Molecular characterization of tomato cultivarsgrown in Saudi Arabia and differing in earliness of fruit development as revealed by AFLP and ISSR

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Abstract: Early fruit development is an important trait defining fresh tomato marketability in kingdom of Saudi Arabia (KSA), which is related to more profit for farmers. Eight commercial cultivars of tomato (*Solanumlycopersicum* L.) grown in Saudi Arabiaanddiffering in fruit developmentdates were characterized on the molecular genetic basis by inter simple sequence repeats (ISSR) and amplified fragment length polymorphism (AFLP) markers. The results indicated a wide range of molecular variation, in which some markers distinguished among different genotypes. In general, both sets of data allowed for the identification of cultivars by means of pairwise differences, cluster analysis and principal component analysis. AFLP and combined data generated resolved trees with bootstrap support. AFLP and ISSR approaches enabled discrimination among the eight tomato cultivars, which represents a valuable data for improvement of this economic crop in the future.
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I. INTRODUCTION

Tomato (*Solanumlycopersicum* L.) is one of the world's major fresh and processed fruits. The plant is native to South America (e.g., Peru and Ecuador) and was first domesticated in Mexico, while introduced to cultivation in the Middle East by the end of the 18th century.China is the most important source of global production, then United States and Turkey. Saudi Arabia produces about 525 thousand tons of tomatoes per year (FAOSTAT 2011, http://faostat3.fao.org/faostat-gateway/go/to/download/Q/QC/E), while the global output in the range of 160 million tons annually reproduced annually on 4.7 million hectares. The average consumption of fresh and processed tomatoes in the Kingdom of Saudi Arabia is 31 kilos per capita/year (Statistics 2007,http://www.saudi-arabia.cropscience.bayer.com/en/Crops/Tomato.aspx).

DNA fingerprinting is a convenient tool forassessing genetic diversity (Park, West, & St. Clair, 2004) (Semagn, Bjørnstad, & Ndjiondjop, 2006) (Mondini, Noorani, & Pagnotta, 2009). The characterizationof various plant genetic resources withmolecular markers offers a unique opportunity todefine significant marker-trait associations of biologicaland agronomic interest (Parmar et al., 2010). Cultivated tomato is a plant species in which biochemical and molecularmarkers such as isozymes and RFLPs yielded limitedamount of information due to the lack of variability, as a consequence of self-pollination incombination with the narrow genetic base of themodern cultivars (Miller & Tanksley, 1990) (Breto, Asins, & Carbonell, 1993)(Alvarez, Van de Wiel, Smulders, & Vosman, 2001). Nevertheless, different types of molecular markers such asSSRs, CAPS, and ESTs have beendeveloped and mapped onto the 12 tomato chromosomes(Broun & Tanksley, 1996) (Frary, Fulton, Zamir, & Tanksley, 2004).DNA fingerprinting techniques, e.g., random amplified polymorphic DNA (RAPD) and amplified fragment length polymorphism (AFLP), are successfully conducted to develop DNA markers in tomato in

which no sequence information is required, and the occurrence of high polymorphism ratio due to multi-locus detection by single marker analysis (Saliba-Colombani, Causse, Gervais, & Philouze, 2000). ISSR (inter-simple sequence repeat) markers arealso used successfully and extensively in the genus Lycopersicon (Tikunov, Khrustaleva, & Karlov, 2003) to study genetic diversity among tomato cultivars and related species and landraces (Smolik, Zielinski, Rzepka-Plevnes, & Adamska, 2006)(Terzopoulos & Bebeli, 2008). It is also used in the assessment of genetic purity (Liu et al., 2007)and in the evaluation of genetic recombination (Toppino et al., 2008).

The present study wasconducted in order to examine the genetic diversityof the eight tomato genotypes broadly cultivatedin KSAusing ISSR and AFLP markers and to detect markers possibly linked to earliness of fruit development.

II. MATERIALS AND METHODS

Plant material

Eight tomato cultivars cultivated in Saudi Arabia were examined (Table 1). These cultivars were originally grown in four different locations in Saudi Arabia and differ in their earliness of fruit development.

Table 1.Code number, name and mean character of the 8 date tomato cultivars.

no.	Name	Earliness	Abbreviation	Fruit shape	Weight
1	Castle rock	Early	CR	Round	100-150 gm
2	Chico III	Normal	Ch	Rectangular	110-130 gm
3	Moneymaker	Relatively early	MM	Round	110-140 gm
4	Pearson	Normal	PN	Flat	250-300 gm
5	Better Boy (VFN-8)	Normal	VFN	Round	150-200 gm
6	Super Marmande	Relatively early	SM	Flat	200-250 gm
7	Super Strain B	Relatively early	SSB	Round	120-140 gm
8	Baraka	Normal	BK	Rectangular	140–160 gm

Genomic DNA extraction and purification

Extraction of total DNA was performed separately from fiveleaves of individual plants using Gene JET Plant Genomic DNA Purification Mini kit (Thermo scientific Co.). To remove RNA contamination, RNase A (10 mg/ml, Sigma, USA) was added to the DNA solution and incubated at 37^{o}C for 30 min. The DNA concentration in different samples was estimated by measuring optical density at 260 nm and 280 nm using NanoDrop 2000 (Thermo Scientific Co., USA).

Inter simple sequence repeat (ISSR)

Eleven primers were utilized for ISSR analyses (Table 2). PCR was performed, according to Dangi (Dangi, Lagu, Choudhary, Ranjekar, & Gupta, 2004), in a total of 25 µl reaction volume and amplification (Veriti® Thermal Cycler, Life Technologies, Applied Biosystem, NY, USA) was programmed to 40 cycles after an initial denaturation cycle for 4 min at 94^{o}C. Each cycle consisted of a denaturation step at 94^{o}C for 1 min, an annealing step at 40^{o}C for 1 min, and an extension step at 72^{o}C for 2 min, followed by extension cycle for 7 min at 72^{o}C in the final cycle. Reactions were done in three replicates per sample (e.g., bulk of five DNA extracts) in order to saturate polymorphism within cultivars and ensure reproducibility of the data.

Table 2. List of ISSRprimers and their nucleotide sequences successfully used in the present study.

No.	Name	Sequence	No.	Name	Sequence
1	814	$(CT)_8TG$	8	HB10	$(GA)_6CC$
2	844A	$(CT)_8AC$	9	HB11	$(GT)_6CC$
3	844B	$(CT)_8GC$	10	HB12	$(CAC)_3GC$
4	17898A	$(CA)_6AC$	11	HB14	$(CTC)_3GC$
5	17898B	$(CA)_6GT$			
6	HB8	$(GA)_6GG$			
7	HB9	$(GT)_6GG$			

Amplified fragment length polymorphism (AFLP)

AFLP analysis was performed using the florescent AFLP Plant Mapping kit (Life Technologies, Applied Biosystem, NY,USA) according to the manufacturer's protocol. Genomic DNA samples were digested with the restriction enzymes *Eco*RI and *Mse*I, followed by ligation of adapters to the digested DNA fragments. Pre-amplification was carried out using ligation and pre-selective amplification module (P/N 402004) with *Eco*RI primer plus one extension base at the 3' position (A) and *Mse*I primer plus one extension base at the 3' position (C). Eight out of 12 combinations of *Eco*RI primers (plus three extension bases) and *Mse*I

primers (plus three extension bases) were successfully used to selectively amplify the DNA fragments matching the primer-extension sequences using selective amplification start-up module (P/N 4303050). The eight combinations were: E-ACA/M-CAG, E-ACT/M-CAT, E-ACC/M-CTA, E-ACC/M-CTG, E-AAC/M-CTC, E-AAG/M-CTT, E-AGG/M-CAC and E-ACG/M-CAA.Reactions were done in three replicates per sample and non-repeatable data were removed.

Detection of PCR products

ISSR products were visualized using agarose gel electrophoresis (1.2% in 1x TBE buffer) followed by staining with ethidium bromide (0.3 ug/ml). Amplicons were visually examined with a UV transilluminator and photographed using a CCD camera (UVP, UK). Data scored as (1) for the presence and (0) for the absence of a given fragment and sizes were estimated by comparison with a 100-bp ladder (Bioron, Germany) using TotalLab Quant (TotalLab Ltd, UK). Fragments recovered by ISSR were considered reproducible and scorable based on the dataset generated from the three separate amplifications for each primer. Binary data matrices were entered into TFPGA (version 1.3) and analyzed using qualitative routine to generate a similarity coefficient. AFLPs were separated by capillary electrophoresis and amplicon sizes were estimated on ABI 3500 DNA genetic analyzer (Life Technologies, Applied Biosystems, NY, USA). Fragments were sized using the GeneScan™ 600 LIZ® Size Standard v2.0 [ROX] size standard. Electrophoregram and tabular data were generated using GeneScan Analysis software version 2.0. A genetic fingerprint was produced using Genemapper 4.1 (Applied Biosystems, NY, USA)for each sample by scoring the presence (1) or absence (0) of a standardized set of markers between 50 and 600 base pairs in size(Rogers, 2008).

Data analysis

Dissimilarity coefficients were used to construct dendrograms using unweighted pair group method with arithmetic average (UPGMA) and sequential hierarchical and nested clustering (Neighbor Joining or NJ) routine using NTSYSpc (version 2.10, Exeter software). Principle component analysis (PCA) data was generated using JMP11 software (SAS Institute Inc., CN, USA). Similarity matrices from ISSR and AFLP dataset were compared based on the TFPGA, the normalized Mantel statistic (Mantel, 1967), and the PIC (polymorphism information content) was calculated using the following standard formula (Powell et al., 1996) (Smith et al., 1997).

The PIC value provided an estimate of the discriminating power of a marker. Marker index (MI, the product of PIC and the number of polymorphic bands) was calculated for each marker type. In addition, average heterozygosity (H_e) and the effective multiplex ratio (E) were also calculated(Powell et al., 1996).

III. RESULTS AND DISCUSSION

ISSR and AFLP molecular tools were utilized to characterizeeightcommercially available tomatocultivars grown at different locations in Saudi Arabia (Table 1). Only, clear, unambiguous and reproducible amplicons recovered through both techniques were considered for scoring. Each amplicon was considered a single locus. The minimum number of samples to be bulked in order to saturate polymorphism within each cultivar was determined by PCR with the ISSR primer 814 as five or more samples (data shown upon request). Optimal number of primers for ISSR or primer combinations for AFLP required in discriminating among genomic DNAs of different plant genotypes was estimated based on the reproducibility of data and the generated level of polymorphism (75±10%). The required value of genetic distance to classify correlated plants accessions as distinct cultivars waspreviously discussed (Cabrita, Aksoy, Hepaksoy, & Leitão, 2001) (Papadopoulou et al., 2002). In the present study, 11, out of 15, primers for ISSR and eight, out of 12,combinations for AFLP with informative patterns were selected. Selection of ISSR primers and AFLP combinations was based on the number of the recovered amplicons and the reproducibility of the results. Less than 7% intra-plant polymorphism was detected across the two types of analyses for the plants of the same cultivar (data provided upon request). As being dominant markers, pooling (bulked DNA) strategy for ISSR and AFLP analyses is thought to be ideal for saturating such an intra-plant polymorphism with no effects on the accuracy of the obtained results. Mengoni(Mengoni, Gori, & Bazzicalupo, 2000) indicated that 10% of intra-plant polymorphism is statistically insignificant and results can still be trustable.

Amplified products and polymorphism amongdifferent Tomatocultivars

ISSR, usually 16–25 bp long, uses microsatellites as primers in a single-primer PCR reaction targeting multiple genomic loci to amplify mainly the ISSR sequences of different sizes (Zietkiewicz, Rafalski, & Labuda, 1994)(Reddy, Sarla, & Siddiq, 2002).ISSR molecular analysis is based on inter tandem repeats of short DNA sequences proven to be highly polymorphic even among closely-related genotypes, due to the lack of functional genetic constraints in these non-coding DNA regions. The analysis generated a total of 63 ampliconsacross ISSR primers with an average number of sixamplicons per primer (Table 3). The size of the ISSR amplified fragments ranged from 40bp (for primer 17898B) to 4693bp (for primer HB11). The highest number of

amplicons (9) was exhibited by primer HB9, whereas the lowest number (3) was revealed by primers 17898A (Table 3). Meanwhile, the number of polymorphic ISSR amplicons was as high as 39 representing a level of polymorphism of 62% and an average number of polymorphic bands of 3.5 per primer (Table 3). The level of polymorphism for different ISSR primers ranged from 25% (for primer HB12) to 80% (for primers 17898B and HB11). In this context, Aguilera (Aguilera et al., 2011)reported a low level of polymorphism (34%) in ISSR analysis of Braziliantomato cultivars, while Ahmed (Mansour, Teixeira da Silva, Edris, & Younis, 2010)reported high polymorphism in ISSR analysis (100%).

AFLP analysis is based on the PCR amplification of selected restriction fragments of a total genomic DNA digest to combine the reliability of RFLPs with the advantages of PCR methods. Therefore, AFLP permits the development of more accurate comprehensive fingerprints (Vos et al., 1995). In the present study, AFLPanalysis of theselected eightprimer combinations generated a total of 1302 amplicons with a mean number of 162.7 per combination (Table 3). The size of the AFLP amplified fragments ranged from50(for primer combination E-ACC/M-CTA) to 565bp (for primer combination E-ACA/M-CAG). The number of polymorphic amplicons was 998 representing an average of 77% polymorphism(Table 3). The level of polymorphism ranged from 57% (for primer combination E-AAG/M-CTT) to 91% (for primer combination E-AAC/M-CTC). The highest number of amplicons (213) was exhibited by primer combination E-AAC/M-CTC, whereas the lowest number (133) was revealed by primer combinations E-ACC/M-CTG(Table 3). Ning (Ning, Jing-bin, Jing-fu, & Xiang-yang, 2012)used64 AFLP primer combinations to analyze tomato cultivars and generated 1328 bands and revealed 19%-43% polymorphism. These low rates in the percentage of polymorphisms could be attributed to the distances among different tomato cultivars or the use of different primer combinations.

Cultivar-specific molecular markersfor different tomato cultivars

The total number of cultivar-specific markers scored across cultivars and type of marker was as high as 415in which 401of them were generated from AFLP analysis, while only 14 for ISSR (Tables 4 & 5). The highest number of cultivar-specific markers generated from ISSR analysis (3) was scored for primer 814, 844A and HB10, while no cultivar-specific markers were generated for primer 844B, 17898A, HB8 and HB12. The highest number of cultivar-specific markers generated from ISSR analysis for a given cultivar was 4(Baraka cultivar), while the lowest (0) was scored for cultivarBetter Boy (VFN)(Table 4). There are two cultivar-specific markers generated for cultivar Castle rock in which one of them was generated by primer BH11 and the other by primer 17898A, while three cultivar-specific markers generated for cultivar Super Marmand by primers 814, HB10 and HB14.These fiveISSR markers can be further investigated for possible linkage with gene(s) for the earliness of tomato fruit development.

Table 3. Total number of amplicons, monomorphic amplicons, polymorphic amplicons and percentage of polymorphism as revealed by the two types of markers among the eight tomato cultivars.

Marker type	Primer	No. amplicons	No. monomorphic amplicons	No polymorphic amplicons	% polymorphism
ISSR	814	8	2	6	75
	844A	6	3	3	50
	844B	4	2	2	50
	17898A	3	1	2	67
	17898B	5	1	6	80
	HB8	5	2	3	60
	HB9	9	3	6	67
	HB10	6	2	4	67
	HB11	5	1	4	80
	HB12	4	3	1	25
	HB14	8	4	4	50, 100
	Total	**63**	**24**	**39**	**62**
AFLP	E-ACA/M-CAG	174	47	127	73
	E-ACT/M-CAT	142	44	98	69
	E-ACC/M-CTA	158	22	136	86
	E-ACC/M-CTG	133	27	106	80
	E-AAC/M-CTC	213	19	194	91
	E-AAG/M-CTT	157	67	90	57
	E-AGG/M-CAC	141	26	115	82
	E-ACG/M-CAA	184	52	132	72
	Total	**1302**	**304**	**998**	**77**

Table 4. List of positive and negative cultivar-specific markers of the eight tomato cultivars detected through ISSR marker type. The table indicates the type and number of markers along with their molecular weights (MW) in bp.

Marker type	Primer	Number (and MW in bp) of cultivar-specific markers								
		CR*	Ch	MM	PN	VFN	SM	SSB	BK	**Total**
ISSR	814	1 (584)	-2 (947, 321)		**3**	844A	---1 (1866)	--2 (3156,	2310)	**3**
	844B	-	-	-	-	-	-	-	-	**0**
	17898A	-	-	-	-	-	-	-	-	**0**
	17898B	1 (575)	-	-	-	-	-	1 (40)	-	**2**
	HB8	-	-	-	-	-	-	-	-	**0**
	HB9	-	1 (1976)	-	-	-	-	-	-	1
	HB10	-	-	2 (1557, 84)	-	-	1 (760)	-	-	**3**
	HB11	1 (2322)	-	-	-	-	-	-	-	**1**
	HB12	-	-	-	-	-	-	-	-	**0**
	HB14	-	-	-	-	-	1 (669)	-	-	**1**
	Total	**2**	**1**	**2**	**1**	**0**	**3**	**1**	**4**	**14**

*See Table 1.

Table 5. List of positive and negative cultivar-specific markers of the eight tomato cultivars detected through AFLP marker type. The table indicates the type and number of markers along with their molecular weights (MW) in bp.

Marker type	Primer combination	Number (and MW in bp) of cultivar-specific markers								**Total**
		CR*	Ch	MM	PN	VFN	SM	SSB	BK	
AFLP	E-ACA/M-CAG	10 (98,92, 137,160, 312,326 (212, 267, 230,235) 554)	2 (94,281) 135) 199,275, 338,352	3 (55, 56, 78,80, 346, 427)	1 (106) 147, 175 375,388	6 (57,58, 102,565)	6 (41,139, 286,296 399, 444	4 (42,83, 175,196, 481,486	17 94,565) 515,536	
	49	E-ACT/M-CAT	5 (100,168	7 (46,47,	2 (58, 167)	1 (421)	0	7 (49,52,	9 (97,	
160 131,190 227,231 234,237 307, 425	10 (70,214	296, 335 336)	68,73, 104, 146 147)				110,156 174, 183 220)	189,246 274, 276, 298, 305, 318		
		41								
(43,72, 223,229)	E-ACC/M-CTA	23 (56,58, 61,93, 94,105, 117,119, 129, 134, 156, 178, 180,190, 193,194 197,201, 362, 421,	4 (84,101, 137,150)	4 (46,103, 101,123)	10 (23,24, 26,29, 30,33, 34,36, 40,45) 422)	7 (56,79, 132,133 207,210, 319)	6 (48,53, 93,99, 140,242)	3 (61,161, 295)	4	
							61			
(69,135, 179,221 305,322 336,349 350,357 398,410 419)	E-ACC/M-CTG	4 (71,111, 204, 248)	10 (48,53, 59,113, 116,146, 152, 154, 182, 211)	2 (87, 134)	2 (192,236)	6 (50,108 109,137 138,312)	5 (60,61, 178,263, 287)	4(161,171 227,381)	13	
		46								
(180,207, 249,258, 265,316, 320,331, 351,368, 377,393,	E-AAC/M-CTC	25 (90,107, 118,123, 126,127, 133,138 146,149 161,166	-	1 (41)	1 (185)	6 (41,45, 46,48 54,125,	11 (46,49, 58,59, 91,92 108,159, 165,170, 195,333)	8 (36,37, 54,56, 57,141, 259,266)	16	

Table 5. Continued

Marker type	Primer combination	Number (and MW in bp) of cultivar-specific markers								**Total**
		CR*	Ch	MM	PN	VFN	SM	SSB	BK	
173, 189 198,199 463,468) 212,236 237,247 277,294 319,386 449)							394,443,		**69**	
	E-AAG/M-CTT	6 (92,101, 129,130 259,260)	7 (43,45 82, 84, 86, 142, 148)	4 (114,206, 255,266)	-	1 (271)	4 (51,171, 175,229)	3 (242,243, 288)	14	
(52, 102, 118,137, 140, 186, 188,192, 221, 225, 236,258, 296, 433)		**39**								
	E-AGG/M-CAC	7 (58,95, 140,142, 154,200, 211)	8 (43,59, 80, 94, 151,168, 195,216)	1 (133)	2 (102,229)	12 (45,46, 48,53, 54,65, 66,87, 133,137,	1 (155)	5 (77,90, 196,199, 238) 163,183)	8 **44**	
(108,114, 115,190, 207,247, 317,350)										
	E-ACG/M-CAA	13 (60,77, 83,101, 102,115 128, 136, 170 216, 247,280, 283)	8 (40,91, 228,246, 329,343, 351,384)	3 (101,126, 234)	2 (162,272)	3 (112,161, 200)	5 (99,74, 155,194 225)	3 (58,121, 388)	15	
(82,224, 227,235, 242,253, 259,297, 311,328, 336,355, 362,416 466)		**52**								
Total	**93**	**46**	**20**	**19**	**41**	**46**	**39**	**97401**		

The highest number of cultivar-specific markers generated from AFLP analysis for a given primer combination was 69 (primer combination E-AAC/M-CTC), while the lowest number of cultivar-specific markers (39) was generated for primer combination E-AAG/M-CTT. The highest number of cultivar-specific markers generated from AFLP analysis for a given cultivar was 97 (Baraka), while the lowest (19) was scored for cultivarPearson(Table 5). The results of Park (Park et al., 2004), when screening 26 primer combinations using 74 tomato cultivars, indicated that from the 1092 bands scored, 102 AFLP bands (9.3%) were cultivar-specific markers.

In conclusion, we recommend the use of AFLP, especiallyprimer combinations E-AAC/M-CAC and E-ACC/M-CTG, in estimating distances among tomato cultivars due to the generation of high number of amplicons, high percentage of polymorphism and high number of cultivar-specific markers.

Genetic relationships and cluster analysis

The genetic similarities among the eight tomato cultivars based on Nei'smethod (Nei, 1978), within and across both types of markers are shown in Table 6 and Figure 1. The highest pairwise similarity indices resulted from ISSR, AFLP and across type of markers were between VFNand Super Marmande (89%), between Moneymakerand Super strain B (78%), and between Chicoo III and both Persona orVFN (81%). While, the lowest similarity indices were between Monymaker and Baraka (69%), between Castle rock and VFN (62%),and between Castle rock and Baraka (58%). The results of the dendrograms generated based on ISSR, AFLP and across types of markers were in harmony with those of the similarity indices. In addition, the results of similarity indices and dendrograms generated from AFLP or acrosstypes of markers indicated accumulative information towards the partial separation of cultivars based on earliness in fruit development. Based on the wealth of information generated from AFLP as compared to ISSR, it can be concluded that the resulted dendrogram of AFLP data was closer to that resulted across types of markers (Table 6 & Figure 1). In addition, the results of principal component analysis (PCA) plots (Figure 2) were in harmony with those of the generated dendrograms and similarity indices. It is worth mentioning that the dendrogram and PCA plots based on AFLP or combined analyses have discriminated cultivars based on the characteristic of earliness of fruit development in which Cassle rock cultivar (with early fruit development) was separated in a cluster, while Moneymaker, Super Marmande and Super Strain B cultivars (with relatively early fruit development) were separated in a second cluster. The other cultivars that are not early in fruit development were separated in a third cluster. However, ISSR analysis failed to distinguish among cultivars based on earliness in fruit development (Figure 2).No specific markers were detected for tomato fruit shape or weight across both types of markers.

The polymorphism information content (PIC), average of heterozygosity (H_e), the effective multiplex ratio (E), and the marker index (MI) were computed for each assay based on experimental data (Table 7). The data for ISSR and AFLPmarker types for PIC (0.36 and 0.37, respectively) resulted insimilar values indicating the preference of AFLP in molecular characterization. Less than 30 out of the 63 ISSR markers (~43%) exhibited PIC values ranging from 0.75 to 1.0 and over 400 of the 1302 AFLP markers (32%) exhibited PIC values ranging from 0 to 0.25 or 0.75 to 1.0 (Figure 3a,b).Expectedly, AFLPalso revealed higherHe, E and MI values (0.50, 1003 and 496.49, respectively, Table 7) as compared to those for ISSR (0.46, 39 and 18.1, respectively, Table 7) indicating that AFLP is more effective in detecting polymorphism among tomato cultivars. The obtained results in the present investigation agreed with these of Powell (Powell et al., 1996) across both types of markers. The results of ISSR data analysis indicated the suspicion in utilizing this type of marker in detecting genetic relatedness among tomato cultivars. It is possible to improve reliability on ISSR data if more primers were used in characterizing cultivars at the molecular levels. More recently, AFLP markers were identified for important characteristics (Kepiro & Roose, 2010)(De Vos et al., 2013) (Zhang et al., 2013) in plant and animal, that can be utilized in marker-assisted selection (MAS) programs.

It is worth mentioning that both types of markers differ in their ability to differentiate individuals, the mechanism of detecting polymorphism and genome coverage. They can be complementary to each other, although this was not the case in the present study, depending on technical availability. In conclusion, this study has provided sufficient molecular tools that can be used to identify tomato cultivars and marker-assisted selection in breeding program for early fruit development in tomato.

Table 6. Similarity matrixes based on molecular data for the eight tomato cultivars. Orange box indicates the highest values, while green box indicates the lowest.

ISSR	CR*	Ch	MM	PN	VFN	SM	SSB	BK
CR *	1.00							
Ch	0.88	1.00						
MM	0.79	0.86	1.00					
PN	0.86	0.85	0.89	1.00				
VFN	0.77	0.81	0.83	0.80	1.00			
SM	0.78	0.83	0.79	0.78	0.89	1.00		
SSB	0.76	0.78	0.70	0.74	0.80	0.81	1.00	
BK	0.75	0.80	0.69	0.71	0.77	0.76	0.86	1.00

AFLP	CR*	Ch	MM	PN	VFN	SM	SSB	BK
CR *	1							
Ch	0.66	1.00						
MM	0.70	0.75	1.00					
PN	0.69	0.73	0.73	1.00				
VFN	0.62	0.75	0.70	0.75	1.00			
SM	0.73	0.69	0.74	0.76	0.71	1.00		
SSB	0.66	0.75	0.78	0.76	0.73	0.76	1.00	
BK	0.63	0.76	0.71	0.75	0.73	0.70	0.75	1.00

Overall	CR*	Ch	MM	PN	VFN	SM	SSB	BK
CR *	1							
Ch	0.63	1.00						
MM	0.72	0.74	1.00					
PN	0.67	0.81	0.79	1.00				
VFN	0.61	0.81	0.74	0.80	1.00			
SM	0.72	0.68	0.76	0.71	0.69	1.00		
SSB	0.63	0.75	0.78	0.77	0.77	0.76	1.00	
BK	0.58	0.77	0.68	0.77	0.77	0.65	0.72	1.00

*See Table 1.

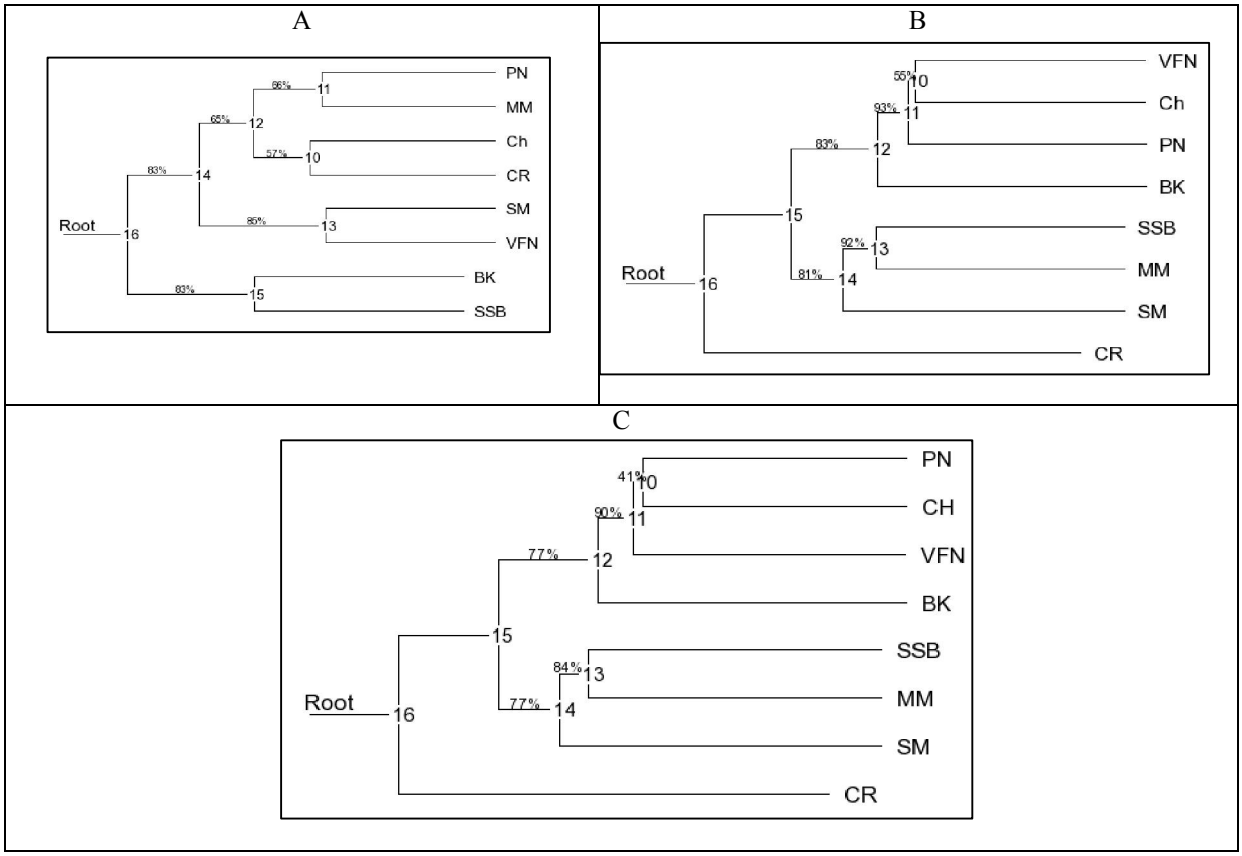


Figure 1. Dendrograms based on algorithm of unweighted pair group method witharithmetic averages among tomato cultivars (see Table 1) within ISSR (a) and AFLP (b)or across types of marker (c).

Table 7. Polymorphism information content (PIC), expected heterozygosity for polymorphic products (He), effective multiplex ratio (E) and the marker index (MI) of each marker type used across differenttomato cultivars.

Marker type	PIC	He	E	MI
ISSR	0.36	0.46	39	18.1
AFLP	0.37	0.50	1003	496.49

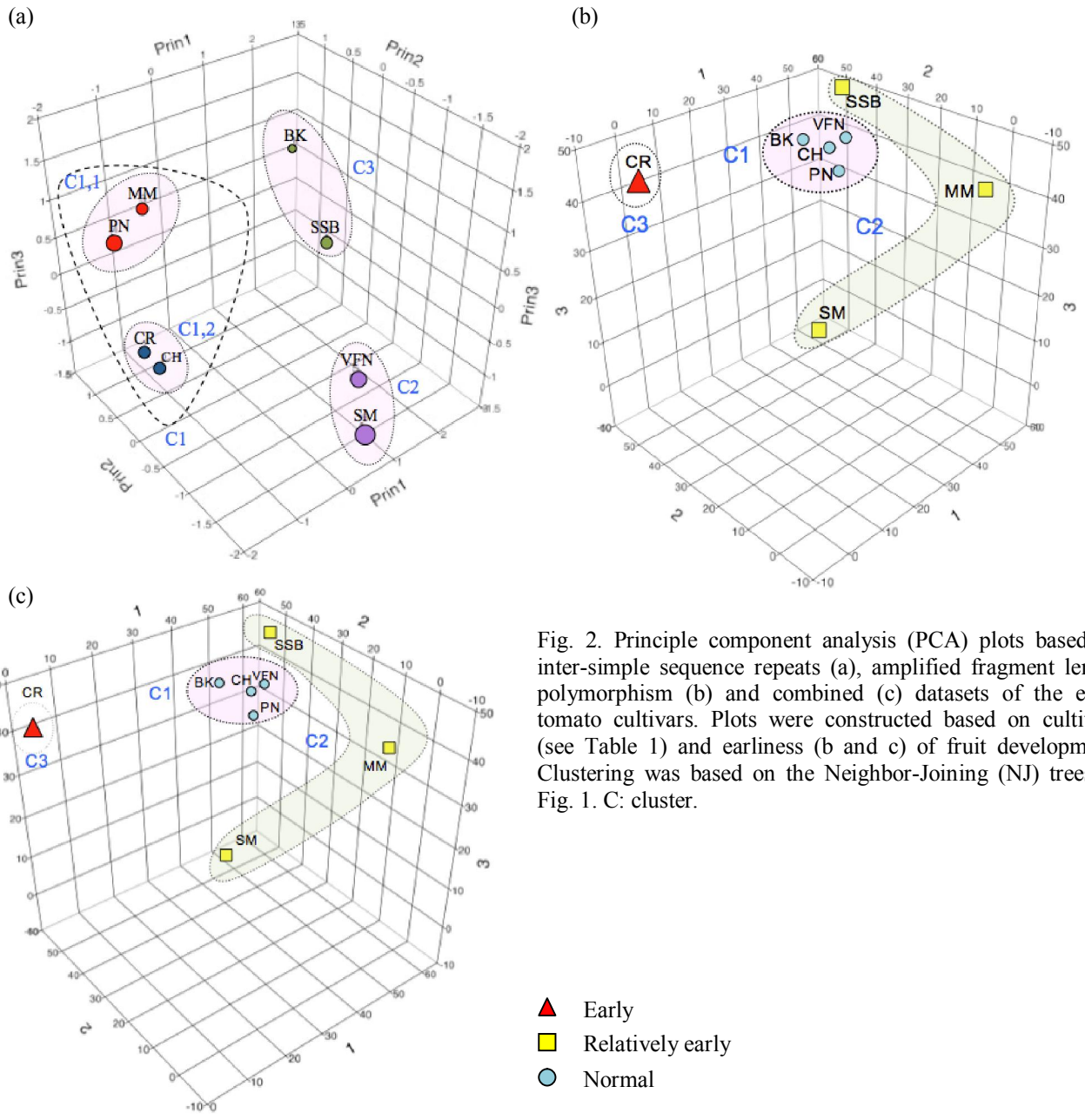
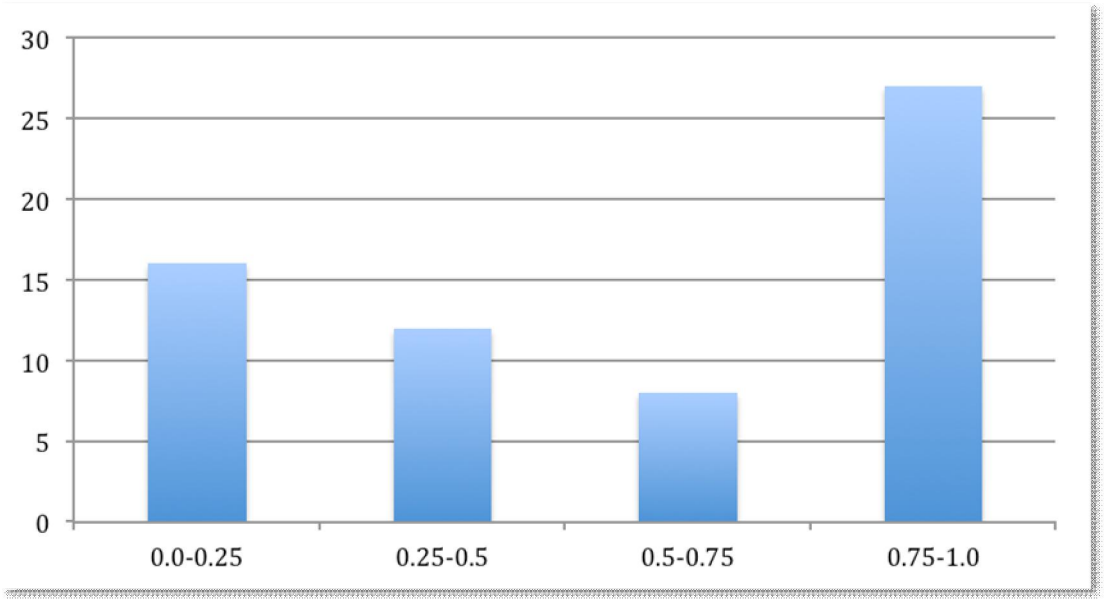


Fig. 2. Principle component analysis (PCA) plots based on inter-simple sequence repeats (a), amplified fragment length polymorphism (b) and combined (c) datasets of the eight tomato cultivars. Plots were constructed based on cultivars (see Table 1) and earliness (b and c) of fruit development. Clustering was based on the Neighbor-Joining (NJ) trees of Fig. 1. C: cluster.

(a)



(b)

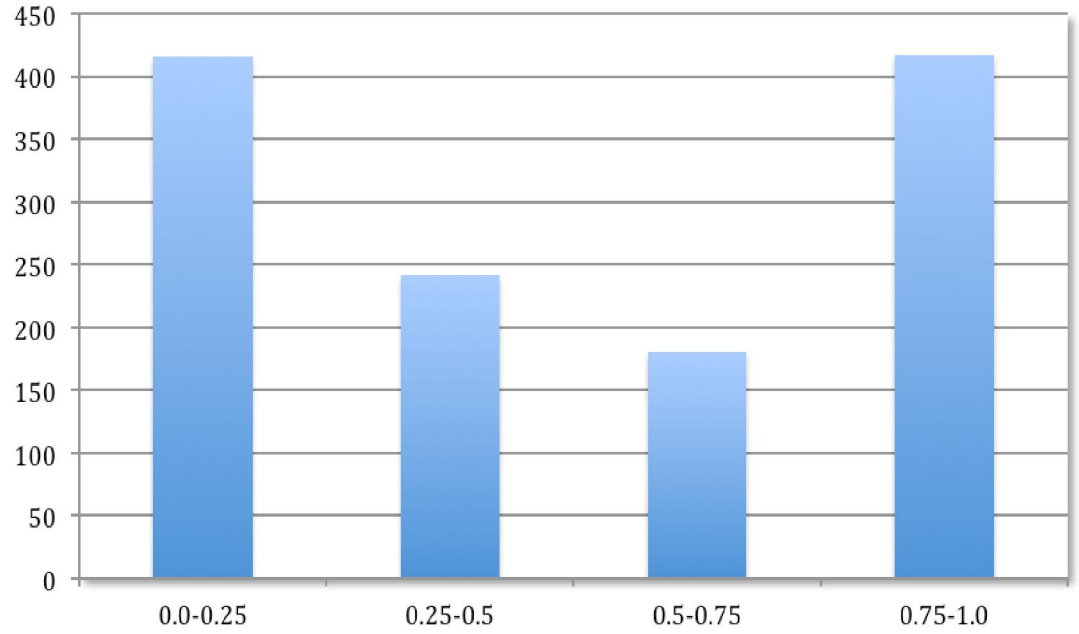


Fig. 3. Distribution of the polymorphism information content (PIC) obtained from inter-simple sequence repeat (a) and amplified fragment length polymorphism (b) datasets.

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