Development of SSR Markers for Variety Identification in Italian Ryegrass (*Lolium Multiflorum* Lam.)

M. Inoue
Forage Crop Research Institute, Japan

N. Yuyama
Forage Crop Research Institute, Japan

H. Cai
Forage Crop Research Institute, Japan

The XX International Grassland Congress took place in Ireland and the UK in June-July 2005. The main congress took place in Dublin from 26 June to 1 July and was followed by post congress satellite workshops in Aberystwyth, Belfast, Cork, Glasgow and Oxford. The meeting was hosted by the Irish Grassland Association and the British Grassland Society.

Proceedings Editor: D. A. McGilloway

Publisher: Wageningen Academic Publishers, The Netherlands

Development of SSR markers for variety identification in Italian ryegrass (*Lolium multiflorum* Lam.)

M. Inoue, N. Yuyama and H. Cai
Japan Grassland Farming & Forage Seed Association, Forage Crop Research Institute, 388-5, Higashiakada, Nasushiobara, Tochigi, 329-2742 Japan. Email: minoue@jfsass.or.jp

Keywords: molecular marker, SSR-enriched library, variety identification, Italian ryegrass

Introduction Italian ryegrass (IRG, *Lolium multiflorum* Lam.) is one of the most important cool-season forage grasses in the world, and is the most widely cultivated annual forage grass in Japan. Simple sequence repeat (SSR) markers have the advantages of being PCR-based, multiallelic and possessing high levels of polymorphism. They are very suitable for variety identification, especially for out-crossing species including IRG. The objective of this study was the development of SSR markers for variety identification in IRG.

Materials and methods An individual of IRG variety, Waseaoba, was used to construct the SSR-enriched genomic library. The repeat motifs used were $(CA)_n$, $(GA)_n$, $(AAG)_n$, $(AAT)_n$. The methods of sequencing and primer design etc. were same as that described by Cai *et al*. (2003). To screen the working primer pairs, a panel consisting of five IRG individuals randomly selected from five different varieties and three individuals representing the closely related species perennial ryegrass (PRG), meadow fescue (MF) and tall fescue (TF) were used.

Results A total of 4,000 clones (1,000 clones from each of four libraries) were sequenced. Of these, 796 unique SSR clones which could be used to design primers were identified and all of the four libraries contained perfect clones with very high frequencies, ranging from 88.6 % to 96.4 % (Table 1). So far, it has been found that out of the 140 primer pairs tested using the screening panel, 96 primer pairs (68.6 %) could amplify polymorphic SSR products and 23 primer pairs amplified PCR products but no polymorphisms in the five IRG individuals used (Figure 1). The rest of the 21 primer pairs were considered to be unsuitable for use because they amplified multi-copy products or amplified no bands.

Table 1 Efficacy of SSR isolation from IRG SSR libraries

Library	Motif	Clones sequenced	Unique SSR clones
A	CA/GT	1,000	151
B	GA/CT	1,000	305
C	AAG/TTC	1,000	148
D	AAT/TTA	1,000	192
Total			796

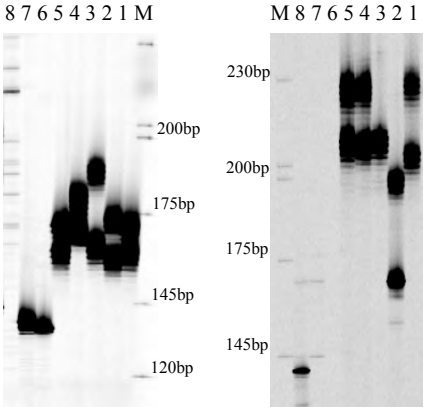

Figure 1 PCR products amplified by the loci B1H16 (left) and B1G14 (right) in the screening panel. Lane 1, IRG 1; lane 2, IRG 2; lane 3, IRG 3; lane 4, IRG 4; lane 5, IRG 5; lane 6, PRG; lane 7, MF; lane 8, TF, M, size marker

Future work After screening the working primers, we will use them to find specific SSR markers to distinguish the most frequently used 12 varieties of IRG in Japan.

References

Cai, H., N. Yuyama, H. Tamaki & A. Yoshizawa (2003). Isolation and characterization of simple sequence repeat markers in the hexaploid forage grass timothy (*phleum pretense* L.). Theoretical and Applied Genetics, 107, 1337-1349.