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INTERSPECIES POLYMORPHISM ANALYSIS OF EST-SSR MARKERS IN WHEAT AND BARLEY

Keywords: EST-SSRs, polymorphism, barley, wheat

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Summary

EST-derived Simple Sequence Repeats (EST-SSRs) are markers derived from transcripts and they are useful for assaying the functional diversity in intra- and interspecies germplasm collections of different crop species. Because of their high level of transferability EST-SSRs are useful marker type in comparative mapping projects. In present study a set of 116 wheat derived EST-SSRs was used for wheat-barley interspecies polymorphism analysis as an introduction phase forward to physical mapping of markers in barley genome. In wheat cultivar Chinese Spring 94.83% of applied EST-SSRs were succesfully amplified, while in barley cultivar Betzes amplification fidelity was 89.66%. Detected transferability was 87.27% which confirmed that wheat EST-SSR markers are highly transferable across barley genome. Among 66 detected interspecies polymorphic loci, only 26 loci may be considered as potential candidates for physical mapping in barley genome using wheat-barley chromosome arm addition lines.

Introduction

Comparative genetic mapping is the cornerstone of understanding biological systems and processes across cereal species in order to isolate genes of agronomic interests. Several studies have demonstrated that among cereals local co-linearity is often deviated but even in the absence of co-linearity, data from comparative studies offer the possibility of increasing the number of markers in a targeted region without the need to develop additional markers from the species of interest. Procedures for comparative mapping are well established (Devos & Gale, 2000) and use of comparative mapping derived resources lead to the isolation of several resistance genes and QTLs up to now.

Among the different kinds of molecular markers that are available, expressed sequence tag (EST)-SSRs have received significant attention because of the following advantages over other DNA-based markers: they can be rapidly developed from EST databases at low cost, they detect variation in the expressed portion of the genome, so that gene tagging should give 'perfect' marker-trait associations, they produce higher quality patterns and show a high level of transferability to closely related species (Varshney *et al.*, 2005). Transferability of wheat, barley and rice derived EST-SSRs into different cereal crop species has be demonstrated (Thiel *et al.*, 2003; Yu *et al.*, 2004; Zhang *et al.*, 2005, Castillo *et al.*, 2008).

The aims of the study were analysis of wheat-barley interspecies polymorphism using a set of 116 wheat derived EST-SSRs and evaluation of their transferability to barley.

Material and Methods

Plant material: Wheat cultivar Chinese Spring and barley cultivar Betzes were used in interspecies polymorphism analysis.

DNA extraction, PCR amplification, polymorphism detection: Total genomic DNA was extracted from grains (Plaschke *et al.*, 1995). A set of 116 EST-SSRs (Zhang *et al.*, 2005) was analysed (Tab. 1). Microsatellites were amplified in 20 µl PCR reaction containing: 30 ng DNA, 1xPCR buffer, 2 mM $MgCl_2$, 0.2 nM of each dNTP, 10 pmol of each EST-SSR primers, and 2 units of Taq polymerase. The following thermal profile was used for EST-SSR amplification: initial denaturation for 5 min at 94° C, 12 cycles of 30 s at 94°, 30 s at 62° C, 30 s at 72°C, followed by 35 cycles of 30 s at 94°, 30 s at 56° C, 30 s at 72°C, and final extension for 10 min at 72° C. PCR products were size separated by standard 2% agarose gels and visualised by ethidium bromide.

Results and Discussion

A set of 116 wheat EST-derived microsatellites were tested in order to detect: a level of successful amplification in wheat and barley genome, a level of interspecies polymorphism and transferability.

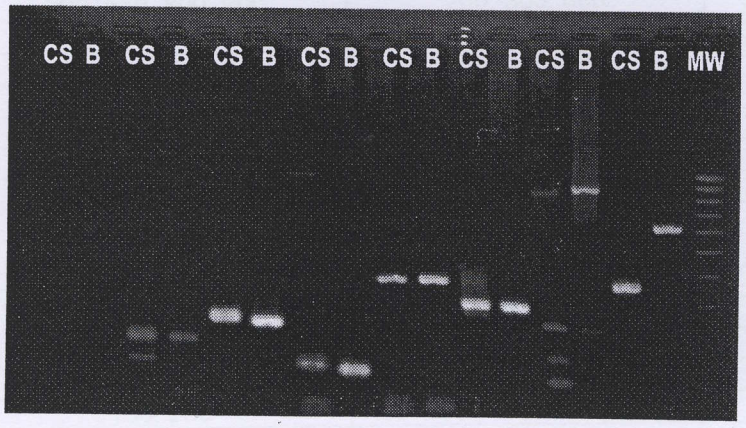

Figure 1. Agarose gel electrophoresis of PCR products obtained for Chinese Spring (CS) and Betzes (B)
(from the left to right: cfe 210, 211, 212, 214, 219, 220, 224, 225, MW 100bp)

In wheat cultivar Chinese Spring 94.83% of applied EST-SSRs were succesfully amplified. The size of the obtained PCR products in wheat genome were in range 100-900 bp (Fig. 1). In approximately 15% of cases product size was larger then expected. Larger size of obtained products may be a consequence of an intron

presence or internal duplication within a gene, or a presence of additional unspecific amplification products. Using a set of 240 EST-SSR markers Zhang *et al.* (2005) also detected differences between expected and observed size in amplification products.

Amplification fidelity in barley cultivar Betzes was 89.66%, resulting in high wheat-barley transferability level (87.27%) and indicating high level of homology between those related genomes. Our results confirmed that wheat derived EST-SSRs are highly transferable into related cereal crops and are in agreement with preveiously reported data (Eujayl *et al.*, 2002, Gupta *et al.*, 2003, Zhang *et al.*, 2007). Interspecies polymorphism was detected in 66 out of 110 succesfully amplified loci in both species. Two types of polymorphisms were detected: size difference of a single amplification product between wheat and barley, and presence/absence of extra amplification products in wheat or barley. Based of these types of polymorphism 26 EST-SSR loci were selected as candidates for physical mapping in barley genome using wheat-barley addition lines (Tab 1).

Table 1. List of 26 analysed EST-SSRs wich are recommended for physical mapping (**CS** - stands for Chinese Spring, B – Betzes; SA - successful amplification; OPS – obtained product sizes, TEPS / theoretically expected product sizes, **P** – stands for polymorphism observed (candidate markers for mapping in addition lines)

	SA		OPS (bp)		TEPS (bp)	P
	CS	B	CS	B		
cfe35	+	+	130, 150, 200, 280, 550, 680	100, 180, 450, 900	119	+
cfe40	+	+	300	100	180	+
cfe53	+	+	180, 400	150, 280, 400	104	+
cfe56	+	+	100, 300, 800	100, 300, 350, 400	156	+
cfe60	+	+	200	200, 300, 350	136	+
cfe63	+	+	150, 280, 360	280, 300, 350	149	+
cfe77	+	+	200, 800	150, 300, 550	146	+
cfe79	+	+	200, 550, 800, 900	200, 300, 600	220	+
cfe82	+	+	280	280, 400	254	+
cfe90	+	+	180	180, 400, 700	159	+
cfe98	+	+	250, 350, 400, 500, 800	250, 350, 400, 600	139	+
cfe101	+	+	300	280, 400	286	+
cfe119	+	+	350	100	130	+
cfe126	+	+	350	250, 300, 350, 480, 600	224	+
cfe136	+	+	100, 250	100, 700	160	+
cfe137	+	+	150, 200	100, 150, 400	267	+
cfe175	+	+	250, 380	180, 200, 250, 300	216	+
cfe225	+	+	350	600	349	+
cfe229	+	+	330, 500, 600	250, 300, 450	298	+
cfe248	+	+	200, 230	150, 200	202	+
cfe255	+	+	100, 230	180, 430	146	+
cfe272	+	+	180	180, 700	132	+
cfe278	+	+	200, 250, 280, 500, 800	150, 200, 280, 300	178	+
cfe288	+	+	280, 330	150, 300	210	+
cfe294	+	+	250, 550	250, 300, 350	250	+
cfe299	+	+	230	330, 380	113	+

Conclusion

Tested wheat EST-derived microsatellites showed high level of amplification efficiency in wheat and barley genome confirming their robustness and superior performance over genomic SSRs. Wheat EST-SSR markers showed high transferability level and they are good candidates for different marker-based breeding strategies in barley. Different types of detected interspecies polymorphism can be used for genetic and physical mapping of wheat EST-SSRs in barley genome.

References

Castillo A., Budak H., Varshney R. K., Dorado G., Graner A., Hernandez P. (2008): Transferability and polymorphism of barley EST-SSR markers used for phylogenetic analysis in *Hordeum chilense*. BMC Plant Biology 8: 97.

Devos K. M., Gale M. D. (2000): Genome Relationships: The Grass Model in Current Research. Plant Cell, 12: 637-646.

Eujayl I., Sorrels M.E., Baum M., Wolters P., Powel W. (2002): Isolation of EST-derived microsatellite markers for genotyping the A and B genome of weat. Theor Appl Genet 104:399-407.

Gupta P.K., Rusting S., Sharma S., Singh R., Kumar N., Balyan H.S. (2003): Transferable EST-SSR markers for the study of polymorphism and genetic diversity in bread wheat. Mol GEn Genomics 207:315-323

Plaschke J., Ganal M. W., Roder M. S. (1995): Detection of genetic diversity in closely related bread wheat using microsatellite markers. Theor. Appl. Genet. 91, 1001-1007.

Thiel T., Michalek W., Varnshey R.K., Graner A. (2003): Exploiting Est database for the development and characterization of gene-derived SSR-markers in barley (*Hordeum vulgare*). Theor. Appl. Genet.106:411-422.

Varshney R.K., Graner A., Sorrells M.E. (2005): Genic microsatellite markers in plants: features and applications. Trends in Biotechnology 23: 48-55.

Zhang L.Y., Bernard M., Leroy P., Feuillet C., Sourdille P. (2005): High transferability of bread wheat EST-derived SSRs to other cereals. Theor. Appl. Genet. 111, 677-687.

Zhang L.Y., Bernard M., Ravel C., Balfourier F., Leroy P., Feulliet C., Sourdille P. (2006): Wheat EST-SSRs for tracing chromosome segments from wide range of grass species. Plant Breeding 126: 251-258.

Yu L.K., La Rota M., Kantety R.V., Sorrells M.E. (2004): EST derived SSR markers for comparative mapping in wheat and rice. Mol. Genet. Genomics 217:287-290.