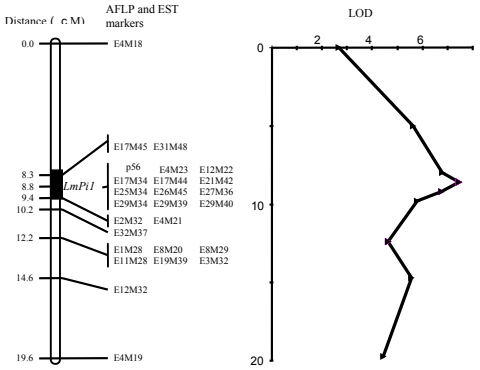

Figure 2 Linkage map showing the position of the QTL for the resistance gene *LmPi1*.

Conclusion The results indicate that the p56 marker could be used for the introduction of the *LmPi1* resistant allele into susceptible germplasm to enhance the resistance to ryegrass blast in Italian ryegrass breeding.

Reference

Mizuno K, Yokohata Y, Oda T, Fujiwara T, Hayashi K, Ozaki R, Kobashi K, Ashizawa H, Ushimi T (2003) Breeding of a new variety 'Sachiaoba' in Italian ryegrass (*Lolium multiflorum* L.) and its characteristics. (in Japanese) Bull Yamaguchi Agric Expt Stn 54:11-24