African Journal of Biotechnology Vol. 12(7), pp. 665-669, 13 February, 2013
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB12.2838
ISSN 1684–5315 ©2013 Academic Journals

Full Length Research Paper

Inter-simple sequence repeat (ISSR) markers in the evaluation of genetic polymorphism of Egyptian *Capsicum* L. hybrids

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Accepted 17 January, 2013

DNA samples of six hybrids of *Capsicum annuum* and *Capsicum frutescens* were analyzed with ten inter-simple sequence repeat (ISSR) primers, which produced 52 polymorphic bands out of 87 bands with polymorphism average of 60%. ISSR patterns scored five distinguishable species-specific bands; two for *C. frutescens* and three for *C. annuum* and 16 unique bands for hybrids individually that were considered as molecular markers for Egyptian hybrids. The genetic dendrogram among the materials constructed by the unweighted pair-group method with arithmetic average based on the Dice coefficient showed that the six hybrids can be separated into two groups with genetic distance at 0.25. The first cluster included hybrids of *C. frutescens* and the other grouped those of *C. annuum* indicating narrow genetic base among the tested hybrids in both species. This study revealed considerable genetic diversity in the different *Capsicum* hybrids for conservation of genetic resources and efficient crop breeding programs.

Key words: *Capsicum*, inter-simple sequence repeat (ISSR), hybrids, genetic polymorphism, molecular markers.

INTRODUCTION

The genus *Capsicum* (pepper) includes a group of economic plants that are grown as spices, vegetables and colouring agents throughout the world and rated as the world's most demanded spice crop. Sweet pepper (*Capsicum annuum* L) is a member of the solanaceous family. It is one of the most important, popular and favorite vegetable crops cultivated in Egypt for local consumption and exportation and are commonly called "filfil akhdar", where "filfil" means pepper and "akhdar" means green (El-Bassiony et al., 2010). *Capsicum frutescens* is also widespread throughout tropical and subtropical regions, such as Asia, Africa, and the Pacific Islands. Improvement in any crop plant refers to positive heritable changes brought about in its genome to enhance economic output. Molecular markers have greatly enhanced the scope of detailed genetic analysis and improvement of crop plants. These markers act as excellent tools to study genetic diversity, genome mapping, choice of parental lines for crossings, marker-assisted selection, variety identification and hybrid purity in the seed industry (Hammer et al., 2003; Mathew, 2006). To date, different kinds of biochemical and molecular markers such as SDS-PAGE, isozymes, RFLP, RAPD, AFLP and ISSR have been successfully used to complement traditional morphological studies of *Capsicum* (Lefebvre, 2005; Moscone et al., 2007; Patel et al., 2011; Lijun and Xuexiao, 2012). Inter simple sequence repeat (ISSR) requires very small amount of template and is convenient in result recording and highly reproducible (Zietkiewicz et al., 1994). In previous study, slight intra and inter specific biochemical variations were detected in the six hybrids of *C. frutescens* and *C. annuum* (Ahmed, 2012); therefore application of molecular ISSR markers was conducted here for more characterization and discrimination among these hybrids for future crop breeding programs.

Table 1. Number and types of the ISSR bands as well as the total polymorphism percentages generated in six *Capsicum* hybrids.

Primer code	Sequence	Monomorphic band	Polymorphic band		Total band	Polymorphism (%)
			Unique	Shared		
HB 1	$(CAA)_5$	4	0	1	5	20
HB 2	$(CAG)_5$	6	2	2	10	40
HB 4	$(GACA)_4$	4	2	2	8	50
HB 8	$(GA)_6GG$	4	0	5	9	55
HB 9	$(GT)_6GG$	4	1	2	7	43
HB10	$(GA)_6CC$	3	1	3	7	57
HB11	$(GT)_6CC$	3	2	3	8	63
HB13	$(GAC)_3GC$	4	1	3	8	50
HB14	$(CTC)_3GC$	2	4	7	13	85
HB15	$(GTG)_3GC$	1	3	8	12	92
Total		35	16	36	87	60

MATERIALS AND METHODS

Plant materials

Seeds of the six new Egyptian hybrids; khairat, yoser 1, yoser 4 of *Capsicum frutescens* and kotof 1, kotof 2, kotof 3 of *Capsicum annuum*; were produced and supplied by Agricultural Research Center (Horticulture Research Department), Dokki, Giza, Egypt.

ISSR analysis

Leaves from the six hybrids three weeks old greenhouse-cultivated plants per hybrid were pooled together for DNA isolation. DNA extraction was performed using protocols of Dellaporta et al. (1983). Ten primers were independently used in PCR reactions as described by Williams et al. (1990). Codes and sequences of these primers are listed in Table 1. Amplifications were performed in a total reaction of 30 µL containing: 2.5 µL dNTPs (8 mM), 0.3 µL Taq DNA polymerase (5 U/ µL), 3.0 µL 10 X buffer with 15 mM $MgCl_2$, 2.0 µL Primer (10mM), 2.0 µL Template DNA (50 ng/ µL) and up to 30 µL H_2O. PCR amplification was programmed to fulfill 30 cycles after an initial denaturation cycle for 2 min at 94°C. Each cycle consisted of a denaturation step at 94°C for 30 s, an annealing step at 44°C for 45 s and an elongation step at 72°C for 90 s. The primer extension segment was done for 7 min at 72°C in the final cycle. PCR- product of 15 µL was resolved in 1.5 % agarose gel electrophoresis with 1×TAE running buffer. The run was performed at 80 V for 180 min using Biometra gel electrophoresis submarine (20 × 10 cm).

Data analysis

Bands of ISSR technique were visualized on UV- transilluminator and photographed by Gel documentation system (Biometra Bio Doc Analyze, 2000). Differences in bands intensity among profiles of the different samples were not considered. The binary data generated were used to estimate levels of polymorphism by dividing the polymorphic bands by the total number of scored bands. Band size was estimated by comparing with 1 kb ladder (Invitrogen, USA) using gel analyzer Ver. 3 program. Data generated by ISSR primers was used to compile a binary matrix for cluster analysis. Genetic similarity among accessions was calculated according to Dice similarity coefficient (Dice, 1945) and used to construct a dendrogram using unweighted pair group method with arithmetic average (UPGMA) using SPSS-11 program.

RESULTS

Ten ISSR primers produced polymorphic, as well as, monomorphic bands when applied to the six *Capsicum* hybrids. Photos of the produced banding patterns are shown in Figure 1. The primers scored 52 polymorphic bands out of 87 bands with polymorphism average of 60% (Table 1). The number of total bands varied from 5, with HB1, to 13, with primer HB14 with fragment size ranging from 287.6 to 2819.67 bp. Primer HB1 recorded the lowest polymorphism percentage (20%), whereas, the highest was 92% for HB15 primer. Hybrids of *C. frutescens* were characterized by two bands at 373.9 bp, with HB2, and 572.7 bp with HB13, while bands with molecular size of 1476.0 and 706.6 bp with HB8, and 668.3 bp with HB13 distinguished those of *C. annuum*. These five distinguishable bands were considered as species-specific bands and could be used as molecular markers for discrimination between the two *Capsicum* species as indicated in Figure 1. Although, less than 50% of the bands were polymorphic within the two species, 52 polymorphic bands, in general, revealed considerable intra and inter specific variations into the two *Capsicum* species. Among them 16 bands were detected as unique bands (Table 2).

The similarity coefficient values ranged from 0.709 to 0.883 using dice coefficient (Table 3); showed a close relationship between khairat and yoser 1 of *C. frutescens* (0.883) and least genetic similarity between yoser 1 of *C. frutescens* and kotof 2 of *C annuum* (0.709) hybrids. The genetic relationships among *Capsicum* hybrids were analyzed by UPGMA method (Figure 2). The dendrogram separated the hybrids into two major groups with genetic distance 0.25. The first cluster included hybrids of *C.*

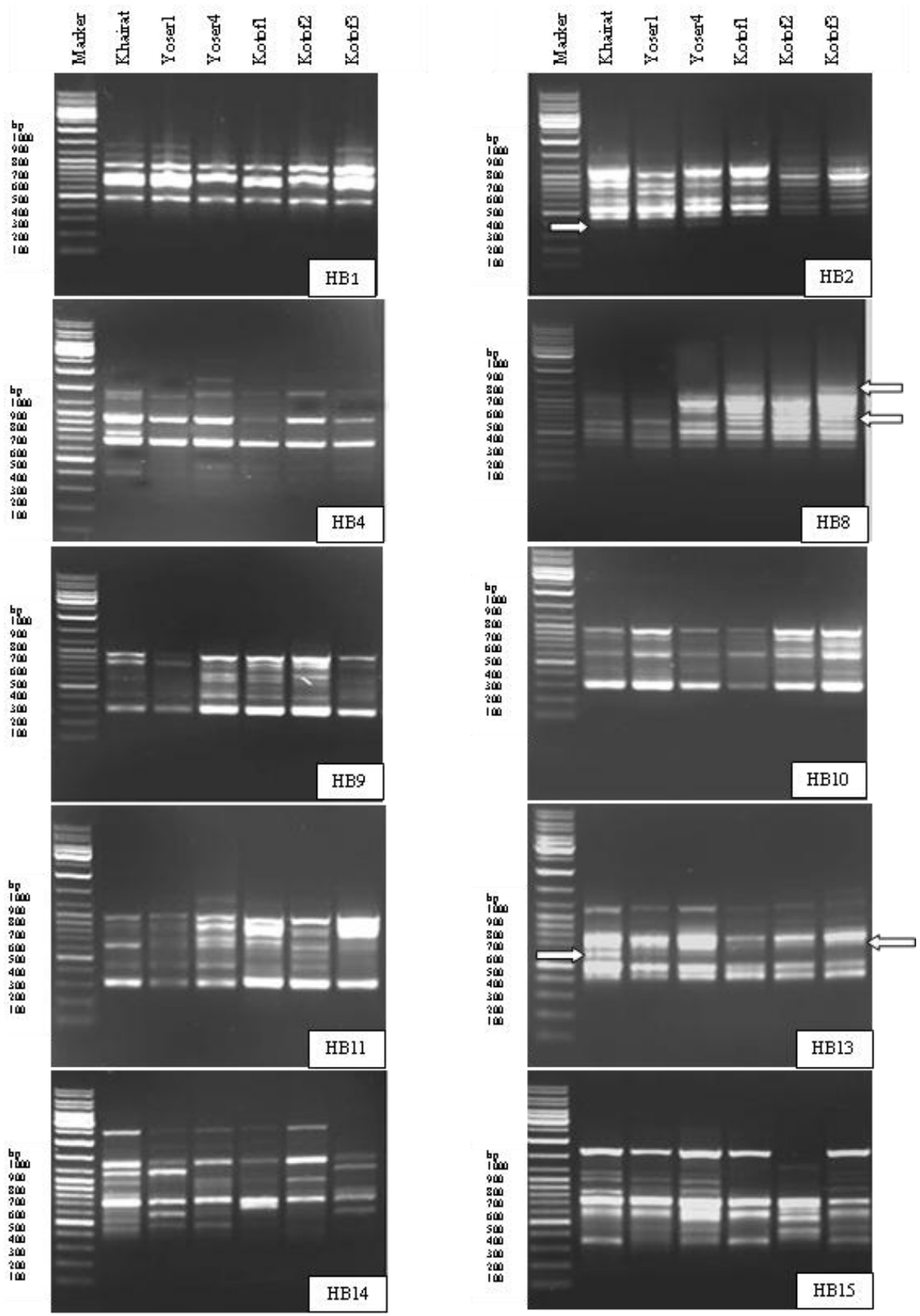

Figure 1. ISSR profiles of the six *Capsicum* hybrids generated by ten primers. Arrows indicate five species-specific bands discriminating between the two *Capsicum* species.

Table 2. Unique bands of different *Capsicum* hybrids.

Species	Hybrid	Number of specific band	Primer	Band size (bp)
C. annuum	Kotof 1	1	HB 9	456.1
		1	HB 14	652.1
	Kotof 2	1	HB 10	635.9
		1	HB 11	487.4
		2	HB 15	558.7, 513.0
C. frutescens	Kotof 3	2	HB 2	1573.9, 1474.1
		1	HB 13	1581.9
	Khairat	1	HB 4	746.1
		2	HB 14	484.3, 415.4
	Yoser 4	1	HB 4	1889.9
		1	HB 11	1379.9
		1	HB14	963.6
		1	HB 15	1461.2

Table 3. Matrix of the genetic similarity of six *Capsicum* hybrids based on ISSR data analysis.

Hybrid	Khairat	Yoser 1	Yoser 4	Kotof 1	Kotof 2	Kotof 3
Khairat	1.00					
Yoser 1	0.883	1.00				
Yoser 4	0.836	0.817	1.00			
Kotof 1	0.779	0.736	0.803	1.00		
kotof 2	0.752	0.709	0.777	0.821	1.00	
Kotof 3	0.800	0.796	0.758	0.817	0.790	1.00

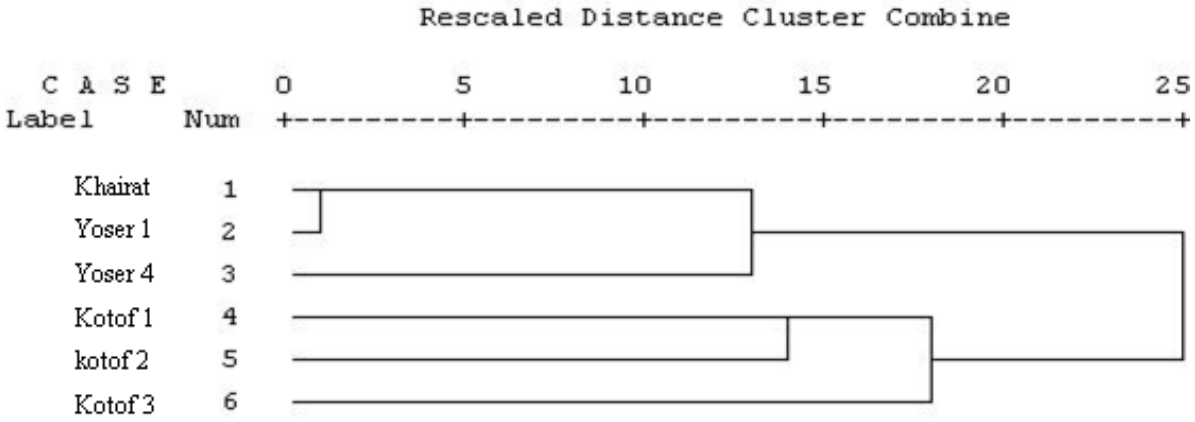

Figure 2. UPGMA phenogram showing genetic diversity of the six *Capsicum* hybrids based on ISSR bands.

frutescens and the other grouped those of *C. annuum.*

DISCUSSION

Right from the first attempt (Cao, 1994), molecular markers have contributed substantially for the improvement of *Capsicum*. In the present study, ten ISSR primers succeeded to produce 52 polymorphic bands out of 87 bands with polymorphism average of 60%, and with considerable intra and inter specific variations into *Capsicum* hybrids. The results obtained are in

consistency with those obtained by Gyulai et al. (1999), Kumar et al. (2001), Kochieva et al. (2004), Mongkolporn and Dokmaihom (2004) who successfully used ISSR along with AFLP and RAPD for determining genetic variation, phylogenetic relationships among different species and genetic purity tests in hybrids within the genus *Capsicum*.

Results of ISSR patterns (Figure 1) scored five distinguishable species-specific bands and 16 unique bands for hybrids individually, which could be used as molecular markers for Egyptian hybrids. These findings were in accordance with those of Wang and Fan (1998) and Patel et al. (2011) regarding the high degree of polymorphism among *Capsicum* germplasm through ISSR markers. Moreover, Lijun and Xuexiao (2012) labeled five cultivated pepper species with 1 to 3 specific bands for each species and used them as molecular markers. Also, Ilbi (2003) identified four RAPD markers that were found to be cultiver-specific markers for three hybrid pepper varieties, which further support the present consideration.

The similarity coefficient values and the UPGMA dendrogram revealed narrow genetic base among the tested hybrids in both species. This is likely due to the fact that their parental breeding lines used to develop these hybrids were the same or were very close to each other, bearing in mind that pepper is a self-pollinated crop. This finding confirm the results of Kumar et al. (2001); Ilbi (2003); Yang et al. (2005); Akbar et al. (2010) and Lijun and Xuexiao (2012) who detected that the genetic variations among five *Capsicum* species were mainly inter-specifically rather than intra-specifically. In conclusion, the results show that ISSR markers detected a considerable genetic diversity in the studied pepper hybrids, which could be used in conservation of genetic resources of new Egyptian hybrids and crop breeding through marker assisted selection (MAS).

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