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RAPD Marker Conversion into a SCAR Marker for Rapid Identification of Johnsongrass [*Sorghum halepense* (L.) Pers.]

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Abstract

Johnsongrass [*Sorghum halepense* (L.) Pers.] is a malignant weed in the world, threatening biodiversity at invaded habitats in more than 50 countries. Because of similarity in morphological characters, *S. halepense* and its relatives, *S. almum*, *S. nitidum*, *S. propinquum*, *S. sudanense*, and *S. bicolor*, etc. was difficult to identify. As a supplementary methodolgy for morphology identification, a molecular detection method was established. Sequence Characterized Amplified Regions (SCAR) marker is a recent established, reliabile, and stable molecular marker based on RAPD maker, an effective way for germplasm identification. In this study, one specific band of S.halepense was screened by 163 pairs of RAPD primers. According to the sequences of the band, a pair of special SCAR primers SH1/SH2 was designed and verified by 65 Sorghum DNA samples from all over the world. The results showed SCAR primers SH1/SH2 can be used to distinguish S.halepense and its relatives rapidly with three exceptions of Australia geotypes.

Keywords: breeding, convert, RAPD marker, SCAR marker, *Sorghum halepense*

Introduction

Sorghum halepense (L.) Pers. is perennial grass with the ribbed leaf sheath, conspicuous midrib, the large purplish panicles, and the extensively creeping rhiaomes (Holm *et al*., 1977). It originated from hybridization of Sorghum arundinaceum and Sorghum propinquum through chromosome doubling. Chromosome: 2n=4×= 40. *Sorghum halepense*, native to Mediterranean region, is one of the ten hardness weeds in the world that reduces in more than 30 crop species and various fruits and threatens biodiversity in invaded habitats in no less than 50 countries (Holm *et al*., 1977). The striking invasiveness and minacity of *sorghum halepense* are mainly on: 1) high reproductive capacity; 2) being an alternate host of various pathogen species, which intimidates the cultivations of local crops; 3) evident allelopathic effects and toxicity to livestock; 4) broad resistances to groups of herbicides; and 5) readiness of crossing with relative species naturally. And the potential hybrids in the one hand may gain even higher invasiveness. In the other hand, gene flow may cause crop species gene-polluted (Jang *et al*., 2009).

Caryopsis is the principal mean of the spread of *sorghum halepense*. Huge numbers of caryopses are readily to distribute with wind, water, birds and other animals. More importantly, its seed dissemination frequently attains by hitchhiking on goods traded allover the world, in particular, by mixing in forage, crop seeds and raw grains, such as sorghum (*Sorghum bicolor*), soybean (*Glycine max*) and maize (*Zae mays*). Therefore, as the key content of its control, caryopsis detection and identification should be the core task in *Sorghum halepense* quarantine. However, *Sorghum halepense* bears similarity to its relative species and the seeds may be deformed and abrased during storage, loading and unloading. It is very difficult to distinguish them morphologically from each other (Yin *et al*., 2004).

Molecular biological methods, especially, molecular markers have obvious advantages for identifying *Sorghum halepense* from seeds of its relative species. Therefore, molecular markers take play an important role in both of genetic diversity and identification of sorghum species. Varieties of molecular markers such as RFLP (Guo *et al*., 2005), RAPD (Li *et al*., 2007), ISSR (Fang *et al*., 2008), etc. have been applied in sorghum germplasm identification and analysis of genetic diversity research.

The RAPD markers which were established by Williams *et al*. (1990) can give polymorphism information covering entire genome by a number of different primers. Because of its features - simple, rapid, efficient, and its short cycle, RAPD markers are widely used in genetic diversity and breeding of tea and other plant in recent years (Chen *et al*., 1999). However, the application of these RAPD markers has been restricted to the laboratories in which they were identified. The RAPD technique has been known for its reproducibility problems mainly among different laboratories (Kesseli *et al*., 1992; Penner *et al*., 1993). SCARs (Kesseli *et al*., 1992) have been proposed as an alternative procedure to increase the reproducibility of the RAPD technique. SCAR marker is a new molecular markers based on RAPD maker (Paran and Michelmore, 1993). For its reliable, stable, long-term using, SCAR marker molecular is a new effective way for identification of germplasm. A large number of SCAR markers have now used on soybean, wheat, corn, mulberry, tobacco and other plants on genetic breeding research, however, this technology had not yet been applied in *Sorghum halepense*. The objective of this study was to find the special segment with random primer in genomic DNA of *Sorghum halepense*, and then convert the RAPD maker to a SCAR marker in order to improve the stability of molecular identification of *Sorghum halepense*.

Materials

Plant materials

Sixty five Sorghum genus seeds were obtained from Yin Liping, Technical Center of Plant and Animal Quarantine, Shanghai Entry-Exit Inspection and Quarantine Bureau, and those specimens were collected from Australian Tropical Crops and Forages Collection - Queensland Department of Primary Industries and Fisheries, Institute of Botany - the Chinese Academy of Sciences, The Chinese Academy of Agricultural Sciences and Botanical Gardens over the world. To estimate the differences, thirteen species including *S. halepense* and its relatives *S. almum*, *S. bicolor*, *S. hybrid*, *S. vulgare*, *S. saccharatum*, *S. nitidum*, *S. arumdinaceum*, *S. verticilliflorum*, *S. drummondii*, *S. sudanse*, Silk sorghum, *S. propinum* were used (Tab. 1).

Reagents and random primers

The reagents were purchased from Shanghai Sangon Biological Engineering Technology & Service Co. Ltd, TaKaRa Biotechnology (Dalian) Co. Ltd and Tiangen Biotech (Beijing) Co. Ltd. The random primers were synthesized by Shanghai Sangon Biological Engineering Technology & Service Co. Ltd.

Methods

Extraction of Genomic DNA from seeds of S.halepense and its relative species

Total DNA was extracted from seed powder using the method improved by our laboratory (Chen *et al.,* 2009). Based on the reported chloroplast gene matK-trnK sequences of S.bicolor, a pair of universal primers were designed, CP3: 5' GTGTTGACGAGATTTCTGTT 3', CP4 5' GGGTTGCTAACTCAATGGTA 3'. The universal primers were used to identify whether the DNA extracted from the seeds can be used as the template.

RAPD-PCR amplification

The DNA bulks were amplified by the RAPD technique (Williams *et al*., 1990) with 163 random decamer primers with more than three repeats. Each amplification reaction of 20 ul contained 2 μL 10 × PCR buffer (TIANGEN, China), 0.4 mM Mix-dNTP (Tiangen, China), 0.25 mM of primer, one unit of Taq DNA polymerase (Tiangen, China) and 25 ng of DNA. Each amplification cycle consisted of the following step: 30 s at 94°C, 40 s

Tab. 1. Accessions list of samples used in the study

Code	Origin	Code	Origin	Code	Origin	Code	Origin
Shal1	U.S.A.(Texan,)	Shal18	U.S.A(Peiolia)	Sbic2	Unknown	Snit4	Australia
Shal2	U.S.A(Peiolia)	Shal19	Australia	Sbic3	Argentina	Saru1	Australia
Shal3	U.S.A	Shal20	U.S.A	Sbic4	Briazil	Sver1	Unknown
Shal4	U.S.A(Peiolia)	Salm1	Argentina	Sbic5	France	Sdru1	Ethiopia
Shal5	U.S.A(Peiolia)	Salm2	Argentina	Sbic6	U.S.A	Sdru2	Kenya
Shal6	U.S.A(Peiolia)	Salm3	Argentina	Sbic7	Unknown	Sdru3	Portugal
Shal7	U.S.A	Salm4	Argentina	Sbic8	Unknown	Sdru4	Zaire
Shal8	U.S.A(Peiolia)	Salm5	Argentina	Sbic9	China(Hebei)	Ssud1	China(Hunan)
Shal9	China(Nanjing)	Salm6	Australia	Shyb1	Unknown	Ssud2	China(Nanjing)
Shal10	China(Nanjing)	Salm7	Ethiopia	Svul1	Unknown	Ssud3	Argentina
Shal11	China(Nanjing)	Salm8	Argentina	Ssac1	Unknown	Ssud4	China(Hunan)
Shal12	China(Nanjing)	Salm9	Argentina	Ssac2	Unknown	Silk1	Australia
Shal13	Argentina	Salm10	Argentina	Ssac3	China(CAAS)	Spro1	China(Guangxi)
Shal14	Australia	Salm11	Australia	Ssac4	Unknown	Spro2	China(Fujian)
Shal15	China(Xiamen)	Salm12	Australia	Snit1	Australia		
Shal16	U.S.A	Salm13	Australia	Snit2	China(Lianyungang)		
Shal17	U.S.A(Peiolia)	Sbic1	Unknown	Snit3	Australia		

Shal1~Shal20: S.halepense; Salm1~Salm13: S.almum; Sbic1~Sbic9: S.bicolor; Shyb1: S.hybrid; Svul1: S.vulgare; Ssac1~ Ssac4: S.saccharatum; Snit1~ Snit4: S.nitidum; Saru1: S.arundinaceum; Sver1: S.verticilliflorum; Sdru1~ Sdru4: S.drummondii; Ssud1~ Ssud4: S.sudanse; Silk1: Silk1: Silk Sorghum; Spro1~ Spro2: S.propinum at 36°C and 80 s at 72°C. After 40 cycles, samples were submitted to 6 min at 72°C and finally kept at 4°C. Amplification products were separated on 1.5% agarose gels containing 10 mg/ml ethidium bromide, immersed in TAE. The DNA bands were visualized under UV light and photographed with the aid of the BIO-RAD Gel DOC-2000. Three representative templates from sorghum genus were selected for screening, and then we selected several polymorphic primers from 163 pairs of RAPD primers, expanding the template to seven for further screening in order to select the target specific segment.

Cloning and sequencing the RAPD specific fragment of S.halepense

The RAPD marker band of S.halepense specific was cloned in vector pMD18-T. Competent DH5α Escherichia coli cells were transformed and mini-prep DNA from white colonies was sequenced with M13 universal primers flanking the insert.

Design and validation of SCAR primers

The sequence data was used to design and synthesize two specific primers of *S. halepense* each containing 18-19 nucleotides including the sequence of the original RAPD primer listed in 1.2.2. The SCAR primers were verified by 65 sorghum DNA samples. The thermocycler was programmed for 35 cycles: 30 s at 94°C, 40 s at 55°C, and 80 s at 72°C.

The RAPD cluster analysis of several sorghum samples

A total of 19 RAPD primers (S101, S117, S125, S127, S133, S134, S137, S147, S151, S164, S178, S187, S194, S201, S224, S226, S227, S248, S253) were selected to amplify one *S. bicolor* sample (Sbic9), four S.halepense samples (Shal7, Shal12, Shal15, Shal19) with different geographical origin and 12 *S. almum* samples (Salm2-Salm13). The cluster of the fingerprints was analysed by SPSS software.

Results and analysis

Cloning and sequencing the RAPD specific fragment of S. halepense

After screening with the three representative sorghum sample, 8 primers were selected from 163 primers. The RAPD-PCR electropherogram amplication of the three samples with eight different primers in primary screen was shown in Fig. 1. Adding another three *S. halepense* samples with different geographic origin and one *S. almum* sample which is the nearest relative species of *S. halepense*, a further screening was carried out from the 8 primers obtained in the first screening. The results showed that a specific band could be amplified by primer S151 when the sample was *S. halepense*. The electropherogram was shown in Fig. 2. Sequenced by Sangon Co. Ltd. (Shanghai), the actual size of the fragment was 510bp, named JGL0924, shown in Fig. 3. Inputing the sequence to NCBI (national center biotechnology information) database for nucleotide BLAST analysis, results showed no high homological sequence in the existing sequences in GeneBank.

SCAR-PCR amplification

According to the sequence, one pair of *S. halepense* specific primers were designed based on the random primer S151. The sequences of the SCAR primers were shown in Tab. 2. The electropherogram of PCR amplification with CP3/CP4 in Fig. 4, 5, 6 showed positive results indicating that the genomic DNA of all sorghum genus samples was available in this study. The verification results of the specific primers SH1/SH2 were shown in Tab. 3. The amplification results of 20 *S. halepense* samples had the specific bands. Except three Australia *S. almum*, the amplification results of other 10 *S. almum* samples, 9 *S. bicolor* samples, 1 *S. hybrid* sample, 1 *S. vulgare* sample, 4 *S. saccharatum* samples, 4 *S. nitidum* samples, 1 *S. arundinaceum* sample, 1 *S. verticilliflorum* sample, 4 *S. drummondii* samples, 4 *S. sudanse*, 1 Silk Sorghum and 2 *S. propinum* samples were

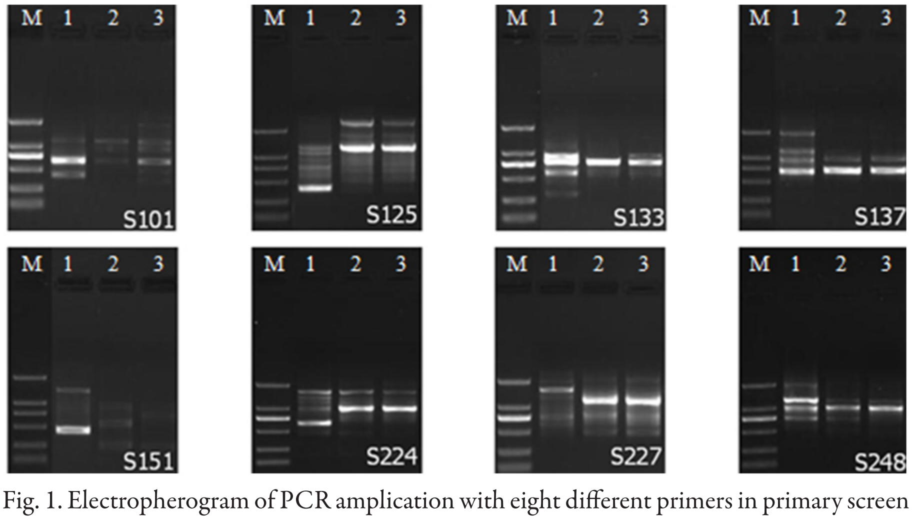

Fig. 1. Electropherogram of PCR amplication with eight different primers in primary screen
M: Marker(DL 2000); 1: Ssud1; 2: Sbic1; 3: Shal9

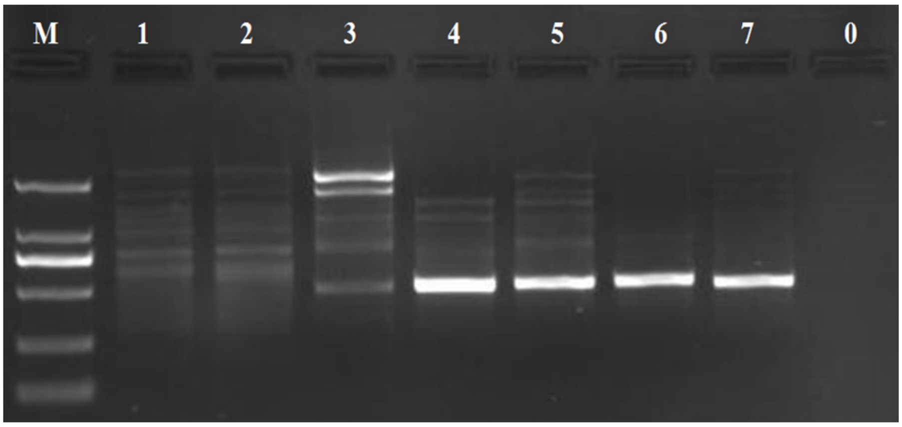

Fig. 2. PCR amplication electropherogram of RAPD primer S151

M: Marker (DL 2000); 1: Ssud1; 2: Sbic1; 3: Salm3; 4: Shal1; 5: Shal2; 6: Shal12; 7: Shal15

GAGTCTCAGGGTATGATCTGTTCGTCTTCCCTCAATTTTACGTCGCTCTGTCTCTGTTTG
TTTCAGTAGCTCTGTCGTGCAGGTTCAATAGTTGTGTCATACCTGTTTTAGTAGGTCTGT
TTAGTTGTTTCAGAAGATATCTGTTGTCTGTTCCTGTTATGTTTAAGTTCTGCGTATATGC
CTCTGTTCTACAGTTCTGTTGTTTCGCTAGTATCTGTTTTTCTGTTATCTGTTTCGATAGT
TGTATCATGTTTGGTGCTGTTGCAGATAATTATATACCATGCTATGCTTTGATATTTGATTT
GCTTTATGGATCATGGTTACACGTCGTAATTATGATTCATGTCATATTTATATTTATCAATA
ATATCATATATGTCATCATCATATTGTTAATTTATGGAATTAAAATGACATGGAAATTGCCT
AATATCCTAACAGCAGCTGTCCACAAACCTGCATGCGTCTTGCCGCTAAGCGTGGATTT
CGTCTGCTGCGCACCTGAGACTC

Fig. 3. Nucleotide sequences of the JGL0924 (The framed part is the sequences of RAPD primer S151)

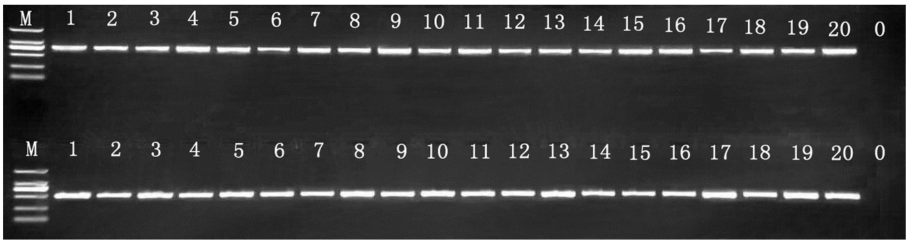

Fig. 4. Electropherogram of PCR amplication with CP3/CP4 and SH1/SH2 (1)

Upper line: universal primers (CP3/CP4), Lower line: primer SH1/SH2

M, DL2000 (TAKARA); 1~20, Shal1~Shal20; 0， negative control

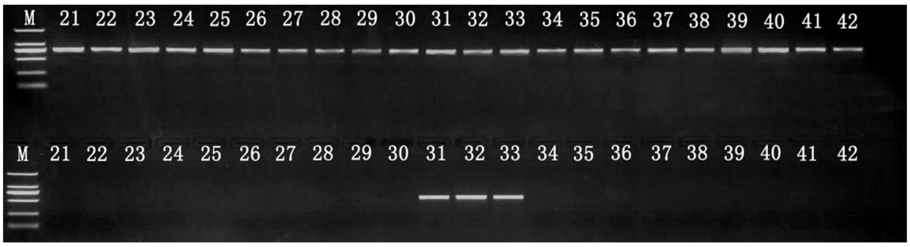

Fig. 5. Electropherogram of PCR amplication with CP3/CP4 and SH1/SH2 (2)

Upper line: universal primers (CP3/CP4), Lower line: primer SH1/SH2

M, DL2000 (TAKARA); 21~33, Salm1~Salm13 and 34~42, Sbic1~ Sbic9

Tab. 2. Sequences of Primers for SCAR

Primer code	Sequence
SH1	5'-AGATTGAGTCTCAGGTGC-3'
SH2	5'-GAGTCTCAGGGTATGATCT-3'

negative. The results were repeatable and stable. The primers could be used to detect S.halepense except samples from Australia, improving the stability of molecular identification.

The results of the RAPD sorghum samples cluster analysis

Nineteen RAPD fingerprints of 17 sorghum genus samples obtained, two pieces of fingerprints were shown in Fig. 7 as examples. All the bands on the fingerprints were cluster analyzed. The dendrogram of 17 sorghum samples based on variation in RAPD fingerprints were shown in Fig. 8. The dendrogram was divided into two main groups. One group included *S. halepense* and *S. almum*, and the other group was *S. bicolor*. In the first group, four *S. halepense* samples and three *S. almum* samples were clustered together as same sub-group, and the rest of *S. almum* samples were clustered as another sub-group. It could be found in the *S. almum* sub-group the genetic distance of the 5 *S. almum* samples from Australia and Ethiopia was very short, so the 5 *S. almum* samples were gathered together. Other four *S. almum* samples from Argentina were gathered together. The analysis results showed that the genetic material of *S. almum* was significantly different with the geographical distribution. Another three *S. almum* samples and four *S. halepense* samples were gathered together, indicating insignificant difference between *S. almum* and *S. halepense*.

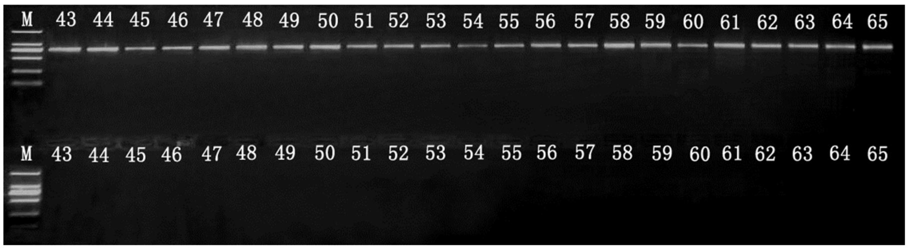

Fig. 6. Electropherogram of PCR amplication with CP3/CP4 and SH1/SH2 (3)

Upper line: universal primers (CP3/CP4), Lower line: primer SH1/SH2

M, DL2000 (TAKARA); 43, Shyb1; 44, Svul1; 45~48, Ssac1~Ssac4; 49~52, Snit1~ Snit4; 53, Saru1; 54, Sver1; 55~58, Sdru1~ Sdru4; 59~62, Ssud1~ Ssud4; 63, Silk1; 64~65, Spro1~ Spro2

Tab. 3. Verification results of the SCAR primers SH1/SH2 for *S. halepense*

Code	Result	Code	Result	Code	Result	Code	Result
Shal1	+	Shal18	+	Sbic2	-	Snit4	-
Shal2	+	Shal19	+	Sbic3	-	Saru1	-
Shal3	+	Shal20	+	Sbic4	-	Sver1	-
Shal4	+	Salm1	-	Sbic5	-	Sdru1	-
Shal5	+	Salm2	-	Sbic6	-	Sdru2	-
Shal6	+	Salm3	-	Sbic7	-	Sdru3	-
Shal7	+	Salm4	-	Sbic8	-	Sdru4	-
Shal8	+	Salm5	-	Sbic9	-	Ssud1	-
Shal9	+	Salm6	-	Shyb1	-	Ssud2	-
Shal10	+	Salm7	-	Svul1	-	Ssud3	-
Shal11	+	Salm8	-	Ssac1	-	Ssud4	-
Shal12	+	Salm9	-	Ssac2	-	Silk1	-
Shal13	+	Salm10	-	Ssac3	-	Spro1	-
Shal14	+	Salm11	+	Ssac4	-	Spro2	-
Shal15	+	Salm12	+	Snit1	-		
Shal16	+	Salm13	+	Snit2	-		
Shal17	+	Sbic1	-	Snit3	-		

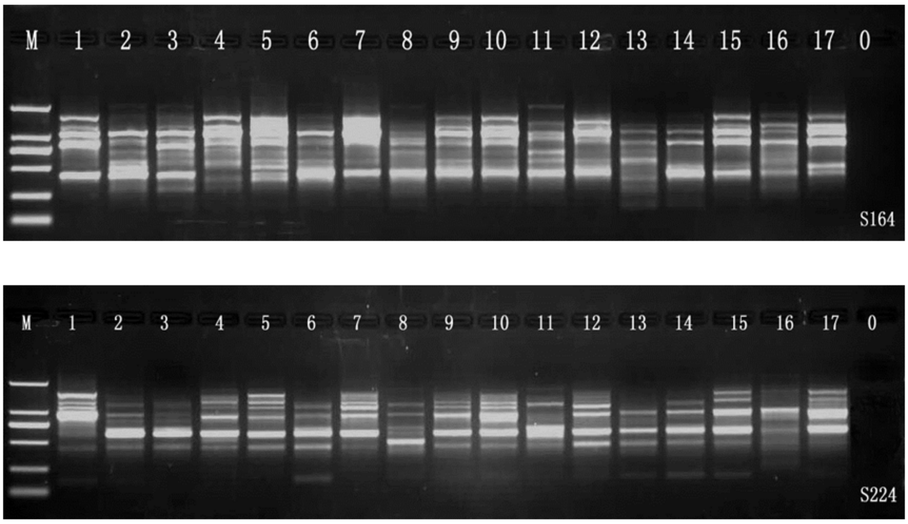

Fig. 7. Electropherogram of RAPD-PCR amplication with two RAPD primer (S164, S224)

Left: primer S164, Right: primer S224

M, DL2000 (TAKARA); 1, Sbic9; 2, Shal12; 3, Shal15; 4, Shal7; 5, Shal19; 6, Salm10; 7, Salm8; 8, Salm3; 9, Salm4; 10, Salm9; 11; Salm5; 12, Salm2; 13, Salm11; 14, Salm12; 15, Salm6; 16, Salm8; 17, Salm7

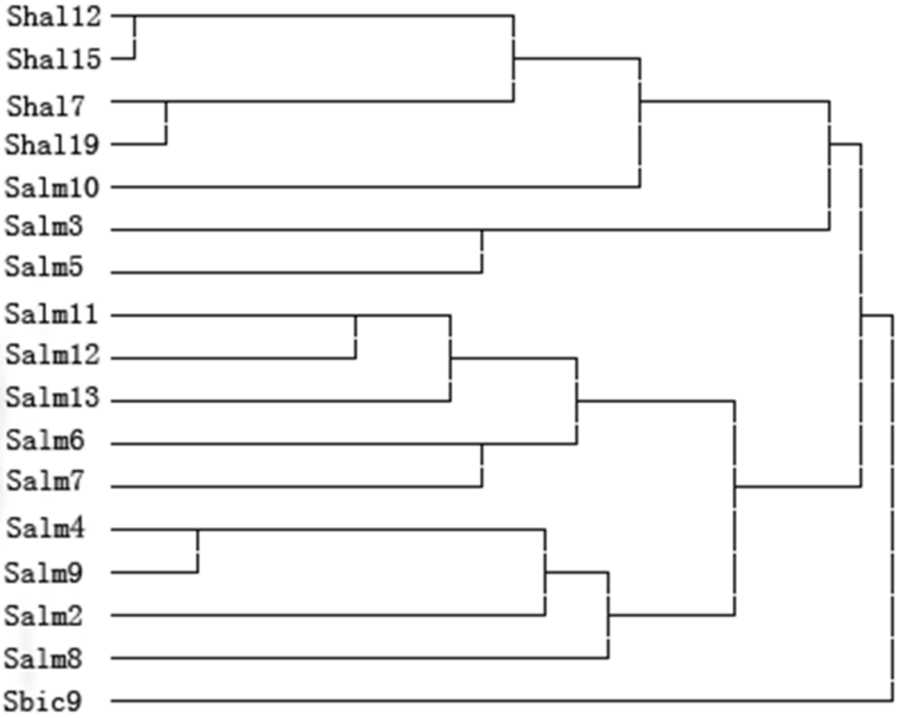

Fig. 8. Dendrogram of 17 sorghum samples based on variation in RAPD Fingerprinting

Discussion

The results showed that the RAPD marker could be successfully converted into a specific SCAR marker for rapid identification of *S. halepense* except Australia genotype of *S. almum*, improving the stability of molecular identification. For the hybridization is common in plantae, there is no strict reproductive isolation between different species hence many intermediate species exist. The SPSS cluster analysis results of the RAPD fingerprints showed that the close genetic relationship of *S. almum* and *S. halepense*, and *S. almum* was hybridization from *S. halepense* with *S. bicolor*, *S. sudanse* or *S. arundinaceum* (Zhang *et al.*, 1993). Therefore, we speculated that the three *S. almum* samples from Australia may be the medium species of *S. halepense* and *S. almum*, and this geotype of *S. almum* just inherited the specific site of *S. halepense* we found in the RAPD marker, leading to a positive result.

From the results we found that the specific band of *S. halepense* amplified by the SCAR primers was distinct.

Comparing with RAPD marker, the sensitivity of the reaction conditions of the SCAR marker was lower. This method reduced the environmental impact on the experimental results, owning higher specificity and repeatability. It reduces the instability of RAPD markers, avoids the complication and high financial cost of AFLP markers. SCAR marker is an ideal tool for molecular identification of species (Yan *et al.*, 2003). Though there are many reports on conversion of RAPD markers into SCAR markers successfully (Paran and Michelmore, 1993), the success rate of SCAR marker conversion is very low, which act as a main obstacle during the conversion process and developing of SCAR markers. The low success rate also results in difficulty in conversion to ensure the specificity of the primers after lengthening the RAPD primers. The next step of our study should include: (1) To further optimize the reaction conditions for specific amplification, increase the annealing temperature to ensure the specificity of the SCAR primers, and shorten the specific amplification primers length to increase the success rate of SCAR marker conversion; (2) To ensure the specificity of SCAR primers, amplify and sequence two adjacent sides of the sequence by reverse PCR, extend the 5 ‘end of RAPD primers to a certain length, resynthesis primers near the 5’ end to avoid covering polymorphism sites; (3) To further verify and analyze through Southern blot or design other primers. Since SCAR markers have stronger reproducibility over RAPD markers, convert the dominant RAPD markers into codominant markers, and owns the STS markers advantages, it improves the rate of gene mapping and molecular mapping. SCAR markers have a potential application in germplasm identification on *S. halepense* etc., more specific SCAR markers for S.halepense should be found in future work.

Conclusions

In this study, sequence characterized amplified regions (SCAR) marker of Johnsongrass was successfully established via conversion of the RAPD marker. This SCAR marker is much more stable, effective and accurate than RAPD markers, which was successfully applied to distinguish Johnsongrass and its relatives. We believe that this technique of conversion from RAPD marker to SCAR marker could also be widely used in fields of bio-detection, including malignant weed discriminating, species relationship analyzing, genetic disease-associated genes screening, genetically modified food detection and so forth.

Acknowledgments

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References

Chen L, Yu FL, Yang YJ, Chen DM, Xu CJ, Gao QK (1999). A study on genetic stability of excellent tea germplasms [*Camellia sinensis* (L.) O.Kuntze] using RAPD markers. J Tea Sci 19:13-16.

Chen Q, Wei SS, Deng ZR, Yin LP, He B, Kong XL (2009). Optimization of DNA extraction from seeds of *Sorghum sudanense* (Piper) Stapf. Not Bot Horti Agrobo 37:256-260.

Fang XE, Chen Q, Yin LP, Wang W (2008). Application of ISSR in genetic relationship analysis of sorghum species. Acta Agron Sinica 34:1480-1483.

Guo QX, Huang KH, Yu Y (2005). Differentiation of *Sorghum halepense* and *S. silk* by PCR-RFLP method. Plant Quarantine 19:330-333.

Holm LG, Plucknett DL, Pancho JV, Herberger JP (1977). The world's worst weeds-distribution and biology. Univ Press of Hawaii, Honolulu.

Li JQ, Wang LH, Zhan QW, Li YF (2007). A study on genetic variation of two species of sorghum by RAPD markers. Acta Prataculturae Sinica 16:140-144.

Jang CS, Kamps TL, Tang H, Bowers JE, Lemke C, Paterson AH (2009). Evolutionary fate of rhizome-specific genes in a non-rhizomatous Sorghum genotype. Heredity 102:266-273.

Kesseli RV, Paran I, Michelmore RW (1992). Efficient mapping of specifically targeted genomic regions and the tagging of these regions with reliable PCR-based genetic markers, 31-36 p. In: American Society of Agronomy (Ed.). Symposium Applications of RAPD Technology to Plant Breeding. ASA, Minneapolis and Madison.

Paran I, Michelmore RW (1993). Development of reliable PCR-based markers linked to downy mildew resistance genes in lettuce. Theor Appl Genet 85:985-993.

Penner GA, Bush A, Wise R, Kim W, Domier L, Kasha K, Laroche A, Scoles G, Molnar SJ, Fedak G (1993). Reproducibility of random amplified polymorphic DNA (RAPD) analysis among laboratories. PCR Methods Appl 2:341-345.

Williams JGK, Kubelik AR, Livak KJ, Rafalski JA, Tingey SV (1990). DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucl Acids Res 18:6531-6535.

Yan NH, Chen J, Ma XR, Deng GB, Yu MQ (2003). Conversion of RAPD marker to SCAR marker of wheat cereal root-knot nematode-resistant gene rkn-mnl. Chin J Appl Environ Biol 9:250-253.

Yin LP, Deng C, Yi JP, Wu H (2004). A study of the homology of *Sorghum halepense* and its relative species. Plant Quarantine 18:232-236.

Zhang JL, Yin LP, Zhang ZX (1993). The weed-*Sorghum almum*. Plant Quarantine 7:122-123.