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Short Communication

DEVELOPMENT OF STS MARKERS FOR WAXY GENE ALLELIC VARIATION IDENTIFICATION IN FOXTAIL MILLET

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ABSTRACT

Waxy gene controls synthesis of amylose, having important application value in crop eating quality improvement. The *waxy* gene of foxtail millet has been cloned and sequenced. All the waxy and low-amylose types arose by the insertions of transposable elements into the *waxy* gene. Based on the sequence variation information caused by transposon insertions, 6 pairs of STS primers were designed to detect 10 allelic variation types of the *waxy* gene in eleven foxtail millet strains. The results showed that *waxy* allelic variation types of nine strains could be determined, they were "Xinji" sticky millet (IV/IV), Green sticky millet (IVa/ IVa), "Shanxi" sticky millet (VII/Wild), "Anyanghong" sticky millet (IV/IV), Jite5 (IV/III), Jigu30 (IV/V), Yellow sticky millet (IVa/ IVa), "Linxian" black sticky millet (IV/ VII), "Xincai" sticky millet (VII/ Wild). Two modern varieties Jigu19 and "Early white waxy" detected three allelic variation types (IVa, III, V), the real reason need be further investigated. No X, VI, VIII variation types were detected in the eleven foxtail millet strains. The developed STS markers were powerful in detecting which of the 10 *waxy* allelic variation types existed in any foxtail millet strain though iodine blue staining amylose determination technology needed when distinguish homozygous variation types from heterozygotes of variation type and wild type because of dominant markers.

Key words: Foxtail millet, Eating quality, *Waxy* gene, Allelic variation, STS marker

INTRODUCTION

Foxtail millet (*Setaria italica* Beauv.) has been regarded as a main food crop for a long historical period in China. With its advantages such as drought and barren resistence, rich and balanced nutrition, foxtail millet becomes an important strategic reserve crop in drought-prone China in recent years **(Diao. 2008)**. While, the poor eating quality of cooked rice leads to limited consumption of foxtail millet. Upgrading eating quality is a key step to achieve industrialization of foxtail millet. *Waxy* gene, which controls synthesis of amylose (Nakayama et al. 1998), was proven playing an important role in crop's eating quality (Tan et al. 1999). The *Waxy* gene of foxtail millet has been cloned and sequenced, consisted of 14 exons and 13 introns (Fukunaga et al. 2002). Inserting of transposons in four regions (intron1, exon3, exon10, intron12) of *Waxy* gene produced 11 allelic variation types, among which, one variation type, Ⅱ did not change amylose content, giving the same non-waxy phenotype as wild type (I). Three variation types, III, VI, IX gave low-amylose phenotype, the remaining seven variation types, IV, IVa, IVb, V, VII, VIII, X, gave waxy phenotype (Kawase et al. 2005). Analysis *Waxy* gene sequences of 130 foxtail millet strains, 3 SNPs were found in coding region of a Korea strain "No.88", while no amylose phenotype change was found (Van et al. 2008). By now, no special molecular markers were developed for *Waxy* gene allelic variation identification in foxtail millet. So six pairs of STS primers were designed to identify 10 allelic variation types of *Waxy* gene in this study, in order to develop special molecular markers for superior low-amylose foxtail millet resources identification and eating quality marker-assisted selection breeding launching.

MATERIALS AND METHODS

11 foxtail millet strains including 7 landraces ("Xinji" sticky millet, "Anyanghong" sticky millet, Green sticky millet, "Shanxi" sticky millet, Yellow sticky millet, "Linxian" black sticky millet, "Xincai" sticky millet), 4 modern varieties (Jigu 30, Jigu 19, Jite 5, "Early white waxy") were grown in the experimental field of the College of Agriculture, Henan University of Science and Technology on 10 May, 2012. The fresh leaves were collected at the five-leaf stage for DNA isolation by CTAB method (Dellaporta et al. 1983).

The STS primers were designed using DNAMAN (6.0) software. The main criteria for primer design were 17～24 nucleotides long, GC content ranging from 35 to 70% and annealing temperature ranging from 45 to 60℃.

The STS primers information was listed in Table 1. Polymerase chain reactions (PCRs) were performed in a 10μl reaction volume containing 30 ng of template DNA, 2.5 pmol of forward and reverse primers, 5μl of 2×Power Taq PCR Master Mix (BioTeke Biotechnology Co. Ltd., Beijing, China) and sterile distilled water. The conditions for amplification were 4 min at 94℃ followed by 34 cycles of 40s at 94℃, 60s at 60℃～52℃, and 60s at 72℃, then with a final extension time of 5 min at 72℃. A total of 5μl PCR production was subjected to electrophoresis at 120 V on 1% agarose gel electrophoresis for 30 min and visualized by ethidium bromide staining.

50 seeds per strain cut longitudinal, then soaked in I_2-KI solution. The staining seeds would appear three colours: blue represents non-waxy phenotype, purple represents low-amylose phenotype, reddish brown represents waxy phenotype.

RESUITS AND DISCUSSION

As shown in Fig. 1, after amplification by STS1 and STS2, amplification fragment combinations and corresponding *Waxy* gene variation types of the 11 foxtail millet strains were successively “Xinji” sticky millet (870/870, IV), Green sticky millet (870/—, IVa), “Shanxi” sticky millet (no amplification, IV, IVa and IV_b excluded), “Anyanghong” sticky millet (870/870, IV), Jite5 (870/870, 2000, IV, III), Jigu30 (870/870, IV), Jigu19 (870/2000, IVa, III), “Early white waxy” (870/2000, IVa, III), Yellow sticky millet (870/—, IVa), “Linxian” black sticky millet (870/870, IV), “Xincai” sticky millet (no amplification, IV, IVa and IV_b excluded). Fig. 2 gave the amplification results of STS3, STS4. After amplification by STS3, only Jigu 30, Jigu19, “Early white waxy” produced expected fragment, indicating V variation type existed in the three strains. After amplification by STS4, “Shanxi” sticky millet, “Linxian” black sticky millet, “Xincai” sticky millet produced expected fragment, indicating VII variation type existed in the three strains. STS5, STS6 gave no expected products, so no X, VI and VIII variation types existed in the 11 strains (the amplification results didn’t give here). Integrating the results above, *Waxy* genotype of 11 foxtail millet strains could be determined: “Xinji” sticky millet (IV/IV), Green sticky millet (IVa/ IVa), “Shanxi” sticky millet (VII/VII), “Anyanghong” sticky millet (IV/IV), Jite5 (IV/III), Jigu30 (IV/V), Jigu19 (IVa/III/V), “Early white waxy” (IVa/III/V), Yellow sticky millet (IVa/ IVa), “Linxian” black sticky millet (IV/ VII), “Xincai” sticky millet (VII/ VII).

Table 1. Six pairs of STS primers for 10 *Waxy* allelic variation types identification.

Marker	Primer sequences	Tm (℃)	Amplification fragment (bp) and corresponding allelic variation type											
			Iva	IV	IX	IVb	III	Ⅲ, Ⅸ	IVa, III	IV, III	V	VII	X	VI	VIII
STS1	AATAGTGTTGCCGATAGGG GGTGCTTCTGACTTGTGAGT	55	870	870	—	1234	—	—	870	870					
STS2	GCTTCGTGTTCTGTCTGAATC GAATCCGCCTGGGAGTA	57	—	870	870	870	2000	870, 2000	2000	870, 2000					
STS3	GAAAATAGTGGGCAAATGC GAAGGTGTGAGCAGTCAAGTA	55									1337				
STS4	CAAGTGTTCAGTGCGTCG CCTCCACAGATTCATCCC	55										1560			
STS5	CGTGTCTTGTTGTCGGAT TGAAGGTGTGAGCAGTCAA	55											1135		
STS6	GGGCTCTTTCTTTTCTTTGT GAGGCGTTGGTTTAGTTACAC	55												534	1557

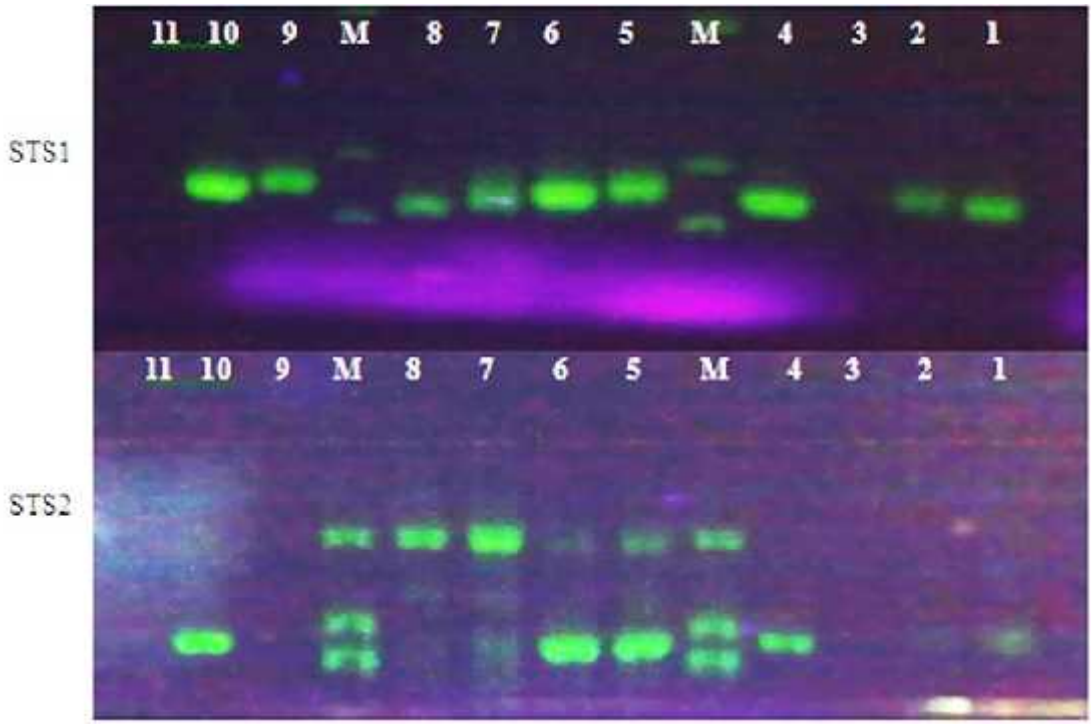

Fig. 1. Amplification results of STS1, STS2 primers in 11 foxtail millet strains (Note: 1-"Xinji" sticky millet, 2-Green sticky millet, 3-"Shanxi" sticky millet, 4-"Anyanghong" sticky millet, 5- Jite5, 6- Jigu30, 7- Jigu19, 8-"Early white waxy", 9- Yellow sticky millet, 10-"Linxian" black sticky millet, 11-"Xincai" sticky millet, M-Marker D2000)

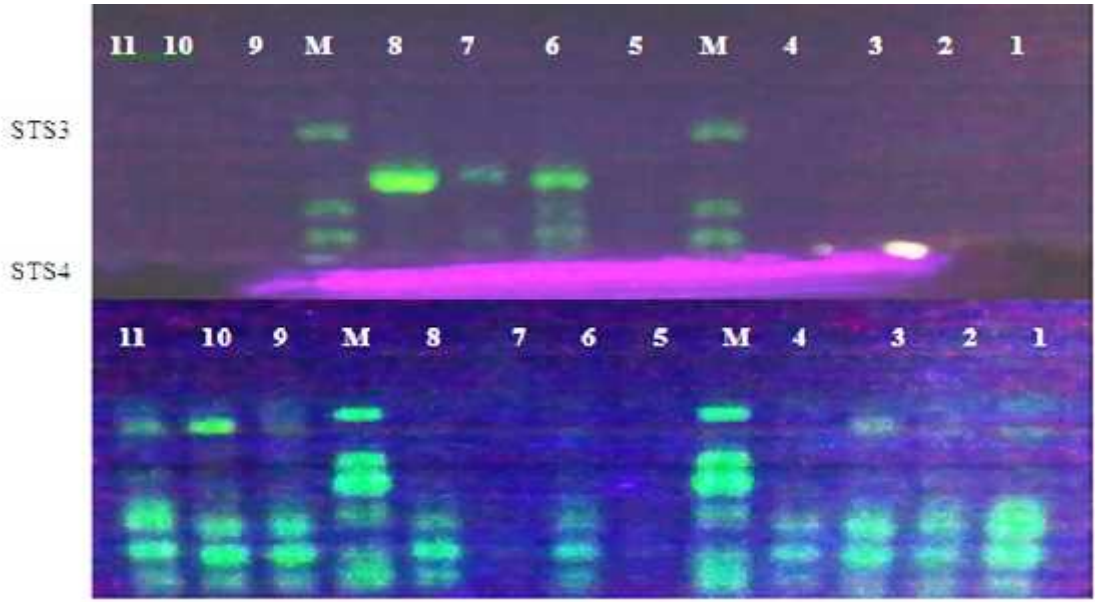

Fig. 2. Amplification results of STS3, STS4 primers in 11 foxtail millet strains

After measuring by I_2-KI method, 5 of the 7 landraces showed waxy phenotype, they were "Xinji" sticky millet, "Anyanghong" sticky millet, Green sticky millet, Yellow sticky millet, "Linxian" black sticky millet. The remaining two landraces "Shanxi" sticky millet, "Xincai" sticky millet showed low-amylose phenotype (Fig. 3, Fig. 4). 3 of 4 modern varieties showed waxy phenotype, they were Jigu 30, Jigu19, "Early white waxy". The last modern variety Jite5 showed low-amylose phenotype. As STS markers in this study were dominant markers, couldn't classify homozygotes and heterozygotes, leading to some inconsistency

between Waxy genotype and amylose phenotype. For example, "Shanxi" sticky millet and "Xincai" sticky millet only detected VII variation type, two possible diploid *Waxy* gene combinations would exist: VII/VII or VII/Wild. Low-amylose phenotype of the two landraces determined their VII/Wild genotype instead of VII/VII.

Fig. 3. "Linxian" black sticky millet, Yellow sticky millet showed waxy phenotype

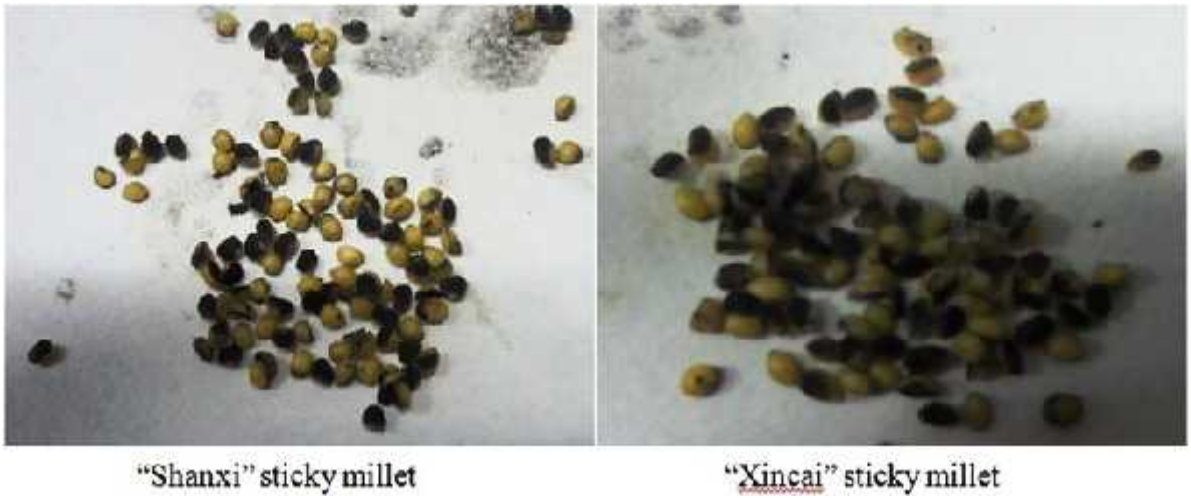

Fig. 4. Low-amylose phenotype of "Shanxi" sticky millet and "Xincai" sticky millet

In this study, *Waxy* allelic variation type of nine foxtail millet strains could be clearly determined, indicating the effectiveness of the developed STS markers. While as dominant markers, the developed STS markers couldn't distinguish homozygotes and heterozygotes, so when detected only one allelic variation type, amylose content should be measured to determine whether *Waxy* genotype was homozygous variation type or heterozygotes of variation type and wild type. Two modern varieties Jigu19, "Early white waxy" detected three *Waxy* allelic variation types, which may be attributed to inserting of transposons in intron1 and exon3 at the same time, the real reason should be further investigated.

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