Amplification profiles of the SSR markers in cultivars of hexaploid wheat and their relatives

Nitta M and Nasuda S
Laboratory of Plant Genetics, Graduate School of Agriculture, Kyoto University, Kitashirakawaoiwake-cho, Sakyo-ku, Kyoto 606-8502 Japan, and NBRP-WHEAT, Japan

ABSTRACT

The National Bioresource Project-WHEAT (NBRP-WHEAT), launched by the Japanese government in 2002, aims to maintain and distribute seed stocks and DNA clones of "Wheat". The second-term NBRP-WHEAT started in 2007. Additionally to its primary roles in handling seed stocks and DNA clones, the second-term NBRP-WHEAT features the collection and characterization of DNA markers, which will make the seed stocks of NBRP-WHEAT more valuable for molecular works, such as, isolation and characterization of genes in wheat.

We started to survey amplification profiles of the publically available SSR markers (*barc*, *cfa*, *cfd*, *cfe*, *cft*, *gdm*, *gwm*, *hbg*, *hbe*, *hbd*, *wmc*, and STM markers) in cultivars of hexaploid wheat and their relatives. The lines to be tested include; eight *Aegilops* species with representative diploid genomes, *Triticum monococcum*, *T. boeoticum*, *T. urartu*, *T. durum*, *T. spelta* and 31 hexaploid wheat accessions. We also took samples of *Hordeum vulgare*, *H. spontaneum*, and *Secale cereale* as outgroup species. The PCR products using template DNA from these lines are separated on a capillary-electrophoresis machine and the amplification profiles are documented as chromatograph of fluorescence intensity and pseudo-electrophoretic-patterns. As pilot experiments, we tested 464 markers on 12 lines thus far and established the methodology for stable amplification of SSRs and reproducible separation of PCR fragments.

INTRODUCTION

In the first-term NBRP-WHEAT activity, more than 1150 seed stocks and 360000 EST clones were collected. These resources are open to he public through the web page (http://www.nbrp.jp/report/reportProject.jsp). To promote the utilization of the seed stocks in various molecular studies, we decided to add genotype information to some of our stocks.

Simple sequence repeat (SSR) is a PCR-based method to detect polymorphisms. SSR is widely used in wheat sciences such as linkage mapping, QTL mapping, marker-assisted selection, and phylogenetic survey.

In our second-term NBRP-WHEAT activity, we started to produce SSR profiles for 48 lines of wheat and its relatives. The objectives of the project are (1) to add genotype information to the stocks, and (2) to find a set of SSR markers suitable for detection of diversity in wheat species. The current status of our project is reported here.

MATERIALS AND METHODS

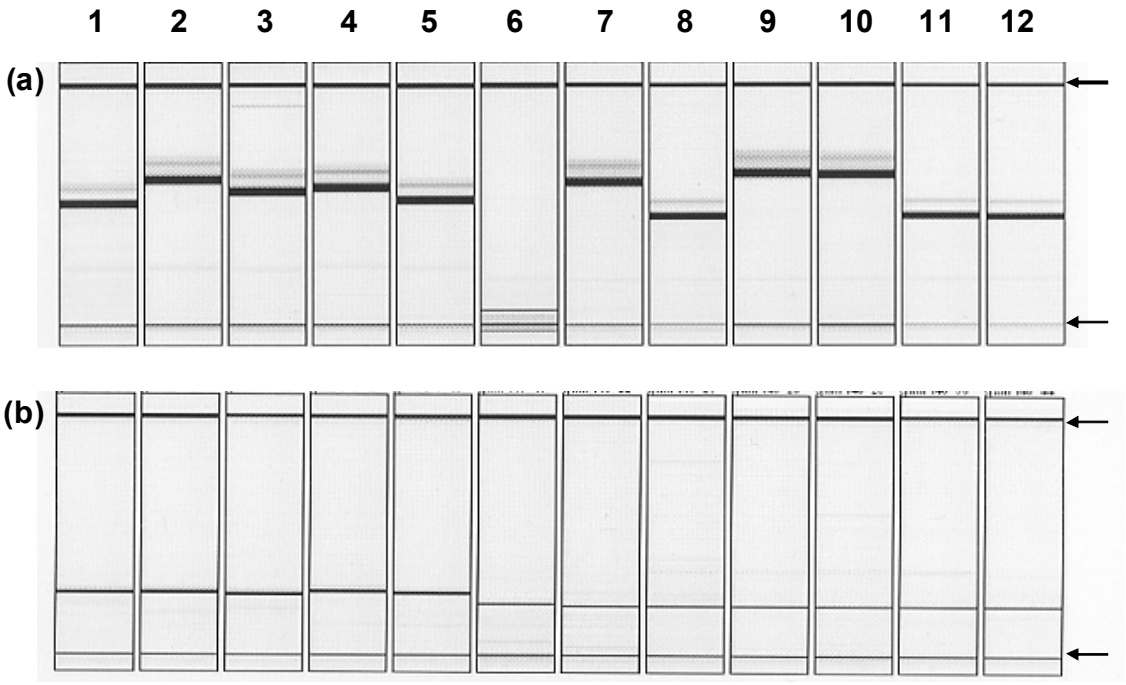
Plant materials: The following materials were used for DNA isolation; eight diploid *Aegilops* species with representative genomes [*Ae. speltoides* (genome: S), *Ae. longissima* (L), *Ae. tauschii* (D), *Ae. umbellulate* (U), *Ae. caudata* (C), *Ae. uniaristata* (N), *Ae. comosa* (M), and *Ae. mutica* (T)], diploid and tetraploid wheat [*T. monococcum*, *T. boeoticum*, *T. urartu*, and *T. durum* cv. Langdon], spelta wheat [*T. spelta* var. *duhamerianum*], and 27 cultivars or germplasms of common wheat (*T. aestivum*) [Chinese Spring, Timstein, Hope, Cheyenne, and others (mainly Japanese cultivars)]. We also included parental lines of the ITMI mapping population (Opata and Synthetic) as controls for the SSR marker. Rye (*Secale cereale* cv. Imperial) and barley *(Hordeum vulgare* cv. Betzes, *H. vulgare* cv. Harunanijyou, and *H. spontaneum*) were used as outgroup species.

SSR and STM primers: We obtained primer information from publications and databases. Currently more than 3000 SSR markers are publically available. After checking duplications, we synthesized a total of 2545 primer sets (1861 SSR primers and 684 STM primers). The markers consisted primary of *barc* (Song et al. 2005), *cfa* and *cfd* (Guyomarc'h et al. 2002; Sourdille et al. 2004), *gdm* (Pestsove et al. 2000), *gwm* (Röder et al. 1995, 1998), *hbg*, *hbe*, and *hbd* (Torada et al. 2006), and *wmc* (Gupta et al. 2002; Somers et al. 2004) markers. Primers for the STM markers were synthesized according to Hayden et al. (2002, 2004).

DNA isolation and PCR: Plant DNA was isolated from young fresh leaves with the DNeasy Plant Mini Kit (QIAGEN) and quantified by spectrophotometer. PCR reaction was carried out with the iCycler (BioRad). We used Takara ExTaq (Takara). The optimum PCR conditions were determined as described in the Results and discussion section. The PCR products were subjected to electrophoresis in a capillary gel-electrophoresis apparatus HAD-GT12 (eGene) with a gel cartridge GCK-5000. Electrophoretic patterns were analysed using the software supplied for the electrophoresis apparatus.

RESULTS AND DISCUSSION

Optimum conditions for PCR: First, we tested reproducibility of SSR amplification using the DNA samples of Opata and Synthetic as templates. 12 markers were randomly selected from *barc*, *cfa*, *cfd*, *gdm*, *gwm*, and *wmc* markers and amplified according to the conditions given by the original literature. However, in our hands, the number and size of amplified fragments



were quite different from those in the literature. We overcame this problem by addition of betaine and DMSO into the reaction mixture at the final concentrations of 1 M and 3%, respectively.

Amplification profiles: So far we tested 464 SSR markers on a subset of 12 lines (*Ae. tauschii*, Norin 26, Norin 61, Chihokukomugi, Sumai 3, Zenkojikomugi, Hanamanten, Abukumawase, *S. cereale* cv. Imperial, *H. vulgar*e cv. Betzes, *H. vulgare* cv. Harunanijo, and *H. spontaneum*), and 272 SSR markes on a different subset of 12 lines (Opata, Synthetic, *Ae. longissima*, *Ae. umbellulate*, *Ae. uniaristata*, *T. durum* cv. Langdon, *T. spelta*, Chinese Spring, Timstein, Hope, Cheyenne, and Chogokuwase). In total, we obtained 8292 amplification profiles. Because we set the PCR conditions stringent (high annealing temperature) to avoid amplification of ambiguous bands, the SSR amplification tends to be genome-specific. Figure 1 shows examples of amplification patterns of 12 hexaploid wheats. The marker gwm136 (Figure 1a) showed a highly polymorphic amplification pattern and gwm148 (Figure 1b) showed less polymorphic pattern. We performed the pair-wise comparison between Chinese Spring and Norin 26. By 96 primer combinations, 232 loci were identified in the two lines. Of them, 144 were polymorphic between the lines. Thus, the polymorphism rate was 0.621 between Chinese Spring and Norin 26. This may be an overestimation because we observed many null alleles in one sample.

FUTURE PROSPECTS

In the present investigation, we established conditions for PCR, electrophoresis, and data analysis of the SSR markers. We will perform a polymorphism survey on the selected 48 samples. We are planning to test at least 2000 markers on the 48 samples, resulting in 96000 PCR profiles. The amplification profile will be open to public through the web page of NBRP-WHEAT (http://www.shigen.nig.ac.jp/wheat/komugi/top/top.jsp). By the end of this project, we hope to link wheat accessions with genotyping data. These lines will be very useful for identification of genes of interests. Additionally, we will be able to select SSR markers that show high polymorphism index.

ACKNOWLEDGEMENTS

We thank Drs. Bernd Friebe, Takashi R. Endo, Taihachi Kawahara, Koji Murai, Yoshihiro Matsuoka, Hisashi Tsujimoto, Masaya Fujita, Kenji Kato, Hideho Miura,

Tomohiro Ban, Kazuhiro Nakamura, Hitoshi Matsunaka, and Kazuhiro Sato for providing seeds of the lines analysed in this study. We also thank the Japanese wheat scientist community for valuable suggestions given during selection of representative wheat lines. We especially thank Dr. Masaya Fujita for his coordination to access to the seed stocks kept in the National Agricultural Research Center of Japan. This project is supported by the National Bioresource Project (NBRP), from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

REFERENCES

Gupta P.K., Balyan H.S., Edwards K.J., Isaac P., Korzun V., Röder M., Gautier M.F., Joudrier P., Schlatter A.R., Dubcovsky J., De la Pena R.C., Khairallah M., Penner G., Hayden M.J., Sharp P., Keller B., Wang R.C.C., Hardouin J.P., Jack P., Leroy P. 2002. Genetic mapping of 66 new microsatellite (SSR) loci in bread wheat. *Theor Appl Genet* 105:413–422

Guyomarc'h H., Sourdille P., Charmet G., Edwards K.J., Bernard M. 2002. Characterization of polymorphic microsatellite markers from *Aegilops tauschii* and transferability to the D genome of bread wheat. *Theor. Appl. Genet.* 104:1164–1172

Hayden M.J., Good G., Sharp P.J. 2002. Sequence tagged microsatellite profiling (STMP): improved isolation of DNA sequence flanking target SSRs. *Nucleic Acids Res.* 30:e129

Hayden M.J., Stephenson P., Logojan A.M., Khatkar D., Rogers C., Koebner R.M.D., Snape J.W., Sharp P.J. 2004. A new approach to extending the wheat marker pool by anchored PCR amplification of compound SSRs. *Theor. Appl. Genet.* 108:733–742

Pestsova E., Ganal M.W., Röder M. 2000. Isolation and mapping of microsatellite markers specific for the D genome of bread wheat. *Genome* 43:689–697

Röder M.S., Plaschke J., Konig S.U., Börner A., Sorrells M.E., Tanksley S.D., Ganal M.W. 1995. Abundance, variability and chromosomal location of microsatellites in wheat. *Mol. Genet. Genomics* 246:327–333

Röder M.S., Korzun V., Wendehake K., Plaschke J., Tixier M.H., Leroy P., Ganal M.W. 1998. A microsatellite map of wheat. *Genetics* 149:2007–2023

Somers D.J., Isaac P., Edwards K. 2004. A high density microsatellite consensus map for bread wheat (*Triticum aestivum* L.). *Theor. Appl. Genet.* 109:1105–1114

Song Q.J., Shi J.R., Singh S., Fickus E.W., Costa J.M., Lewis J., Gill B.S., Ward R., Cregan P.B. 2005. Development and mapping of microsatellite (SSR) markers in wheat. *Theor. Appl. Genet.* 110:550–560

Sourdille P., Singh S., Cadalen T., Brown-Guedira G.L., Gay G., Qi L., Gill B.S., Dufour P., Murigneux A., Bernard M. 2004. Microsatellite-based deletion bin system for the establishment of genetic-physical map relationships in wheat (*Triticum aestivum* L.). *Funct. Integr. Genomics* 4:12–25

Torada A., Koike M., Mochida K., Ogihara Y. 2006. SSR-based linkage map with new markers using an intraspecific population of common wheat. *Theor. Appl. Genet.* 112:1042–1051