Development of EST-derived simple sequence repeat (SSR) markers in Italian ryegrass (*Lolium multiflorum* Lam.)

M. Hirata[1], Y. Miura[1], T. Takamizo[2] and M. Fujimori[2]
[1]*Japan Grassland Farming & Forage Seed Association, Forage Crop Research Institute, 388-5 Higashiakada, Nasushiobara, Tochigi 329-2742, Japan, Email: hirata@jfsass.or.jp*
[2]*National Institute of Livestock and Grassland Science, 768 Senbonmatsu, Nasushiobara, Tochigi 329-2793, Japan*

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Introduction Italian ryegrass (IRG, *Lolium multiflorum* Lam.) is an important grass species used for agricultural purposes in Japan. Linkage maps in IRG have been constructed using molecular markers including RFLPs, AFLPs (Inoue *et al*., 2003) and genomic SSRs. The SSR marker is a useful tool for genome analysis because it is PCR-based with a relatively high level of polymorphism, co-dominantly expressed. The EST-SSR markers are expected to be more conserved and have a higher rate of transferability than genomic SSR markers (Scott *et al*., 2000), and the gene functions of ESTs may be predicted. Ikeda *et al*. (2004) have developed an IRG EST database consisting of 54,811 sequences. The aim of this study was to develop EST-SSR markers using this EST database and assign them into a reference map of IRG.

Materials and methods Clones containing a SSR motif (dinucleotide or trinucleotide repeats; ≥8 repeats) were selected from the IRG EST database. Primers were designed with PRIMER0.5 software. Primer combinations that gave a good signal in the parents were used for analysis. Sixty individuals of the reference population produced by a cross between 11S2 and 11F3 were used for mapping. Linkage analysis was performed with JoinMap software (LOD=4.0) using EST-SSR markers, selected AFLP markers and genomic SSR markers.

Results The efficiency of EST-SSRs development is given in Table 1. Of the 54,811 ESTs used, 103 (0.2%) contained SSR motifs. Among them, 78 clones were unique and 67 clones could be used to design primers. Forty-nine clones amplified products of the expected size in the parents. Of them, 38 clones were dinucleotide repeats(19(GA)n, 12(CA)n, 6(TA)n, 1(GC)n) and 11 clones were trinucleotide repeats (4(CGG)n, 3(CTT)n, 1(CAA)n, 1(CAC)n, 1(CAG)n, 1(CTA)n). The average (maximum) number of dinucleotide and trinucleotide repeats were 12.5(43) and 9.1(15), respectively. Twenty-nine clones detected polymorphism among F_1 individuals. In the linkage analysis, EST-SSR markers were distributed across 7 linkage groups and 2-6 EST-SSR markers were mapped into each of the linkage groups (Figure 1).

Table 1 Efficiency of EST-SSRs development

	No. of clones	%
ESTs	54811	
Clones containing an SSR motif	103	0.2
Unique clones	78	76
Clones for primer design	67	86
Amplified clones in parents	49	73
Polymorphic clones in population	29	59
Mapped clones into reference map	21	72

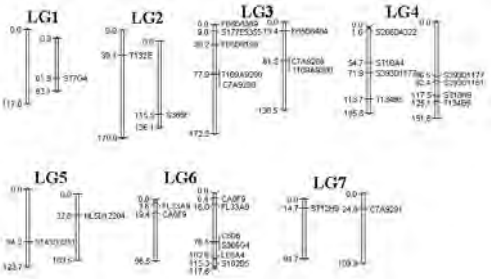

Figure 1 Linkage map of SSR markers and selected AFLP markers in the 11S2 × 11F3 2-way pseudo-testcross F1 population. (only EST-SSRs are shown)

Conclusions We developed 49 EST-SSRs using the IRG EST database. Twenty-nine clones could detect polymorphism among F_1 individuals. Finally 25 markers generated from 21clones were mapped into 7 linkage groups with 2-6 EST-SSR markers per group. The SSR markers developed in this study will be useful as core markers in genome analysis of IRG and related species.

References

Ikeda S, Takahashi W, Oishi H. (2004). Generation of expressed sequence tags from cDNA libraries of Italian ryegrass (*Lolium multiflorum* Lam.). Grassland Science 49, 593-598.

Inoue M, Gao ZS, Hirata M, Fujimori M, Cai HW (2004) Construction of a high-density linkage map of Italian ryegrass (*Lolium multiflorum* Lam.) using restriction fragment length polymorphism, amplified fragment length polymorphism, and telomeric repeat associated sequence markers. Genome 47:57–65

Scott KD, Eggler P, Seaton G, Rossetto M, Ablett EM, Lee LS, Henry RJ (2000) Analysis of SSRs derived from grape ESTs. Theoretical and Applied Genetics 100, 723-726