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研究简报

Application of ISSR in Genetic Relationship Analysis of Sorghum Species

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Abstract: The genetic relationship among six sorghum species, namely, *S. bicolor*, *S. bicolor*×*S. sudanese*, *S. saccharatum*, *S. sudanese*, *S. almum*, and *S. halepense* was analyzed by inter-simple sequence repeat (ISSR) method. The results showed that the diversity of sorghum was high at DNA level. Twenty-two primers selected from 110 ISSR primers could amplify 182 clear and reproducible bands, of which 153 bands were polymorphic, accounting for 84.0%. All the sorghum species studied could be distinctly divided into two major groups with the genetic distance level at 0.25 by cluster analysis based on the Neighbor-Joining method. Some primers produced highly polymorphic band patterns in different species, such as IR 89, IS16, according to them, all the sorghum species used could be identified. The results could be used in classification, identification and evolution of sorghum.
Keywords: Sorghum; ISSR; Genetic relationship

ISSR 在高粱属植物遗传关系研究中的应用

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摘 要: 以高粱、杂交高粱、甜高粱、苏丹草、黑高粱和假高粱 6 种不同高粱属植物为实验材料, 利用 ISSR 分子标记分析其遗传关系。结果表明, 这 6 种高粱属植物在 DNA 水平上具有较高的遗传多样性。从 110 条 ISSR 引物中成功筛选到 22 条多态性高、稳定性好的引物, 共扩增出 182 条带, 其中差异性条带 153 条, 多态条带比率(PPB)为 84.0%。基于遗传距离系数的 Neighbor-Joining 聚类分析可以将 6 种高粱分为两大组, 相互之间的遗传距离为 0.25。另外可以根据一些多态性高的引物所扩增得到的品种特异性条带区分所有不同高粱种, 如引物 IR89、IS16 等。这些为研究高粱属植物的分类、鉴定和进化提供了分子生物学方面的理论依据。
关键词: 高粱属植物; ISSR; 遗传关系

Sorghum is an annual or perennial herbaceous plant with more than 50 species, possibly possessing considerable complex genetic relationship, while most of them are economic crops which are known for their nutritious value of grains. However, in recent years, some species have declined rapidly for the reason of being impacted by some malignant weeds, such as Johnsongrass or Blacksorghum[1-3]. Morphological and enzymatic markers have typically been used to identify this weeds and study the genetic relationship of sorghum[4-6], However, the similarities in morphology make it difficult to identify some species' seeds. At the same time, some characters of seeds may be destoryed during transportation.

ISSR is defined as the amplification of regions between adjacent microsatellites using a simple sequence repeat (SSR) primer. Compared with other molecular markers, ISSR uses longer primer allowing for higher annealing temperature that results in greater reproducible bands[7]. Because of the high polymorphism and stability, ISSR have been employed successfully in analyzing the genetic relationship of plant species. Chen[8] studied the genetic diversity of 56 individuals of Isoetaceae from China using ISSR; Song[9] successfully established the polymorphic ISSR pattern in aquatic weed *Leersia hexandra*. Other plants such as common wheat[10], melon germless[11], sugarcane[12], *Paeonia lactiflora*[13], *Capsicum frutescens*[14], and *Paeonia suffruticosa*[15] etc. have already been investigated using ISSR markers. Nevertheless till now, there was no report available for the use of ISSR in analyzing sorghum. In the present study, ISSR technology was used to analyze its genetic relationship.

1. Materials and methods

1.1 Plant materials

Six representative sorghum species of 30 individuals

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each (Table 1) were all provided by Shanghai Entry-Exit Inspection and Quarantine Bureau.

Table 1 Sample list

Species	Latin name	Origin	Sample size
Sorghum	*S. bicolor*	Afghanistan	30
Hybrid sorghum	*S. bicolor*× *S. sudanese*	USA	30
Sweet sorghum	*S. saccharatum*	China	30
Sudangrass	*S. sudanese*	USA	30
Blacksorghum	*S. almum*	Australia	30
Johnsongrass	*S. halepense*	USA	30

1.2 DNA extraction

Total genomic DNA was extracted from frozen young leaves following the CTAB procedure described by Scott[16].

1.3 ISSR-PCR

One-hundred and ten ISSR primers (Shanghai Sangon) were used to amplify the genomic DNA. The amplification was performed in a Thermocycler of FGENO2TD PCR (TECHNE, America) and commenced with 12 min at 94°C, followed by 40 cycles of 30 s at 94°C, 30 s at 48°C, 1 min at 72°C, and ended with 12 min at 72°C. Reactions were carried out in a volume of 20 μL containing 1×PCR buffer (TaKaRa, Japan), 2.0 mmol L^{-1} $MgCl_2$, 250 μmol L^{-1} dNTP, 400 nmol L^{-1} primer, 30 ng DNA template, 1.5 U *Taq* polymerase. The amplified products were separated on 1% agarose gel in 1×TAE buffer with the voltage of 80 V for 1h and visualized by staining with ethidium bromide [17].

1.4 Data analysis

Genetic diversity among different species was measured by PPB. The cluster analysis was carried out using the SPSS 10.0 software based on the genetic distance according to Neighbor-Joining method[18].

PPB (Percentage of polymorphic bands) = Number of polymorphic bands / Total amplified bands.

2 Results

2.1 Screening of primers and genetic diversity analysis

Of the 110 ISSR primers screened, 22 primers were selected in our analysis for the clear, reproducible and polymorphic DNA amplification patterns (Table 2). These selected primers generated 182 bands ranging in size from 100 to 2 000 bp, corresponding to an average of 8.27 bands per primer, and 153 bands were divergent, accounting for 84.0%. Every primer selected produced polymorphic bands with PPB value ranging from 63.0% to 100%. Among the 22 selected primers, seven of them gave low PPB values while the others showed higher polymorphic fingerprints. Some primers, such as IS31, IR7, IR35 exhibited the highest polymorphism with PPB values at 100%. The genetic diversity

Table 2 Sequence of reliable ISSR primers and the number of scorable bands of each prime

Prime	Sequence (5′–3′)	Total amplified bands	Number of polymorphic bands	Percentage of polymorphic bands
IS13	AGAGAGAGAGAGAGAGA	9	8	89.00
IS15	AGAGAGAGAGAGAGACG	11	9	82.00
IS16	AGAGAGAGAGAGAGACC	14	11	79.00
IS18	AGAGAGAGAGAGAGAC	11	8	73.00
IS23	CAGCAGCAGCAGCAGT	9	8	89.00
IS24	CAGCAGCAGCAGCAGG	9	8	89.00
IS27	CTCTCTCTTCTCTCTG	6	5	83.00
IS31	GCACACACACACACACA	11	11	100.00
IR7	GAGAGAGAGAGAGAGAT	7	7	100.00
IR30	TCTCTCTCTCTCTCTCA	6	5	83.00
IR35	ACACACACACACACACT	7	7	100.00
IR37	TGTGTGTGTGTGTGTGC	8	7	88.00
IR43	AGAGAGAGAGAGAGAGYC	5	5	100.00
IR45	TATATATATATATATART	7	5	71.00
IR46	TATATATATATATATARC	7	6	86.00
IR47	TATATATATATATATARG	7	7	100.00
IR52	CTCTCTCTCTCTCTCTRC	7	7	100.00
IR59	GTGTGTGTGTGTGTGTYG	7	6	86.00
IR74	CTCCTCCTCCTCCTC	8	5	63.00
IR75	GGCGGCGGCGGCGGCGG	9	7	78.00
IR80	CATACATACATACATA	6	4	67.00
IR89	VBVATATATATATATAT	11	7	64.00
Total		182	153	—
Average		8.27	6.95	84.00

among the six sorghum species was considerably high according to the PPB value of 84%. Otherwise, the results showed that the di-nucleotide repeat primers could result in the ideal band patterns while among 22 primers selected, only four tri-nucleotide repeat primers (IS23, IS24, IR74, IR75) and one tetra-nucleotide repeat primer (IR80) could have clear patterns, which accorded well with the fact that targeted unit di-nucleotides are more abundant in sorghum species.

2.2 Cluster analysis

Based on the Neighbor-Joining method, a cluster analysis was carried out and a dendrogram was generated that represented the genetic relationships among six sorghum species (Fig. 1). In the dendrogram, all the sorghum species were distinctly separated into two major groups, group I (Blacksorghum and Johnsongrass) and group II (sweet sorghum, sudangrass, sorghum and hybrid sorghum) at the genetic distance level of 0.25. The genetic distance level in group I was 0.21, much higher than group II; sweet sorghum and Sudangrass could be clustered together while sorghum and hybrid sorghum into another in group II with the genetic distance level ranging from 0.075 to 0.180.

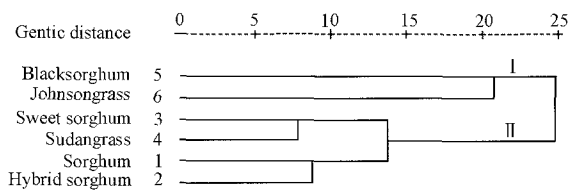

Fig. 1 Dendrogram illustrating genetic relationship among six sorghum species

2.3 Identification of sorghum species

According to the patterns obtained with 22 selected primers, all the sorghum species analyzed could be distinguished, especially the primers IR89 and IS16, as shown in Fig. 2 and Table 3. According to the specific bands in 1 500 bp and 100 bp, Blacksorghum and Johnsongrass could be separated from the other four sorghum species (Fig. 2, left). According to Fig. 2 (right), the Black sorghum had a species-specific band in 400 bp, which could be separated from the Johnsongrass; the hybrid sorghum had a distinctly specific band in 850 bp which could be used to identify it. Meanwhile, Sudangrass was lack of the band in 1 200 bp which could be used to distinguish it. In a word, the different sorghum species could all be well identified by the two primers. Other primers, such as IR30, IR35, IS13, and IS27 shown in Table 4 obtained relatively few bands, but acquired some species-specific bands, which could be used as molecular makers to identify some sorghum species. The other primers resulted in complex bands, which can assist to discriminate with each other. Above all, it was anticipated that ISSR-PCR could be exploited as the basis of molecular techniques for sorghum species identification.

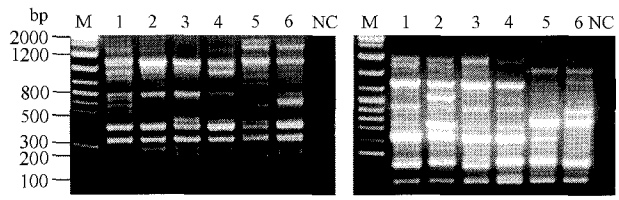

Fig. 2 Amplification of genome in six sorghum species using primer IR89 (left) and IS16 (right)

Table 3 Specific bands of sorghum by IR89 and IS16

Prime	Fragment length (bp)	Sorghum	Hybrid sorghum	Sweet sorghum	Sudangrass	Blacksorghum	Johnsongrass
IR 89	1500	–	–	–	–	+	+
	100	+	+	+	+	–	–
IS16	1200	+	+	+	–	–	–
	1100	+	+	–	–	–	–
	850	–	+	–	–	–	–
	400	+	+	+	+	+	–

"+": specific band; "–": no relevant band.

Table 4 Species-specific bands from different primer amplifications

Primer	Fragment length (bp)	Sorghum	Hybrid sorghum	Sweet sorghum	Sudangrass	Blacksorghum	Johnsongrass
IS27	1200	+	–	–	–	–	–
IS31	1200	+	–	–	–	–	–
IR35	1800	–	+	–	–	–	–
IR37	1100	–	+	–	–	–	–
IR75	250	–	–	–	+	–	–
IS13	1500	–	–	–	–	+	–
IS24	1750	–	–	–	–	+	–
IR30	2000	–	–	–	–	+	–
IR46	1700	–	–	–	–	–	+
IR31	250	–	–	–	–	–	–
IR43	2200	–	–	–	–	–	–
IR45	1500	–	–	–	–	–	–

"+": specific band; "–": no relevant band.

3 Discussion

To the best of our knowledge, this is the first report of genetic diversity in sorghum detected by ISSR primers. The ISSR analysis of six sorghum species revealed a high level of genetic diversity with the PPB value at 84.0% — much higher than detected by morphological traits, allozymes, or microsatellites [4-6]. This phenomenon in sorghum may be interpreted as occasional sexual recruitments in some habitats or the geographic isolation between populations. The diversity level between the different sorghum species revealed by ISSR has great significances in sorghum species conservation and breeding[19-20]. In the study, di-nucleotide repeat primers are more suitable to be used in analyzing the genetic relationship of sorghum than tri-, tetra-, and penta-nucleotide repeat primers, which consist well with the knowledge that targeted unit di-nucleotides are more abundant in sorghum [21]. Meanwhile, poly (AG)-anchored ISSR primers produced more bands in sorghum species than the other di-nucleotide repeat primers which suggests that the frequency of poly (AG) in the sorghum species genome is higher than that of poly (CT), poly (AT), poly (CG), poly (AC), and poly (GT).

Cluster analysis was carried out based on the ISSR data by SPSS 10.0 software, which showed that all sorghum species studied could be clustered into two groups. Group I was composed of Johnsongrass and Blacksorghum, which accorded well with the fact that the Blacksorghum is the hybrid between Johnsongrass and an unknown sorghum species [22]. Interestingly, this two sorghum species are all malignant weeds which will cause serious reduction to crops and bring serious financial loss once invading into an area; Group II was composed of sorghum, hybrid sorghum, sweet sorghum and Sudangrass, all of them are known for its ability to survive under the severe environmental conditions and for the nutritious value of their grains. They are the major economic crops in the world.

ISSR technique was further more applied to identify different sorghum species. According to the patterns obtained with some high polymorphic primers, all sorghum species studied could be successfully discriminated which has important implications for preventing the malignant weeds, such as Blacksorghum and Johnsongrass. Such a method will be a useful identification tool only if a reference sample from the sorghum species is present in the database. In this study, only six sorghum species were analyzed, further investigations will include the analysis of more species to allow the resolution of species status more detailed and to establish a reliable, quick and convenient authentication system for sorghum species [22]. In a word, with the advantages of high polymorphism, reproducibility and convenience, ISSR could offer a quick and reliable alternation in analyzing the genetic relationship of sorghum and identification of some malignant species.

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