DETECTION OF ISSR MARKERS LINKED TO SEED OIL CONTENT OF JOJOBA PLANTS (*Simmondsia chinensis*) CULTIVATED IN EGYPT

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The production of agricultural goods from the desert or semi-arid lands is minimal due to prevailing stress environmental conditions, thus most of the land is unused. Recent advances in modern agricultural farming and biotechnology have facilitated the way for expanding the scope of utilizing those arid lands for human endeavors. In such sense, Jojoba (*Simmondsia chinensis*) is a promising oilseed crop for the economic development of the arid and semiarid land (Bala *et al*., 2015). Jojoba plant is a mono genetic dioecious shrub belonging to *Simmondsiaceae* family. It is native to the southwestern of the United States and northern Mexico. Jojoba oil became widely known in the 18th and 19th centuries. The plant has been cultivated in many countries worldwide, such as Mexico, Argentina, Chile, India, Australia, Tunisia, Palestine, Saudi Arabia and Egypt, due to its promising economic value (Abdel-Mageed *et al*., 2014). Jojoba oil is an unsaturated liquid wax that can be extracted in significant quantities from seeds and it makes up 52 percent of the total weight of the seed. Jojoba seed oil is highly valued for their use in a wide range of pharmaceutical industry, biodegradable lubricants and as a biofuel product (Aburjai and Natsheh, 2003). The oil consists of virtually 98% pure waxes (mainly wax esters, few free fatty acids, alcohols, and hydrocarbon), sterols, and vitamins with few triglyceride esters, so it's widely considered as liquid wax instead of oil or fat (Kramer *et al*., 1983). In the seed-planted fields of the early Jojoba pioneers, high genetic variation in Jojoba plants was a primary cause of failure. However, this variation will also be a key step for developing high yields in the future (Purcell *et al*., 2000). DNA-based genetic markers, such as re-

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striction fragment length polymorphisms (RFLP) and random amplified polymorphic DNA (RAPD) have become more reliable, efficient tools (Nybon, 1994). Also, inter-simple sequence repeat (ISSR) is a type of DNA marker that involves the use of microsatellite sequences directly in the polymerase chain reaction (PCR) for DNA amplification (Wang, 2002; Pradeep *et al*., 2002 and Ibrahim *et al*., 2019). The genetic characterization of the Jojoba plant using modern molecular marker techniques is limited somehow (Bhardwaj *et al*., 2010). Therefore, more studies are needed to better delineate the genetic diversity and its association with different characters. The aim of this study is to explore the genetic diversity of the Jojoba plant associated with oil content and seed weight using ISSR markers.

MATERIAL AND METHODS

Jojoba samples

The plant samples involved a number of sixteen Jojoba clones (one male and fifteen females clones) growing at Go-green for Agricultural Investment and Development company farm, Abo-Ghaleb, Giza, Egypt (GIADC). The selected clones were previously identified by DNA barcoding using *rbcL* gene and the obtained sequences submitted at Genbank with accession numbers (Khalil *et al*., 2020)

Oil extraction and seed weight

Extraction of oil from seeds was performed according to A.O.A.C., (2016) at oils and fats lab, Food Technology Institute, Agricultural Research Center. The seeds under investigation were cleaned (to remove all visible foreign matter), weighed (100 seeds), ground, and soaked in pure n-hexane for 48 hours at room temperature. The miscella was collected, filtered and this process was repeated three times using pure solvent in each time to obtain the most of the oil. The combined miscella was desolventized under vacuum using rotary evaporator at about 40ºC.The obtained oil was dried using anhydrous sodium sulfate and filtered using a Whatman No. 1 filter paper, then filled in dark brown glass bottles and kept at -20ºC.

DNA extraction

Genomic DNA from Jojoba fresh leaves was isolated using DNeasy Plant Mini Kit (QIAGEN, Germany) according to the manufacturer's instructions. DNA quality and quantity were detected by using both NANODROP 2000 (Thermo Scientific, USA), and 1% agarose gel (ethidium bromide staining).

ISSR genotyping

A set of 10 ISSR primers was synthesized and used for the amplification according to Kantety *et al*., (1995). PCR amplification was performed in a 25 ml reaction volume (30 ng template DNA, 0.6 µM ISSR primers, 1 U Taq DNA polymerase, 0.2 mM dNTP mixture, 1X PCR buffer) using GeneAmp PCR System 9700 (Applied Biosystems, USA). After initial denaturation of 5 min at 94ºC, each cycle

comprises 1 min denaturation at 94ºC, 1 min annealing at different annealing temperature (Ta), 2 min extension at 72ºC and a final extension for 10 min at 72ºC. Ta varied according to each primer (Table 1). Finally, PCR-amplified fragments were separated by electrophoresis on a 1.5% agarose gel using 1X TAE buffer (pH 8.0) at room temperature. The gel was visualized with ethidium bromide staining.

Data analysis

For ISSR analysis, only clear and unambiguous bands were visually scored as either present (1) or absent (0) for all samples. Final data sets included both polymorphic and monomorphic bands were recorded. After that, a binary statistic matrix was constructed. Dice's similarity matrix coefficients were then calculated between genotypes using the unweighted pair group method with arithmetic averages (UPGMA). This matrix was used to construct a phylogenetic tree (dendrogram) according to Euclidean similarity index using the PAST software Version 1.91 (Hammer *et al*., 2001).

RESULTS AND DISCUSSION

Seed weight and oil content

The seed weight (100 seeds) and oil content of the selected Jojoba clones are shown in Table (2) and Fig. (1). In several circumstances, seed weight and oil content had a close relationship; as weight increases, so does the amount of oil in the seed. However, it does not, applicable in all circumstances. The weight of 100 seeds in grams ranged from 71.01 to 135.44 and the oil content ranged from 48.16 % to 55.93%. The lowest seed weight was 71.01 gm for clone GIADC1 while, the highest weight was 135.44 for GIADC14. Clone GIADC2 had the highest oil percentage of 55.93 percent, which is relatively high when compared to other seed oils and also previously reported oil percent from Jojoba seeds (Cappillino *et al*., 2003). However, investigation of the Jojoba plant for the presence of oil revealed that the seeds contain almost 50-52% of the seed weight (Gad *et al*., 2021). The Jojoba industry accepts a 50% oil content level in seeds from wild and cultivated Jojoba as the base average from which seed prices have been modified for greater or lower oil levels. (Purcell *et al*., 2000). As noted above, clone GIADC2 has the potential (55.93% oil content) to be a good cultivar in future breeding programs.

ISSR polymorphism among different Jojoba clones

Jojoba is a potential oilseed crop; however, it still needs to be developed commercially and agronomically. An assessment of genetic variability in Jojoba germplasm is essential for exploitation of genetic resources for plant improvement programs. ISSR markers have several unique features such as no prior knowledge of genome sequence, high throughput analysis and generation of high-level polymorphism (Powell *et al*., 1996 and Souframanien and Gopalakrishna, 2004). In this study, 10 ISSR primers

(Fig. 2) were used to investigate the similarity and relationship among the sixteen Jojoba clones. A total of 131 bands were amplified (Table 3) with average of 13.1 bands/primer. The lowest number of bands (10) was produced by the ISSR-20, while the highest number of bands (16) was revealed by the ISSR-4 and -5. The number of monomorphic bands reached 61 with average of 6.1 bands/primer. The maximum number of monomorphic bands (9) was generated by the ISSR-3, while the lowest (1) was produced by the ISSR-9. The total number of polymorphic bands (70) with averaged of 7.0 bands/primer. The lowest number of polymorphic bands (2) was generated by the ISSR-20, while the highest number of polymorphic bands (11) was revealed by the ISSR-1. The percentage of polymorphism was ranged from 20% (ISSR-20) to 91% (ISSR-9). The average level of polymorphism was estimated as 52.6%. The frequency ranged from 0.40 to 0.87 for the ISSR-20 and ISSR-9, respectively. The PIC values ranged from 0.20 (ISSR-20) to 0.37 (ISSR-9 and ISSR-19) with an average of 0.31. The Resolving power values ranged from 4.20 (ISSR-20) to 12.2 (ISSR-9 and ISSR-19) with an average of 8.8. The Heterozygosity index values ranged from 0.23 (ISSR-20) to 0.49 (ISSR-4 and ISSR-19) with an average of 0.39. ISSR marker system was quite informative for assessing the extent of genetic diversity as well as pattern of genetic relationships within the selected plants of Jojoba clones. ISSRs generated relatively higher level of polymorphism in Jojoba, which is in concurrence with the earlier reports in many plant species including blackgram (Souframanien and Gopalakrishna, 2004) and mulberry (Awasthi *et al.*, 2004). The high level of polymorphism observed (91%) with the primers used in this study, indicate a high level of genetic variation among the 16 clones. Similar observation has been reported by Rakoczy-Trojanowska and Bolibok, (2004), who reported highly polymorphic patterns were revealed when using primers based on microsatellite sequences. It seems that the assessment of diversity among these genotypes would be of profound importance for the selection of clones prioritized for the proper utilization as genetic resources in breeding programs.

Genetic similarity and cluster analysis based on ISSR marker

To show the genetic similarity and clustering structure among the sixteen Jojoba clones the UPGMA and Dice coefficient (Table 4 and Fig. 3) were used. The genetic similarity was estimated between 80% and 90%, revealing a high level of similarity. The first high genetic similarity (90%) was detected between (8, 12) and (9, 14), while the lowest genetic similarity (80%) was detected between (5, 8), (6, 7) and (7, 10). The dendrogram showed two main clusters; the first main cluster has grouped three Jojoba genotypes (5, 10 and 15). The second main cluster was divided into two sub-clusters; one sub-cluster contains 10 Jojoba clones (4, 6, 8, 12, 9, 14, male, 13, 1 and 11), while the other sub-cluster contain three Jojoba clones (2, 7 and 3). These findings are comparable to

those reported by Sharma *et al*., (2009) who reported a comparative study of genetic relationships among male and female genotypes of Jojoba using ISSR markers.

Principal coordinate analysis

Principal Coordinate Analysis (PCoA) is performed to compare groups of samples based on phylogenetic. The resulting output file consists of the principal coordinate (PC) axes for each sample. PCoA was carried out to provide spatial representation of the genetic diversity among the selected Jojoba clones based on Dice's similarity matrix (Fig. 4). The multivariate analysis methodology was used to support the grouping results, since the bunch investigation has showed higher resolution for closely related populations. The first PCoA revealed about 15.4% out of all genotypes, while the second PCoA settled 16.6% out of all genotypes. The structure evaluated by the PCoA was in concurrence with the bunching analysis.

Association of ISSR markers with oil content and seed weight

The single locus F-test module in Power maker software was used to conduct the trait-marker association study (Fig. 5). The Characterized locus generated by (ISSR-01, ISSR-08 and ISSR-19) was found to be associated with oil. The highest association (1.8) between oil and ISSR marker primer ISSR-01 was at molecular weight fragment (490bp). While, the lowest association (1.1) revealed by primer ISSR-01 at molecular weight fragment (200bp). Moreover, the association with seed weight was found with more than one locus. The primers (ISSR-01, ISSR-04, ISSR-05, ISSR-09, ISSR-19 and ISSR-20) were found to associate with the weight of 100 seeds. The highest association between the weight and ISSR marker (2.06) primer (ISSR-05) was revealed at molecular weight fragment (1000bp). While, the lowest association (1.12) was revealed with primer (ISSR-09) at molecular weight fragment (240bp). Oil content and seed weight were normally distributed indicate that the traits are controlled by quantitative genes as previously reported by Zheng *et al*., (2008). The results of our study demonstrate the significant potential of the association analysis of oil content with ISSR markers in different Jojoba plants. Finally, as noted above, the findings from the present study should provide some basis for future research in genetic improvement of Jojoba plants in terms of important traits.

SUMMARY

Jojoba has become a significant plant due to its precious seed oil with numerous applications. In an attempt to explore the genetic variation and the association with different traits, ISSR analysis was conducted for the selected Jojoba clones. In the present study, sixteen Jojoba plants (clones) including 1 male and 15 female clone were selected to conduct this experiment. Ten ISSR primers were used in this study. The analysis of oil content in the seeds revealed that one plant (GI-ADC2) contain up to 55.93% oil, which is quite high percentage. Increasing in oil

content and seed weight is not always related to each other. ISSR analysis revealed 61 monomorphic bands, where, the maximum number of monomorphic bands (9) was generated by the ISSR-3, while the lowest (1) produced by the ISSR-9. However, the total number of polymorphic bands was 70. The percentage of polymorphism was ranged from 20% (ISSR-20) to 91% (ISSR-9) with average 52.6%. The dendrogram showed two main clusters; the first main cluster has grouped three Jojoba genotypes (5, 10 and 15). While the second main cluster was divided into two sub-clusters; one sub-cluster contain 10 clones (4, 6, 8, 12, 9, 14, male, 13, 1 and 11) and the other sub-cluster contained three Jojoba clones (2, 7 and 3).The association of ISSR marker with oil content revealed that ISSR-01, ISSR-08 and ISSR-19 were associated with oil content. On the hand, the association of ISSR marker with seed weight revealed that ISSR-01, ISSR-04, ISSR-05, ISSR-09, ISSR-19 and ISSR-20 primers were associated. Finally, molecular marker such as ISSR markers is important in trait-marker association study for improving plant cultivars.

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Table (1): ISSR primers used and their annealing temperature.

No	Name	Sequence (5´-3´)	Annealing temperature (°C)
1	ISSR-1	AGAGAGAGAGAGAGAGYC	51
2	ISSR-3	ACACACACACACACACYT	49
3	ISSR-4	ACACACACACACACACYG	50
4	ISSR-5	GTGTGTGTGTGTGTGTYG	49
5	ISSR-6	CGCGATAGATAGATAGATA	48
6	ISSR-7	GACGATAGATAGATAGATA	51
7	ISSR-8	AGACAGACAGACAGACGC	50
8	ISSR-9	GATAGATAGATAGATAGC	49
9	ISSR-19	HVHTCCTCCTCCTCCTCC	45
10	ISSR-20	HVHTGTGTGTGTGTGTGT	50

Note: Y= C, T; H= A, C, T; V= A, C, G.

Table (2): Oil content and seed weight (100 seeds) of the selected jojoba clones.

Sample NO.	Oil %	Weight of 100 seeds (g)
GIADC1	53.42	71.01
GIADC2	55.93	118.76
GIADC3	53.07	75.03
GIADC4	52.85	120.34
GIADC5	53.09	113.75
GIADC6	51.64	100.11
GIADC7	51.66	106.52
GIADC8	48.98	116.20
GIADC9	49.10	116.58
GIADC10	52.41	102.94
GIADC11	51.97	111.38
GIADC12	51.74	120.91
GIADC13	48.16	81.45
GIADC14	49.70	135.44
GIADC15	52.43	108.29
GIADC16	Male	Male

Table (3): Amplification summary produced by ISSR primers.

Name	**TB**	**MB**	**PB**	**(%P)**	**F**	**PIC**	**R**	**H**
ISSR-1	15	4	**11**	73	0.64	0.35	10.6	0.46
ISSR-3	13	**9**	4	31	0.83	0.24	5.36	0.28
ISSR-4	**16**	6	10	63	0.66	0.35	10.8	**0.49**
ISSR-5	**16**	8	8	50	0.75	0.30	7.87	0.37
ISSR-6	11	3	8	72	0.67	0.35	9.82	0.45
ISSR-7	13	7	6	46	0.70	0.33	9.54	0.42
ISSR-8	11	8	3	27	0.84	0.24	5.27	0.28
ISSR-9	11	**1**	10	**91**	**0.40**	**0.37**	**12.2**	0.47
ISSR-19	15	7	8	53	0.78	**0.37**	**12.2**	**0.49**
ISSR-20	**10**	8	**2**	**20**	**0.87**	**0.20**	**4.20**	**0.23**
Total	**131**	**61**	**70**	**-**	**-**	**-**	**-**	**-**
Means	**13.1**	**6.1**	**7**	**52.6**	**0.71**	**0.31**	**8.8**	**0.39**

Note: Total number of bands (TB), Monomorphic bands (MB), Polymorphic bands (PB), Percentage of polymorphism (% P), Frequency (F) and Polymorphism Information Content (PIC), Resolving Power (R), Heterozygosity Index (H).

Table (4): Genetic similarity matrix among the 16 jojoba clones computed according to Dice's coefficient as revealed by ISSR markers.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	100															
2	89	100														
3	84	90	100													
4	88	89	86	100												
5	84	84	85	86	100											
6	87	83	83	91	86	100										
7	86	91	85	84	82	80	100									
8	86	88	84	90	80	88	86	100								
9	88	86	86	86	86	91	86	92	100							
10	83	82	86	82	86	88	80	82	85	100						
11	87	83	84	86	84	82	84	86	89	81	100					
12	86	86	83	89	84	87	85	93	89	83	88	100				
13	89	86	84	90	88	88	84	87	90	83	84	86	100			
14	87	88	82	91	86	88	86	91	93	84	88	92	88	100		
15	86	84	87	84	89	87	84	86	88	91	83	86	90	88	100	
16	84	86	83	87	83	85	88	88	92	81	89	86	86	91	86	100

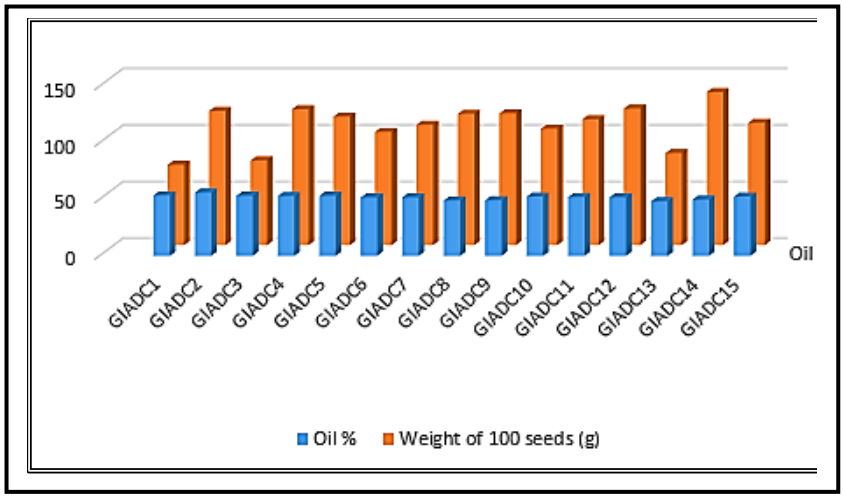

Fig. (1): Oil content (%) and seed wieght (100 seeds) in Grams of the selected jojoba seeds.

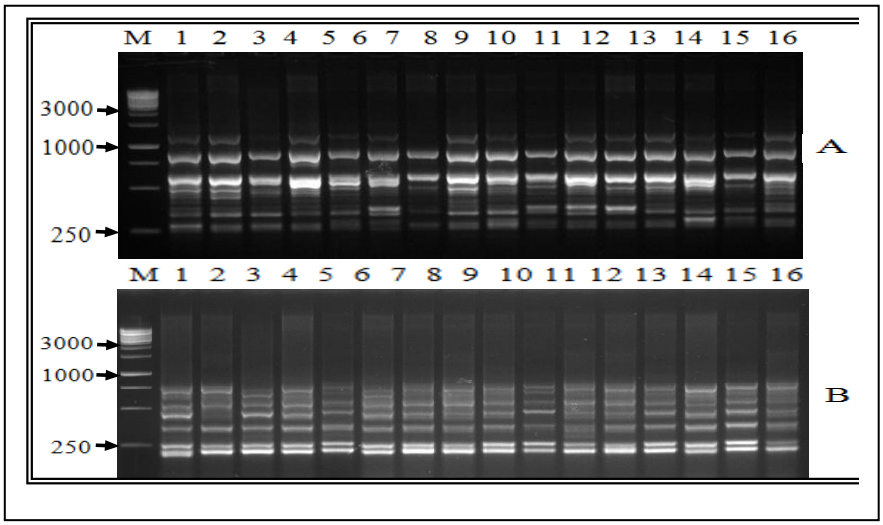

Fig. (2): A representative ISSR profiles of selected Jojoba clones generated by (A): primer ISSR-03 and (B): ISSR-8. M: 1kb DNA ladder (Fermentas, Germany). Lanes 1 – 16: amplified products of Jojoba clones.

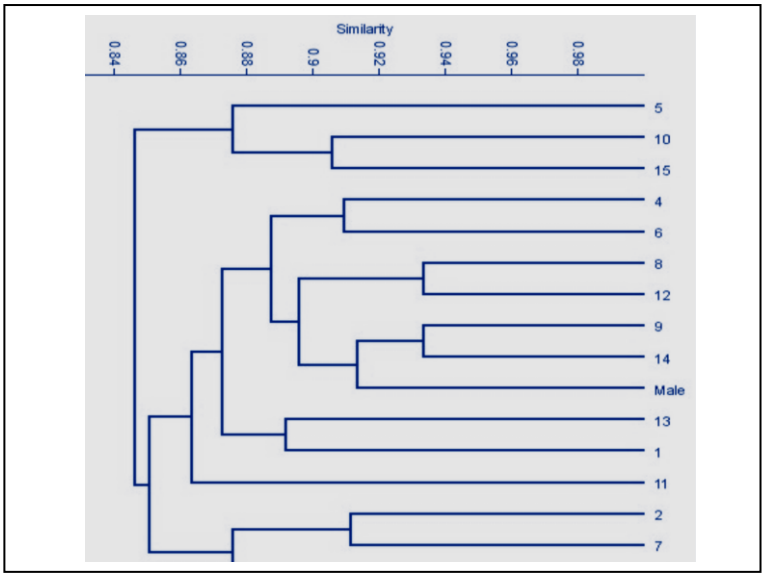

Fig. (3): Dendrogram for the selected Jojoba clones constructed from ISSR data using UPGMA and similarity matrix computed according to Dice coefficient.

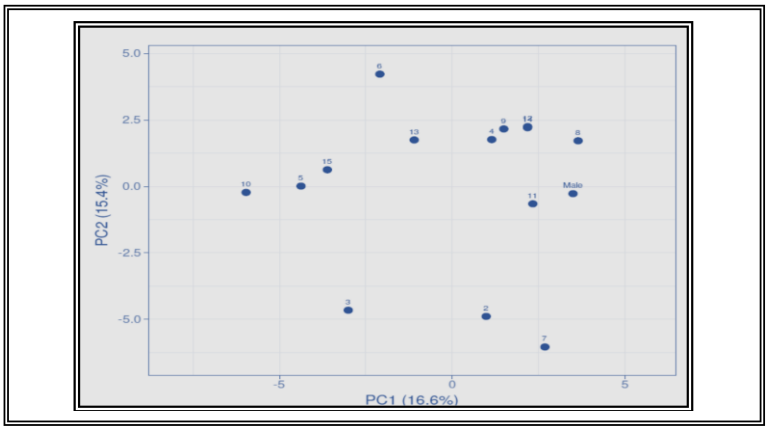

Fig. (4): Principal coordinate analysis based on the calculation of the first three coordinates based on ISSR markers analysis of the studied selected Jojoba clones.

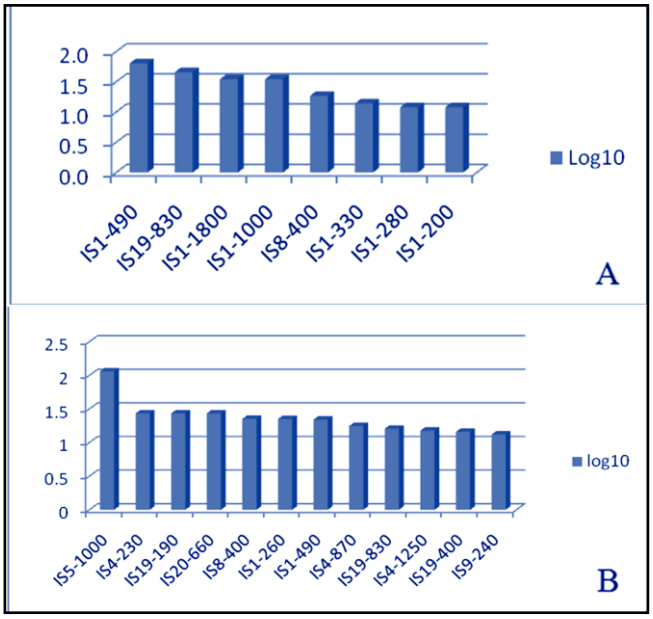

Fig. (5) Association analysis. Oil content (A) and seed weight (B) association with ISSR markers.