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Full Length Research Paper

Construction of genetic map in barley using sequence-related amplified polymorphism markers, a new molecular marker technique

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Sequence-related amplified polymorphism (SRAP) markers, a novel polymerase chain reaction (PCR)-based molecular marker technique, were successfully applied in map construction, cultivar identification, diversity evaluation, comparative genomics and gene location of different plant species. The molecular genetic map of SRAP markers in Steptoe / Morex doubled haploid (DH) population was constructed in this study, using 216 SRAP markers and 312 simple sequence repeat (SSR) markers. Overall, 21 of the 216 SRAP markers generated 78 polymorphic loci, and 98 of 312 SSR markers produced 107 polymorphic loci. Among the 185 loci, 175 loci (70 SRAP loci and 105 SSR loci) were assigned to nine linkage groups. The map covered 1475 cM with a mean density of 8.7 cM per locus. In total, 33 of all the loci (17.84%) showed significant segregation distortion. Moreover, 23 of the 33 loci (69.7%) skewed towards the parent Steptoe, whereas the remaining loci (21.3%) deviated towards the parent Morex and some of these distorted loci tended to cluster at the end of linkage groups, while others were dispersed on linkage groups in a decentralized fashion. The three putative segregation distortion regions (SDRs) were detected on chromosomes 2H, 4H and 5H, respectively. This linkage map indicates its importance in quantitative trait loci (QTLs) mapping, marker-assisted selection (MAS) and integrative analysis for further genetic studies with other linkage maps in barley.

Key words: Barley, sequence-related amplified polymorphism (SRAP), molecular genetic map, simple sequence repeat (SSR), doubled haploid (DH) population.

INTRODUCTION

Barley (*Hordeum vulgare* L.) primarily used as feed of livestock and malts for beer and whisky etc. is a widely variable species cultivated in nearly all regions of the world, and also the fourth most important cereal crop (Zohary and Hopf, 1988; Feng et al., 2006). For scientists as well as commercial breeders, barley is considered a promising crop with a broad genetic potential and has received considerable research attention as a model crop for genetic analysis. Researchers and breeders have increasingly been adopting molecular markers to identify genomic regions influencing traits and to select for desirable phenotypes based on identified marker-trait associations (Langridge and Barr, 2003). Molecular genetic maps of crop species were suggested to play an important role in breeding and genomics research.

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Abbreviations: SRAP, Sequence-related amplified polymorphism; **SDRs,** segregation distortion regions; **QTLs,** quantitative trait loci; **MAS,** marker-assisted selection; **DH,** doubled haploid.

Table 1. List of primer sequences (forward and reverse) used for SRAP marker in this study.

Primer name	Sequence 5’ →3’	Primer name	Sequence 5’ →3’
Me1	TGAGTCCAAACCGGATA	Em9	GACTGCGTACGAATTACG
Me2	TGAGTCCAAACCGGAGC	Em10	GACTGCGTACGAATTTAG
Me3	TGAGTCCAAACCGGAAT	Em11	GACTGCGTACGAATTTCG
Me4	TGAGTCCAAACCGGACC	Em12	GACTGCGTACGAATTGTC
Me5	TGAGTCCAAACCGGAAG	Em13	GACTGCGTACGAATTGGT
Me6	TGAGTCCAAACCGGTAG	Em14	GACTGCGTACGAATTCAG
Me7	TGAGTCCAAACCGGTTG	Em15	GACTGCGTACGAATTCTG
Me8	TGAGTCCAAACCGGTGT	Em16	GACTGCGTACGAATTCGG
Me9	TGAGTCCAAACCGGTCA	Em17	GACTGCGTACGAATTCCA
Em1	GACTGCGTACGAATTAAT	Em18	GACTGCGTACGAATTCAA
Em2	GACTGCGTACGAATTTGC	Em19	GACTGCGTACGAATTCGA
Em3	GACTGCGTACGAATTGAC	Em20	GACTGCGTACGAATTCCT
Em4	GACTGCGTACGAATTTGA	Em21	GACTGCGTACGAATTCCG
Em5	GACTGCGTACGAATTAAC	Em22	GACTGCGTACGAATTAAG
Em6	GACTGCGTACGAATTGCA	Em23	GACTGCGTACGAATTATC
Em7	GACTGCGTACGAATTATG	Em24	GACTGCGTACGAATTTGT
Em8	GACTGCGTACGAATTAGC		

Me1-Me 9, Em1-Em 24 indicate forward primers, reverse primers, respectively.

Genetic linkage map is a basis of quantitative trait loci (QTL) mapping, map-based cloning and molecular marker-assisted selection (MAS) (Wang et al., 2008).

In barley, about 71 molecular genetic maps including random amplified polymorphic DNA (RAPD), restriction fragment length polymorphism (RFLP), amplified fragment length polymorphism (AFLP), simple sequence repeat (SSR) as well as diversity arrays technology (DArT) markers etc. have been acknowledged. Sequence-related amplified polymorphism (SRAP), a novel polymerase chain reaction (PCR)- based molecular marker technique newly developed by Li and Quiros (2001), have simplicity, reliability, moderate throughput ratio and facile sequencing of selected bands as well as richer information than RAPD, AFLP, inter-simple sequence repeat (ISSR) and SSR etc. (Li and Quiros, 2001; Budak et al., 2004, 2005; Ferriol et al., 2003; Zheng et al., 2008).

The linkage maps of SRAP markers in *Brassica* (Li and Quiros, 2001) and cotton (Lin et al., 2009) have been successfully constructed. In barley, the utilization of SRAP markers in the evaluation of genetic diversity of the developed qingke (hulless barley) cultivars from the Qinghai-Tibet plateau regions of China was first reported (Yang et al., 2008, 2010). Ayten et al. (2010) also exhibited a high level of genetic diversity from barley germplasm developed for scald disease resistance based on SRAP markers. However, the map construction of SRAP markers in barley has not been reported. In this study, the combined map of SRAP and SSR markers in barley was constructed to evaluate the potential of SRAP markers in genetic map construction.

MATERIALS AND METHODS

Plant materials and DNA extraction

In total, 150 doubled haploid (DH) lines derived from a cross (Morex × Steptoe) and their parents, kindly offered by Dr. Sato (Okayama University, Japan), were planted at barley experiment fields in Ya’an, Sichuan, China in 2009 to 2010. At tilling period, fresh young leaves of these materials were used to extract genomic DNA using cetyltrimethylammonium bromide (CTAB) protocol with some modifications (Del Sal et al., 1989). The isolated genomic DNA was stored at -20°C for use. DNA concentration was adjusted to 50 ng/µl for PCR amplification.

SSR and SRAP profiling

Overall, 312 SSR primers pairs covering the whole barley genome (http://wheat.pw.usda.gov/cgi-bin/graingenes/) were screened for polymorphism between parents of the DH population, and these primers were synthesized by Shanghai Invitrogen (China). The PCR reaction system and the detection of PCR products were carried out employing the methods of Liu et al. (2011). In all, 33 SRAP primers, including nine forward and 24 reverse primers (Table 1) designed following Li and Quiros (2001), were synthesized (Shanghai Invitrogen, China). A total of 216 primer combinations were used to search for polymorphism between the parents. Informative primer combinations were used to genotype the mapping population. The PCR amplification was conducted in an MJ Thermo Cycler in a total volume of 10 µl, including 50 ng of genomic DNA, 1×PCR buffer containing 1.5mM $MgCl_2$, 0.2 mM dNTPs, 0.3 µM of each primer, and 0.5 U of Taq polymerase. The PCR reaction condition was as follows: an initial denaturing step at 94°C for 5 min, followed by five cycles at 94°C for 1 min, 35°C for 1 min and 72°C for 1 min, subsequently followed by 35 cycles at 94°C for 1 min, 50°C for 1 min and 72°C for 1 min, with a final extension step at 72°C for 10 min (Wang et al., 2008). The PCR products were separated on 6 or 8% denatured polyacrylamide gels and visualized by silver staining.

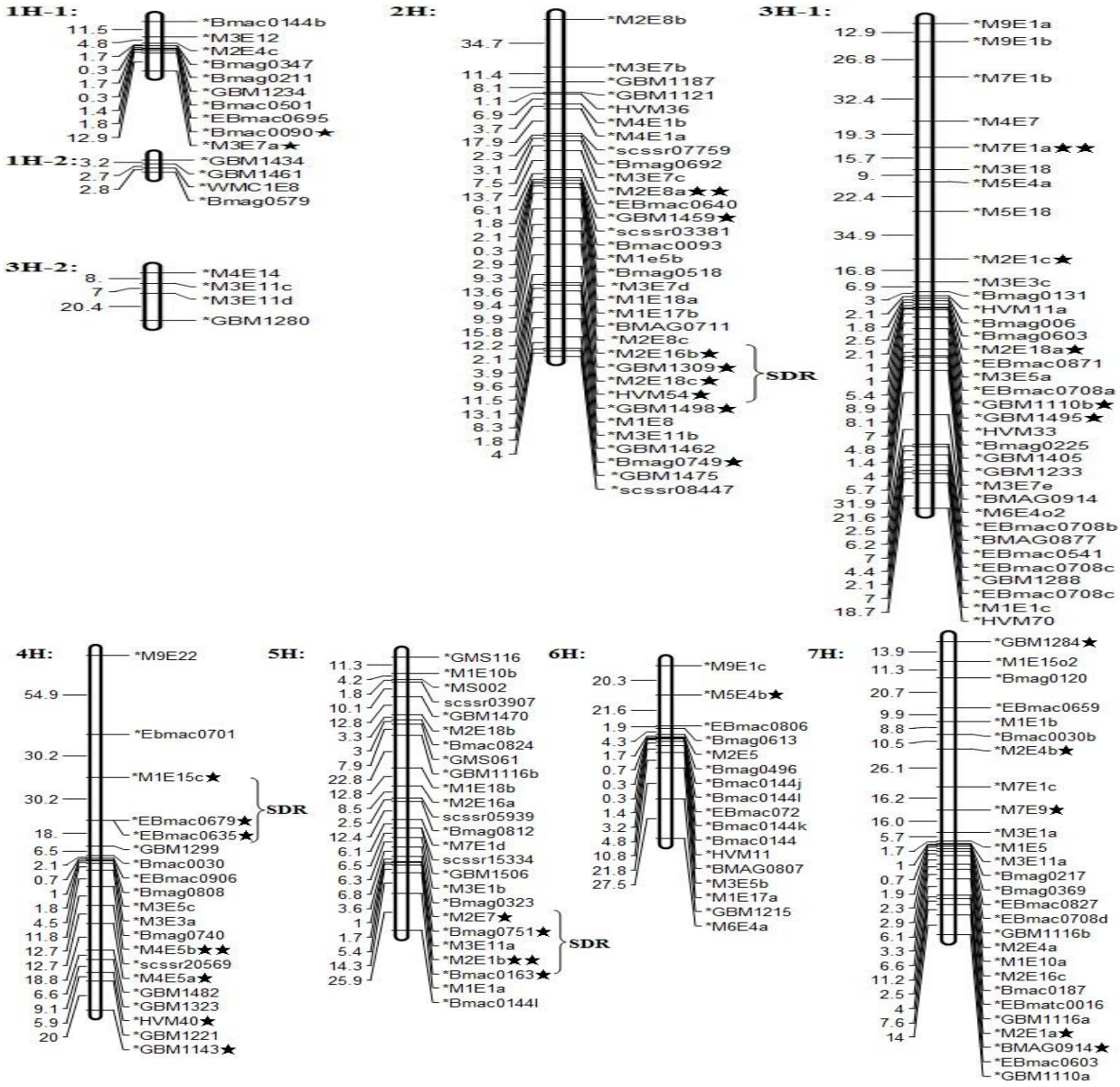

Figure 1. The linkage maps constructed using SSR and SRAP markers in DH population. M-E- represent SRAP marker. ★ and ★★ indicate significant levels of distorted segregation at 5 and 1%, respectively.

Data analysis

The genetic linkage was analyzed using the mapping software Mapmaker 3.0 based on the segregation data (Lander et al., 1987), with LOD = 3.0. The Kosambi map function was used to construct genetic linkage map (Kosambi, 1944) and the graphic representation of the linkage group was drawn by Mapdraw software. The segregation ratio across the mapping population was tested against a 1:1 ratio using Chi-square test. The segregation of markers that did not fit the 1:1 ratio ($P<0.05$) was treated as distorted. SPSS 17.0 was used for Chi-square test.

RESULTS

A total of 528 markers, including 216 SRAP markers and 312 SSR markers were employed to screen the polymorphism between parents of the DH lines. Overall, 21 of 216 SRAP markers generated 78 polymorphic loci, while 98 of 312 SSR markers produced 107 polymorphic loci. Among the 185 polymorphic loci, 175 loci (70 SRAP loci, 105 SSR loci) were assigned to nine linkage groups, which were assigned to corresponding chromosomes by SSR markers with known chromosome locations in barley. The map covered 1475 cM, with a mean density of 8.7 cM per locus (Figure 1).

The phenomenon of markers' segregation distortion was also investigated. The result indicates that 33 of all the loci (17.84%) showed segregation distortion, of which 26 loci showed significant segregation distortion at 0.05

probability level, while seven loci were significant at 0.01 level. Moreover, 23 of the 33 loci (69.7%) skewed towards the parent Steptoe, whereas the remaining loci (30.3%) deviated towards the parent Morex. Also, some of these distorted loci tended to cluster at the end of linkage groups, while others dispersed on linkage groups in a decentralized fashion. The three putative segregation distortion regions (SDRs) were detected on chromosomes 2H, 4H and 5H, respectively (Figure 1).

DISCUSSION

Since the advent of molecular marker and linkage mapping technologies, the number of marker loci placed on genetic maps is exponentially increasing (Varshney et al., 2007). In barley, molecular genetic maps, including RAPD, RFLP (Graner et al., 1991; Karakousis et al., 2003), AFLP (Heun et al., 1991; Becker et al., 1995; Karakousis et al., 2003), SSR (Karakousis et al., 2003), as well as DArT (Wenzl et al., 2007; Hearnden et al., 2007) markers etc. have been acknowledged. However, it is difficult for the QTLs detected by the linkage map constructed by the present markers, far from the linked markers, to meet the marker-assisted selection (Varshney et al., 2007). Therefore, the molecular map construction using new molecular markers is an important work in barley molecular genetics and breeding research.

The SRAP markers, designed by Li and Quiros (2001) has been described as being simple and reliable to operate, with its multiplexing capacity delivering many markers from a single assay (Zhang et al., 2011).This type of markers was successfully applied in map construction (Lin et al., 2009; Zhang et al., 2011), diversity evaluation (Ferriol et al., 2003; Baduk et al., 2004; Sun et al., 2006; Yang et al., 2010) comparative genomics (Li et al., 2003), gene location and cultivar identification (Li et al., 2006). In barley, SRAP markers were only used for diversity analysis and genetic relationships (Yang et al., 2010, Dizkirici et al., 2010). Until now, there is no report about the utility of SRAP markers in the linkage map construction of barley. Moreover, the map constructed in this study is just a framework to see whether the SRAP markers system could be used in barley. An appealing aspect of the SRAP system in this study is that it was able to amplify more polymorphic loci. Overall, 21 of 216 SRAP markers generated 78 polymorphic loci and 98 of 312 SSR markers produced 107 polymorphic loci, indicating that SRAP is a more efficient technique. Moreover, the report from Li et al. (2008) indicated that the average number of polymorphic bands detected by each SRAP primer combination was much higher than that revealed by RAPD and ISSR primers (Liu et al., 2008). Another appealing aspect of the SRAP system is that it was also able to amply the whole genome of barley. In total, 70 of 78 SRAP loci were mapped on nine linkage groups, which were assigned to corresponding chromosomes by SSR markers with known chromosome locations in barley. Consequently, the SRAP marker system is suitable for linkage and quantitative trait loci mapping in barley.

This is the first report on SPAP markers-based linkage mapping in barley. Therefore, future utility of this map for barley should be further integrated into all the maps from S/M DH population to indicate its importance in quantitative trait locus mapping, MAS and integrative analysis for additional genetic studies with other linkage maps in barley.

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REFERENCES

Becker J, Vos P, Kuiper M, Salamini F, Heum M (1995). Combined mapping of AFLP and RFLP markers in barley. Mol. Gen. Genet. 249:65-73.

Budak H, Shearman RC, Gulsen O, Dweikat I (2005). Understanding ploidy complex and geographic origin of the *Buchloe dactyloides* genome using cytoplasmic and nuclear marker system. Theor. Appl. Genet. 111:1545-1552.

Budak H, Shearman RC, Parmaksiz I, Dweikat I (2004). Comparative analysis of seeded and vegetative biotype buffalograsses based on phylogenetic relationship using using ISSRs, SSRs, RAPDs, SRAPs. Theor. Appl. Genet. 109:280-288.

Del Sal G, Manfiloeti G, Schneider C (1989). The CTAB-DNA precipitation method: a common mini-scale preparation of template DNA from phagemids, phages or plasmids suitable for sequencing. Bio Tech. 7:514-520.

Dizkirici A, Zeki K, Güren HE, Budak H (2010). Barley germplasms developed for scald disease resistance exhibited a high level of genetic diversity based on SRAP markers. Turk. J. Biol. 34:271-279.

Feng ZY, Liu XJ, Zhang YZ, Ling HQ (2006). Genetic diversity analysis of Tibetan wild barley using SSR markers. Acta Genet. Sin. 33:917-928.

Ferriol M, Picó B, Nuez F (2003). Genetic diversity of a germplasm collection of *Cucurbita pepo* using SRAP and AFLP markers. Theor. Appl. Genet. 107:271-282.

Graner A, Jahoor A, Schondelmaier J, Siedler H, Pillen K, Fischbeck G, Wenzel G. Herrmann RG (1991). Construction of an RFLP map of barley. Theor. Appl. Genet. 83:250-256.

Hearnden PR, Eckermann PJ, McMichael GL, Hayden MJ, Eglinton JK, Chalmers KJ (2007). A genetic map of 1,000 SSR and DArT markers in a wide barley cross. Theor. Appl. Genet. 115:383-391.

Heun M, Kennedy AE, Anderson JA, Lapitan NLV, Sorrells ME, Tanksley SD (1991). Construction of a restriction fragment length polymorphism map of barley (*Hordeum vulgare*). Genome 34:437-477.

Karakousis A, Gustafson JP, Chalmers KJ, Barr AR, Langridge P (2003). A consensus map of barley integrating SSR, RFLP, and AFLP markers. Aust. J. Agric. Res. 54:1173-1185.

Kosambi DD (1944). The estimation of map distances from recombination values. Ann. Eugen. 12:172-175.

Lander ES, Green P, Abrahamson J, Barlow A, Daly MJ, Lincoln SE, Newberg LA (1987). MAPMAKER: an interactive computer package for constructing primary genetic linkage maps of experimental and

natural populations. Genomics 1:174-181.
Langridge P, Barr AR (2003). Preface. Aust. J. Agric. Res. 54:1-4.
Li G, Gao M, Yang B, Quiros CF (2003). Gene for gene alignment between the *Brassica* and *Arabidopsis* genomes by direct transcriptome mapping. Theor. Appl. Genet. 107:168-180.
Li G, Quiros CF (2001). Sequence-related amplified polymorphism (SRAP), a new marker system based on a simple PCR reaction: its application to mapping and gene tagging in *Brassica.* Theor. Appl. Genet. 103:455–461.
Li L, Zheng XY, Lin LW (2006). Analysis of genetic diversity and identification of cucumber varieties by SRAP. Mol. Plant Breed. 4:702-708.
Lin ZX, Zhang YX, Zhang XL, Guo XP (2009). A high-density integrative linkage map for *Gossypium hirsutum*. Euphytica 166:35-45.
Liu XJ, You JM, Guo LL, Liu XC, He YJ, Yuan JE , Liu GX, Feng ZY (2011). Genetic analysis of segregation distortion of SSR markers in F_2 population of barley. J. Agric. Sci. 3:172-177.
Liu LW, Zhao LP, Gong YQ, Wang MX, LM Chen, Yang JL, Wang Y, Yu FM, Wang LZ (2008). DNA fingerprinting and genetic diversity analysis of late-bolting radish cultivars with RAPD, ISSR, and SRAP markers. Sci Hortic. 116:240-247.
Sun SJ, Gao W, Lin SQ, Zhu J, Xie BG, Lin ZB (2006). Analysis of genetic diversity in Ganoderma population with a novel molecular marker SRAP. Appl. Micro. Biol. 72:537-543.
Varshney RK, Marcel TC, Ramsay LR, Russell J, Röder MS, Stein N, Waugh R, Langridge P, Niks RE, Graner A (2007). A high density barley microsatellite consensus map with 775 SSR loci. Theor. Appl. Genet. 114:1091-1103.
Wang JS, Yao JC, Li W (2008). Construction of a molecular map for melon (*Cucumis melo* L.) based on SRAP. Front. Agric. China 2:451-455.
Wenzl P, Li HB, Carling J, Zhou MX, Raman H , Paul E, Hearnden P, Maier C, Xia L ,Caig V, Ovesná J , Cakir M, Poulsen D, Wang JP, Raman R, Smith KP, Muehlbauer GJ, Chalmers KJ, Kleinhofs A, Huttner E, Kilian A (2006). A high-density consensus map of barley linking DArT markers to SSR, RFLP and STS loci and agricultural traits. BMC Genomics 7:206.
Yang P, Liu XJ, Liu XC, Wang XW, He SP, Li G, Yang WY, Feng ZY (2008). Genetic diversity analysis of the developed qingke (hulless barley) varieties from the plateau regions of Sichuan province in China revealed by SRAP markers. Hereditas (Beijing) 30:115-122.
Yang P, Liu XJ, Liu XC, Yang WY, Feng ZY (2010). Diversity analysis of the developed qingke (hulless barley) cultivars representing different growing regions of the Qinghai-Tibet Plateau in China using sequence-related amplified polymorphism (SRAP) markers. Afr. J. Biotechnol. 9:8530-8538.
Zhang F, Chen SM, Chen FD, Fang WM, Chen Y, Li FT (2011). SRAP-based mapping and QTL detection for inflorescence-related traits in chrysanthemum (*Dendranthema morifolium*). Mol. Breed. 27:11-23.
Zheng J, Zhang ZS, Chen L, Wan Q, Hu MC, Wang W, Zhang K, Liu DJ, Chen X, Wei XQ (2008). Intron-targeted intron-exon splice conjunction (IT-ISJ) marker and its application in construction of upland cotton linkage map. Agric. Sci. China 7:1172-1180.
Zohary D, Hopf M (1988).Domestication of plants in the old world : the origin and spread of cultivated plants in west Asia, Europe, and the Nile Valley. third ed. Clarendon Press, Oxford, United Kingdom, pp. 13-15.