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The Phylogenetic Analysis and Identification of *Sorghum halepense* and Related Species Based on the Chi-B Partial Sequence

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Abstract

Chi-B partial sequences of *Sorghum halepense* and its relatives were amplified with primer D16/D18 derived from Chi-B of the *Sorghum* genus. The results showed that the length varied from 567 bp to 607 bp, while phylogenetic relationship analysis by "DNASTAR" software showed that all materials were separated into three sections. The first section included *S. halepense, S. almum* and *S. silk* with a similarity of 96.9~100%, the *S. bicolor, S. propinquum* and *S. arumdinaceum* species were in sections of two with 93.0~95.3% similarity, the genetic distance between *S. nitidum* and the others was longer than in the case of the others, with a similarity of 69.0~71.0%. With accession-special primer D51/D55, *S. halepense* and *S. almum* could be identified by PCR.

Keywords: *S. halepense*, Chi-B, phylogenetic analysis

Introduction

Johnsongrass, *Sorghum halepense* (L. Pers.) is one of the ten hardness weeds in the world, which is ranked as the second most dangerous species according to the Chinese Import Act. Its relatives, such as *Sorghum almum*, *S. silk*, *S. propinquum*, *S. arumdinaceum*, *S. bicolor* and *S. nitidum,* are similar regarding the morphological traits of the seed, and thus, it is hard to classify them clearly. For the different inspection positions and harmness of *S. halepense* and its relatives, it is necessary to introduce a new molecular biological method to analyse and identify them.

As for the plant, since the clone of the endochitinase gene had been first reported in *Phaseolus culgaris* (DeWet, 1978), the study on the chitinase gene was never stopped. The clarification and sequence of chitinase were reported in almost all model plants, such as tobacco, potato, *Arabidopsis thaliana*, carrot, barley etc. The plant gene *Chi-b*, encoding the basic chitinase, has reportedly evolved from the same archaic ancestor (Doggett, 1976). A study on chitinase showed that the evolvement of *Chi-b* could have occurred in several manners, like gene transfer, replication, or deletion (Ouyang et al., 2001).

Until now, the study on the Chitinase gene has been focused on gene transfer for improving the disease resistance traits of the plant, while the study on the phylogenetic analysis or identification by *Chi-b* has not been reported. In this study, we report the sequence of *Chi-b,* which proves that a difference appears in *S. halepense* and its relatives, and the quarantine species Johnsongrass and Columbus grass could be classified by PCR using the *Chi-b* sequence.

Materials and methods

Plant materials

Seeds of the Sorghum Genus were obtained from Yin Liping, Technical Center of Plant and Animal Quarantine, Shanghai Entry-Exit Inspection and Quarantine Bureau and all the 35 seeds were analyzed for *Chi-b* variation. To estimate the difference, ten accessions including *S. halepense* and its relatives *S. almum*, *S. arumdinaceum*, *S. bicolor*, *S. nitidum*, *S. propinum*, *S. saccharatum*, *S. silk*, *S. vulgare*, *S. sudanense* with 3 sample sequences from Genbank were used (Table 1).

Sequencing

Total DNA was extracted from seed powder using the method of Moller et al. (1992), diluted ten times and used as template for PCR amplification. Based on the reported *Chi-b* DNA sequence of *S. bicolor*, *S. halepense* and *S. arumdinaceum*, 19-bp conserved regions in 199-217bp and 817-835bp DNA sequence were chosen as universal primers D16/D18. And the special primers D51/D55 were chosen 19-bp region in 251-269bp and 20-bp region in 451-470bp DNA sequence (Table 2).

PCR amplification

10mM Tris-HCl, 50mM KCl (pH 8.3, 1.5mM, MgCl2, 0.1mM of dATP, dGTP, dCTP, dTTP respectively, 100nM primers 10-20ng of genomic DNA, and 0.5U of Taq DNA polymerase were used. For universal amplification, after an initial heating step at 94°C for 3 min, samples were incubated for 30 circles of 94°C for 30 sec, 60°C for 30 sec, and 72°C for 1 min. And for D51/D55 amplification,

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Table 1 Accession list of samples used in the study (including NCBI sequences)

Code	Accessions	Origin	Code	Accessions	Origin
Salm 1	S. almum	U.S.A.	Shal 4	S. halepense	Argentina (peiolia)
Salm 2	S. almum	U.S.A. (peiolia)	Shal 5	S. halepense	Sesame from India
Salm 3	S. almum	Argentina (kesta)	Shal 6	S. halepense	U.S.A. (peiolia)
Salm 6	S. almum	Australia	Shal 7	S. halepense	Nanjing, China
Salm 7	S. almum	Australia	Shal 8	S. halepense	Australia
Saru 1	S. arumdinaceum	Australia	Shal 9	S. halepense	Australia
Sbic 1	S. bicolor, TR30	CAAS, China	Snit 1	S. nitidum	Lianyungang, China
Sbic 2	S. bicolor,WSW	CAAS, China	Spro 1	S. propinum	Lianyungang, China
Sbic 3	S. bicolor, SCS	CAAS, China	Spro 2	S. propinum	Australia
Sbic 4	S. bicolor	Afghanistan	Ssac 1	S. saccharatum	CAAS, China
Shal 1	S. halepense	Argentina (duchess)	Ssac 2	S. saccharatum, M81-E	CAAS, China
Shal 12	S. halepense	KSYSship, U.S.A.	Ssac 3	S. saccharatum, JA3058	CAAS, China
Shal 17	S. halepense	Soybean from Bwship, Argentina	Ssilk 1	S. silk	Australia
Shal 18	S. halepense	Soybean from Argentina	Svul 1	S. vulgare	U.S.A
Shal 2	S. halepense	Texan, U.S.A.	Svul 2	S. vulgare	Unknown
Shal 21	S. halepense	Soybean from DKLship, Argentina	VxS	S. sudanense	Unknown
Shal 25	S. halepense	Soybean from Argentina	AY048375	S. bicolor	NCBI
Shal 26	S. halepense	Soybean from Uruguay	AF402939	S. halepense	NCBI
Shal 3	S. halepense	U.S.A. (peiolia)	AF402938	S. arumdinaceum	NCBI

samples were incubated for an initial heating step at 94°C for 3 min, followed 35 circles of 94°C for 30 sec, 55°C for 30 sec, and 72°C for 1 min. All reactions were completed by incubation at 72°C for 5 min. The PCR products with 6×loading buffer were electrophoresed on 1.5% agarose gels with 1×TAE buffer to confirm that the length was in the range of the estimated sequence.

Sequence analysis

All the universal PCR products were sequenced in Sangon Co. Ltd. (Shanghai) and 38 sequences, including previewed 3 Genbank's sequences, were aligned with software ClustalX1.8. Data were analyzed using DNASTAR on a DELL PC. Deletions were considered to be single evolution events and were weighted the same as nucleotide substitutions. The phylogenical tree based on the Chi-b partial sequence was obtained by N-J method using MegAlign program.

Identification of Johnsongrass and Golumbusgrass

6 accessions of *S. halepense*, 5 accessions of *S. almum* and their relatives were amplified with primers D51/D55 that were based on the sequence alignment of *Chi-b*, then a PCR method for the classification of quarantine weeds was set.

Results and discussion

Length variations of the Chi-b region

There were 219 variants (191 nucleotide substitutions and 28 deletions) in the entire *Chi-b* region obtained (Figure 1). Out of these, the 102 variants of *S. nitidum* were singleton. The lengths of the accessions *S. halepense* were 590-594 bp, *S. almum* and *S. silk* had similar lengths of the *Chi-b region*. *S. vulgare*, *S. saccharatum* and *S. Sudanese* were 607 bp, longer than those of 595 bp for *S. propinum*, 591 bp for *S. arumdinaceum* and 567 bp for *S. nitidum*. The lengths of *S. bicolor* accessions were ranged from 596-607 bp. The differences between the *Sorghum* Genus were not centralized in one region and distributed along the whole *Chi-b* gene sequence. The difference among geographic accessions of *S. halepnese* and *S. almum* resided in 45 variations (34 substitutions, 11 deletions). Out of which, the accession shal 7 and salm 3 had the biggest variations, respectively (8 in shal 7 and 8 in salm 3). There is a bigger variety region between 179 bp and 393 bp, where there are 18 different bases and 8 deletions (Figure 1).

Table 2 Primers of PCR

	Nucleotide sequence (5'-3')	Location/bp	Amplification/bp
D18[a]	GTCACCGACGCATTCTTCA	199-217[C]	594
D16[b]	ATTCTTCCCGTTGCACTCG	817-835[C]	
D51[a]	AGGGCAAGAACTTCTACAC	251-269[C]	219
D55[b]	GATCATACTGATCGATGTTT	451-470[C]	

a: forward primer; b: reverse primer; c: Nucleotide location in AF402939

The genetic distance analysis

An estimate of genealogical relationships among the *Sorghum* Genus was obtained by the N-J method, based on nucleotide variation in the *Chi-b* region (Figure 1). There are clearly four distinct clusters. The first cluster included all the *S. halepense* and *S. almum*. For the *Chi-b* region, the nucleotide distance between *S. halepense* and *S. almum* was 0.0-1.5, so they were very similar genetically. The second and the third included other species except *S. nitidum*. The latter belonged to cluster IV. The nucleotide distance between clusters was 10.3-11.0, 9.0-9.7, 21.3 for cluster I and cluster II, I and III, respectively I and IV. The average distance between cluster II and III was 1.6-3.3, and they were much more similar. Cluster IV included only *S. nitidum* and presented a larger diversity than others in the *Chi-b* region. Thus, the Chi-b region could tell the difference within the *Sorghum* Genus (Figure 1, Table 2).

Application of the Chi-b region

According to the diversity of *Chi-b* between Johnsongrass or Golubusgrass and their relatives, primers of D51/D55 were designed to identify the *Sorghum* Genus. The PCR results showed that all the samples of Johnsongrass and Golubusgrass were positive, while other species and the blank sample were negative (Figure 2).

The phylogenic relationship of *S. halepense* and its relatives: the relationship between Johnsongrass and Golubusgrass is so close that the result *on Chi-b* gene diversity isn't clear. In this respect, they have 96.6-99.0% genetic similarity, it is thus considered that *S. halepense* contributed to *S. almum*, which accorded to the estimate by the cell genetic method (Moller et al., 1992). The diversity and the sequence results among *S. almum*, *S. arundinaceum* and *S. Sudanese* show that although cultivated species were considered to be more distantly related to *S. almum*, *S. halepense* may contribute more to *S. almum* gene pool.

It has been suggested that *S. silk* was obtained from *S. halepenese*. Others have considered it a product of the hybridization of *S. halepenese* and a cultivated species. The diversity between *S. halepense* and *S. silk* for the *Chi-b* gene was only 1.0-1.5 while that between *S. arundinaceum* and *S. silk* was even larger, so it is considered that *S. silk* was obtained more likely from *S. halepense* than other cultivated species. As a potential weed, *S. silk* must be inspected.

As the results of the sequence analysis on the *Chi-b* region showed the similarity between *S. halepense* and *S. bicolor* is 82.5-83.7%, while diversity with *S. arundinaceum*

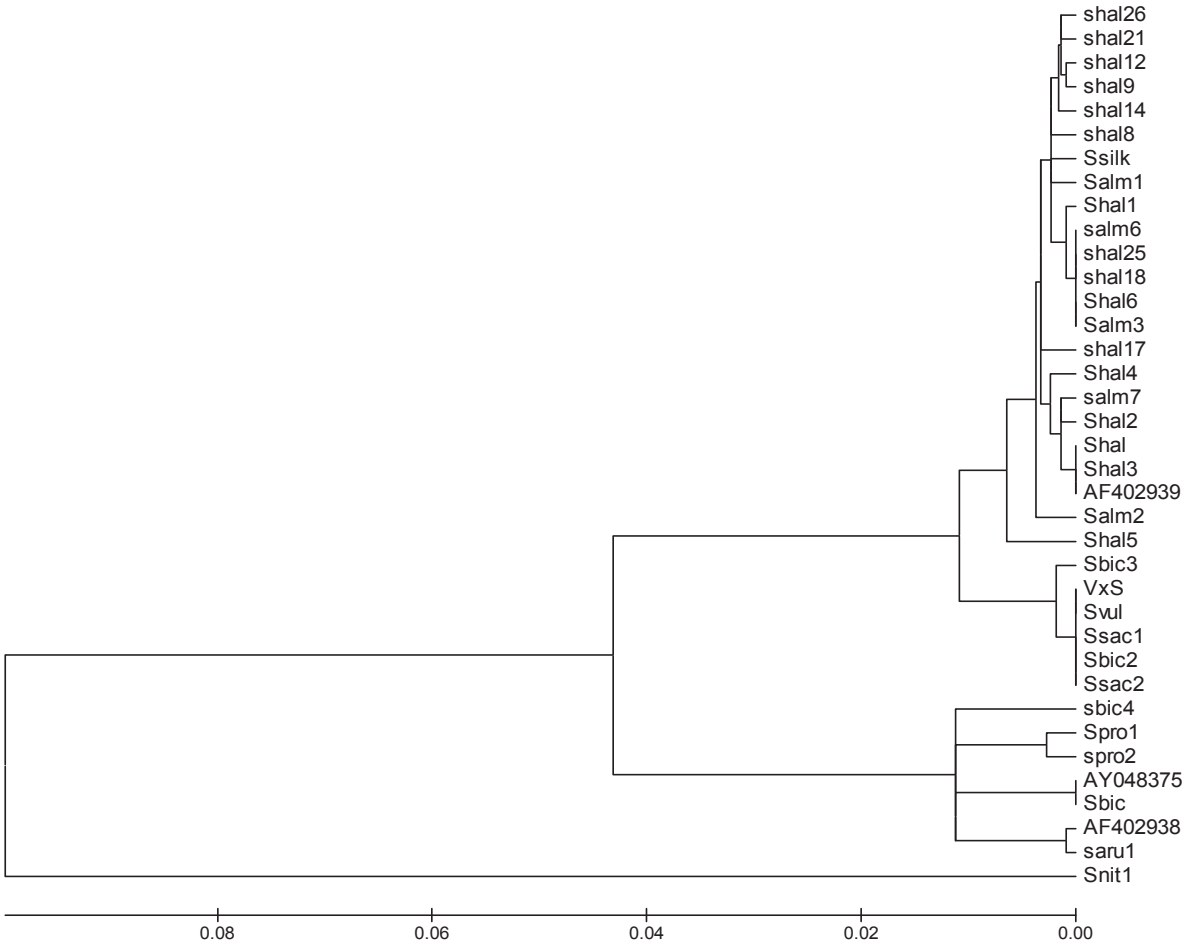

Figure 1 The phylogenetic tree of accessions based on variation in chitinase-b genes

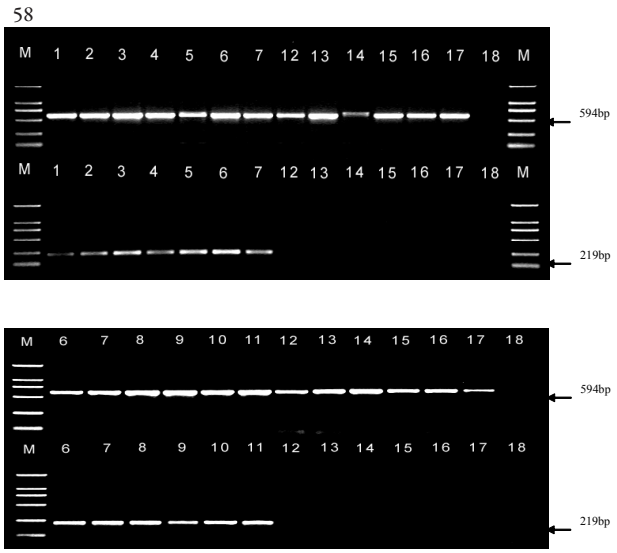

Figure 2 Identification *S. halepense* and *S. almum* by PCR, Upper line: common primer, Lower line: special primer, M, DL2000; 1~6, different *S. halepense*; 7~11, different *S. almum*; 12, *S. versicolor*; 13, *S. bicolour*; 14, *S. nitidum*; 15, *S. arundinaceu*m, 16, *S. propinum*; 17, *S. Sudanese*; 18, blank control

is 10.3-11.0. The diversity distance of the *Chi-b* region between *S. halepense* and its relatives like *S. bicolor*, *S. arundinaceum* and *S. propinum* was so high that a nucleotide difference still exits, for the accessions in section *Eusorghum*. Though Ragab (1994) considered that *S. halepense* and *S. bicolor* were more similar by RFLP, *S. propinum* was more similar with *S. bicolor* than *S. halepense* by *Chi-b* analysis, according to the analysis by ways of traditional morphological classification, kafirin-HPLC, isoenzyme, and ITS sequence analysis (Smith and Smith, 1988; Tomohiro and Takao, 1995; Yin et al., 2004).

Identification weed: by the PCR method, the inspected species *S. halepense* and *S. almum* could be identified from a small quantity of weed DNA. It is suited for inspection work, as it overcomes the morphological disadvantages, such as the lack of intercept and capture, mature in homogeneity, or abrasion of seeds. The inspection period of identifying weed was shortened to 1-2 days. The method that predigested the inspection equipments and reduced the skilful background was fit to be extended as a common inspection means.

References

Broglie, K. E., J. J. Gaynor, R. M. Broglie, 1996, Ethylene- regulated gene expression: molecular cloning of the genes encoding an endochitinase from *Phaseolus culgaris*. Proc. Natl. Acad. Sci. USA 83, 6820-6824.

DeWet, J. M. J., 1978, Systematic sand evolution of sorghum sect, *Sorghum* (*Gramineae*). Am. J. Bot. 65, 477-484.

Doggett, H., 1976, Sorghum. In Evolution of crop plants, N.W. Simmonds, Longman, London, 112-117.

Garber, E. L., 1950, Cytotaxonomic studies in the genus *Sorghum*. Univ. Calif. Publ. Bot. 23, 283-362.

Guo, Q. X., K. H. Huang, 1995, The research of the related species of *Euchlaena*. Fujian Agric. Sci. andTech., 8-9.

Moller, E. M., G. Bahnweg, H. Sandermann, 1992, A Simple and Efficient Protocol for the High Molecular Weight DNA from Filamentous Fungi, Fruit Bodies, and Infected Plant Tissues. Nucleic. Acids. Res 20, 6115-6116.

Ouyang Shw, Zhao Kj, Feng Lx, 2001, The Structure and Function, Classification and Evolution of Plant Chitinases. Chinese Bulletin of Botany 21(4), 30-34.

Ragab, R. A., 1994, Construction of sorghum RFLP linkage map using sorghum and maize DNA probe. Genome 37(4), 590-594.

Smith J. R. C., O. S. Smith, 1988, Variation in Kafirin and aclohosobubke glutekin chromatograms of sorghum inbred lines revealed by reversed-phase high-performance liquid chromatography. Theor. Appl. Genet. 76, 97-107.

Tomohiro, A, T. Takao, 1995, Structural classification of plant chitinase: two subclasses in class I and class II chitinase. Biosic Biotech Biochem, 59(2), 336-338.

Yin L. P., C. Deng, L. Kang, 2004, Phylogenetic Relationship of *S. halepense* and Related Species Based on rDNA Sequence. Plant Quarantine 18(5), 262-265.