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Short Communication

Development of A High Sugar Trait-Related SCAR Marker in Sweet Sorghum

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Abstract

To effectively screen and characterize high sugar traits in sweet sorghum, we developed a sequence-characterized amplified regions (SCAR) marker, which had a 3.25 cM of linkage distance with the high sugar trait in sorghum. A total of 210 random primers was used to screen the polymorphism between a pair of parent lines, sweet sorghum LTR102 (as the female in this experiment), and common grain sorghum 654 (as the male), wherein 48 primers could produce at least one polymorphic fragment. The primer S336, which also produced a polymorphic fragment between the high sugar bulks and normal bulks of F_5 progenies, was further analyzed. The genomic DNA of LTR102 was amplified by S336 to produce an 1119 bp of fragment. We sequenced this DNA, and synthesized a pair of SCAR primers (named as $S336_{1119}$) specific to the DNA. The co-segregation between the SCAR marker and the high sugar trait was analyzed, and a 3.33% of recombinant rate was detected in F_5 individuals, corresponding a 3.25 cM of linkage distance. This would provide an assistant means for sweet sorghum breeding.

Keywords: Sweet sorghum; Bulked segregant analysis; Random amplified polymorphic DNA; Sequence characterized amplified regions (SCAR); Marker-assisted selection

Introduction

Sorghum (*Sorghum bicolor* L. Moench) is a major cereal crop mainly cultivated in the arid and semi-arid regions of the world (Smith and Frederiksen, 2000). The name "sweet sorghum" is used to distinguish those varieties of sorghum, which accumulate high concentrations of soluble sugars like sucrose, glucose and fructose in the stem juice, from the common grain sorghum (Rao *et al.,* 2013). Although sweet sorghum varieties have important agronomic traits and invaluable economic values, the high sugar metabolism-related genes have been little known so far. Thus, development of molecular markers tagged the high sugar trait is significant to the selection of fine varieties and clone of high sugar-related genes.

DNA molecular marker technology has been documented to be a very powerful tool in crop genetic modification, especially for the multiple gene-driven as well as the recessive gene-controlled traits (Winter and Kahl, 1995). For instance, the markers of restriction fragment length polymorphism (RFLP), random amplified polymorphism DNA (RAPD) have been successfully applied in sorghum genomic marking (Bhattramakki *et al*., 2000; Haussmann *et al*., 2002). In a previous study, we developed a SCAR marker, which successfully signed the head smut resistance in sorghum (Li *et al*., 2012).

In this study, to search for polymorphic fragments, we employed a group of RAPD primers to amplify the genomic DNA of sweet sorghum and common line, as well as their F_5 near-isogenic lines, and obtained a primer which produced an 1119 bp of polymorphic DNA in the parents and F_5 progenies. Based on the resulted DNA sequence, a pair of SCAR primers were designed and synthesized, and then successfully tagged the high sugar trait of sorghum with a 3.25 cM of linkage map distance. This may provide an alternative strategy for the selection of new sweet sorghum varieties, and cloning of high sugar-related genes.

Materials and Methods

Sweet sorghum LTR102 (as the female parent in this study) and common grain sorghum 654 (as the male parent) were supplied by Liaoning Academy of Agricultural Sciences. To establish near-isogenic lines, we created F_5 plants from the cross of LTR102 × 654. Bulked segregant analysis (BSA) was used to identify putative markers tagged the high sugar trait. For the high sugar bulk, DNA was extracted and pooled from F_5 individuals with high sugar contents and for the low sugar bulk, DNA was mixed from low sugar F_5 individuals. In this study, the high sugar individual was defined as the total sugar content in stalk juice over 16%, and the low sugar plant as the sugar content below 12%. The sugar contents in plants were determined according to the method described by Yuan *et al*. (2008).

For polymorphic analysis, a total of 210 RAPD primers, obtained from the company of Shanghai

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Shenggong, China, were used to amplify the parents and two sets of bulks. The genomic DNA was extracted from leaf tissues of parent lines and F_5 individuals using the cetyltrimethylammonium bromide (CTAB) method (Doyle and Doyle, 1990). The EN 61010-1 DNA Thermal Cycler (USA) was used to perform PCR, which was established by mixing 17 μL of ddwater, 2.5 μL of 10 × buffer, 2.5 μL of 25 mM $MgCl_2$, 0.5 μL of 10 mM dNTP, 0.5 μL of 5 U/μL Taq enzyme, 1 μL of 10 μM primer, and 1 μL of DNA extract (500 ng/μL). The thermal cycles were programmed for an initial denaturation of 3 min at 94ºC, followed by 35 cycles of 30 s at 94ºC, 20 s at 38ºC, and 80 s at 72ºC; and a final 10 min extension at 72ºC. PCR products were detected in 1.4% agarose gels.

For SCAR marker creation, firstly the genomic DNA of LTR102 was amplified by PCR with primer S336 using the same reaction mixture and the same program as above mentioned. RAPD primer S336 is TCCCCATCAC. The resulted DNA fragment was extracted from the gel using DNA recovery kit (Sunbiotech, Beijing, China), and sequenced (performed in Takara Biotechnology Co., Ltd, Dalian, China). Based on the DNA sequence, we designed SCAR primer $S336_{1119}$, forward 5´-CTGTGTGATGTAG-CCTC-3´, and reverse 5´-ATCCTAAACCCATCAGTG-3´. Using these primers, the co-segregation analysis between the SCAR marker and the high sugar trait was conducted on the F_5 near-isogenic populations including 87 high sugar individuals and 33 low sugar individuals by PCR. The reaction program was the same as above mentioned, and the reaction mixture was composed as follow: 17 μL dd water, 2.5 μL 10 × buffer, 2.5 μL 25 mM $MgCl_2$, 0.5 μL 10 mM dNTP, 0.5 μL 5 U/μL Taq enzyme, 0.25 μL 10 μM forward primer, 0.25 μL 10 μM reverse primer, and 0.3 μL DNA templates (100 ng/μL). PCR products were separated in 1.4% agarose gel. The recombinant rates and map distances were calculated according to the Kosambi mapping function (Kosambi, 1944). The percentage of recombination (r)=recombinant individuals/total tested individuals × 100. Linkage map distance (cM) = $1/4 \times \ln (1+2r)/(1-2r)$.

Results

The RAPD primers were tested on a survey of polymorphic DNA production in the parent lines and F_5 bulks. Among them 48 primers amplified at least one polymorphic DNA fragment between the parent lines, as well as the two F_5 bulks (data not shown). We selected the primer S336, which produced an 1119 bp of polymorphic DNA fragment (Fig. 1), to create SCAR markers. Firstly, this DNA was sequenced (data not shown), then a pair of SCAR primers were synthesized based on the DNA sequence in the principle avoiding hairpin structure and appropriate G+C content.

The co-segregation analysis in a total of 120 F_5 individuals was performed using the SCAR primers. One high sugar and 3 low sugar individuals respectively in 87 high sugar group and 33 low sugar group displayed recombination (Table 1), and corresponding linkage map distance was 3.25 cM.

To confirm the identity of SCAR marker, the genomic DNA of parent LTR 102, 654 and representative F_5 plants were amplified by primer $S336_{1119}$, and as expected, the marker well tagged the high sugar trait (Fig. 2).

Discussion

Due to the quantitative trait (QTL) controlled by complex multiple genes, the high sugar metabolism-related genes in sweet sorghum have been little known so far. However, increasing studies by molecular marker-assisted techniques on the sugar-related QTLs have been emerging in sweet sorghum. For instance, a genetic map of sugar components marked with AFLP (amplified fragment length polymorphism), SSR (simple sequence repeat), and EST (expressed sequence tag)-SSR markers was developed, and several QTLs were detected using composite interval mapping (CIM) (Shiringani *et al*., 2010). Based on the RFLP, AFLP, and SSR markers, Bain *et al*. (2006) constructed a genetic linkage map spanning 983.5 cM and aligning to the 10 linkage groups of sorghum, in which two brix-related QTLs were sited on SBI-04 and SBI-10. Another nice stem sugar-related linkage map was constructed by Ritter *et al*. (2008), where QTL alleles responsible for enhanced sucrose and sugar contents were located on SBI-01, SBI-05 and SBI-06. Using AFLP technology, differential markers between sweet and grain sorghum were found to mainly distribute on SBI-08 and SBI-10 (Ritter *et al*., 2007). Natoli *et al*. (2002) used the methods of simple interval mapping (SIM) and CIM to fix 1 and 2 sucrose-related QTLs on SBI-03 and SBI-05, respectively. These works and other related works are helpful to understand the genetic mechanism associated with the sugar metabolism in sorghum, and the increasing identification of QTLs may accelerate sweet sorghum breeding by marker-assisted selection.

However, it may be more convenient and efficient for fine variety selection using a simple molecular marker tightly linked to the purpose trait-related major gene. In this context, the SCAR marker developed in the present study may be useful to sweet sorghum selection and breeding, due to its tight linkage with the high sugar trait, as well as the more faithful, stable, repeatable, easy-to-use, and inexpensive compared to other molecular markers. Furthermore, since the completed genome sequence of sorghum was published (Paterson *et al*., 2009), the studies associated with the molecular marker-related events have been more effective, because the marker identification and the primer design have been easier. Based on the genomic sequence, in theory any valuable SCAR markers can be easily mapped to a corresponding linkage group, which will greatly aid in the map-based cloning of genes of interest.

Table 1: Co-segregation of the $S336_{1119}$-amplified polymorphic fragment in F_5

Primer	F_5 individuals	Total plants tested	Polymorphic fragment		Percentage of recombination %
			Present	Absent	
$S336_{1119}$	High sugar	87	86	1	3.33
	Low sugar	33	3	30	

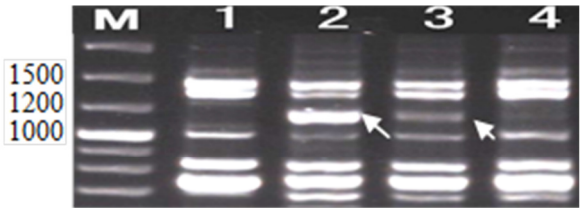

Fig. 1: PCR results of RAPD primer S336. M: DNA ladder, 1: male parent, 2: female parent, 3: high sugar pool, 4: low sugar pool. The arrows indicated the polymorphic DNA with 1119 bp

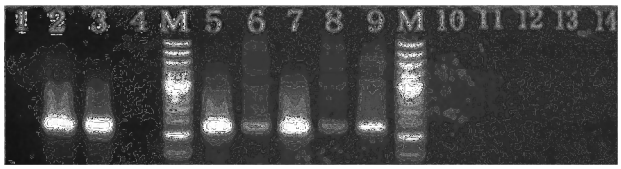

Fig. 2: PCR detection of parent lines and representative F_5 individuals with SCAR primers. M: DNA ladder, 1: male parent, 2: female parent, 3: high sugar pool, 4: low sugar pool, 5 – 9: high sugar F_5 individuals, and 10 – 14: low sugar F_5 individuals

In conclusion, we developed a SCAR marker with a 3.25 cM of linkage map distance with the high sugar trait in sorghum. To our knowledge, this is the first SCAR marker tagging the high sugar property in sorghum.

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