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Genetic Variation of Some Banana Cultivars Grown in Egypt Using Inter Simple Sequence Repeat

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ABSTRACT

Banana is a very important fruit crop in Egypt after citrus, grapes, and mangos. High morphological and horticultural variations were recorded between the banana cultivars worldwide. In this study, the genetic diversity between seven common banana cultivars grown in Egypt was evaluated using inter-simple sequence repeat (ISSR) markers. Total DNA extracts were prepared and used as templates for PCR reaction in the presence of six primers. These primers generated 51 DNA fragments containing 50 polymorphic bands and one monomorphic band. The ISSR-PCR primers were varied in their amplified DNA fragments which ranged between 5 to 11 bands. The fifty DNA polymorphic fragments differentiated between the seven banana cultivars under investigation which belong to different banana groups. Similarities between these cultivars ranged from 27.57 to 80.18%, which proved the genetic variations between these cultivars and also confirmed the morphological and horticultural differences between them. The highest similarity of 80.18% was found between Williams and Baladi (Unknown) cultivars, while the lowest similarity was between Maghrabi and Baladi banana cultivars. A dendrogram of phylogenetic relationships confirmed the genetic and morphological diversity between the seven banana cultivars. The experimental results revealed the importance of ISSR markers in distinguishing between the banana cultivars.

INTRODUCTION

Bananas (*Musa* sp.) are the fourth most important fruit crop in Egypt after citrus, grapes and mangos due to their taste along with their nutritive, availability throughout the year and medicinal value. Genetic variability of banana cultivars is one of the most important factors in banana improvement programs (Swain *et al.*, 2016).

The diversity between banana cultivars or species became an important interest to be investigated (Rajamanickam and Rajmohan 2010) and could be supported by DNA fingerprinting tools.

Several techniques were applied to assess the taxonomy and determination of genetic diversity of bananas, *i.e.*, morphological characters (Simmonds 1990), isozymes (Bhat *et al.,* 1992), cytogenetics (Osuji *et al.,* 1997, Osuji *et al.,* 1998), spacers (Lanaud *et al.,* 1992), amplified fragment length polymorphism (AFLP) (Loth *et al.,* 2000, El-Khishin *et al.,* 2009, Safhi *et al.,* 2023) restriction fragment length polymorphisms (RFLP) (Gawel and Jarret 1991, Gawel *et al.,* 1992, Carreel *et al.,* 2002, Kiran *et al.,* 2015), random amplified polymorphic DNA markers (RAPD) (Kaemmer *et al.,* 1992, Bhat *et al.,* 1995, Pillay *et al.,* 2001, Masoud *et al.,* 2008, Venkatachalam *et al.,* 2008, Brown *et al.,* 2009, Poerba and Ahmad 2010), inter simple sequence repeat (ISSR) (Godwin *et al.,* 1997, Venkatachalam *et al.,* 2008, Poerba and Ahmad 2010, Lu *et al.,* 2011, Babu *et al.,* 2018, Wanvisait *et al.,* 2019, Wahyudi and Rifliyah 2020, Hasan *et al.,* 2021), microsatellites (Grapin *et al.,* 1998).

Rout *et al.* (2009) differentiated between different varieties of bananas collected from different banana genetic resources based on ISSR analysis using 15 primers. Poerba and Ahmad (2010) used ISSR to assess the genetic diversity between 18 banana cultivars using five primers which generated 26 DNA fragments with sizes ranging from 350 to 2000 bp. A percentage of 92.86% of these fragments were polymorphic fragments.

Kiran *et al.* (2015) evaluated the genetic diversity of 10 banana cultivars in Odisha using RAPD-PCR tool by five random oligonucleotides selected from 15 primers due to their abilities to produce visible and reproducible DNA polymorphisms. These primers generated 24 DNA fragments with sizes ranging from 300 to 3000 bp and 75% of them were recorded as polymorphic fragments. Rayan *et al.* (2016) showed that the four banana cultivars of Basrai, Hindi, Grand-Naine and Williams were successfully cultivated in new reclaimed sandy soil irrigated via a drip system in Sohag Governorate, Egypt. These cultivars were varied in their growth characters (height of pseudostem, number of green leaves per bunch, length and width of leaves length) and periods of cropping cycle (time needs for bunches emerging, weight of bunches and hands and fingers per bunch). Babu *et al.* (2018) used ISSR tool to evaluate the genetic diversity within eight banana cultivars (Basrai dwarf bale, Hanuman bale, Kari Bale, Kalyani bale, Nanjanagud Rasbale, Rajapuri Bale, Sakkare bale and Sugandhi Bale) by 20 ISSR primers. Eight primers produced 30 monomorphic fragments while 12 produced 42 polymorphic with an average of 3.5 fragments per primer. They noted the presence of a huge genetic dissimilarity among the eight banana cultivars which reflect their different genomic constitution.

Genetic variations and relationships among 21 commercially important banana cultivars (Robusta and Williams) of South India were evaluated *via* 12 ISSR-PCR primers (Venkatachalam *et al.,* 2008) generating 56.73% polymorphic fragments. The genetic similarity coefficients in ISSR RAPD analysis ranged from 18 to 84.62%. They showed that eight banana cultivars were highly distinct from one another.

Kharadi *et al.* (2014) identified the genetic variation of six banana cultivars by ISSR-based genetic analysis using 7 primers generated 56 scorable DNA fragments classified as 39 (69.64%) polymorphic and 17 (30.36%) monomorphic fragments. The analytical results showed a dendrogram of the banana cultivars indicated the presence of close relationships among Mahalaxmi, Robusta, Williams, Sona and Grand Naine cultivars which belong to genome group AAA.

Borborah *et al.* (2020) conducted a comparative analysis of the genetic variation of some non-commercial cultivars of Musa L. from Assam, India using ISSR marker using 7 primers. A total number of

62 DNA fragments were amplified, and 56 fragments showed polymorphism. The index of the genetic similarity showed genetic similarity between the banana cultivars ranged from 0.28 to 0.77 with 0.51 on average.

In Pakistan, Noor *et al.* (2022) used ISSR markers to assess the genetic diversity of 14 local banana varieties using 45 primers. Forty out of the 45 primers resulted in a total of 121 DNA polymorphic fragments contributing to a ratio of 47.87 polymorphism. 11 out of 14 local banana varieties used were uniquely identified by 54 polymorphic ISSR DNA fragments of different sizes.

Wahyudi *et al.* (2022) examined the clustering and genetic variation by ISSR markers of the genomes of different banana cultivars in East Java using five ISSR-PCR primers. High genetic richness with polymorphic loci of 96.97% and Shannon index of 0.587 of the genetic diversity among banana genome groups were shown. They revealed that ISSR was a powerful marker for the identification of banana cultivars. Slightly genetic variation was considered more conserved within the bananas genome groups.

The present study aimed to use ISSR markers to differentiate between the seven banana varieties cultivated in Egypt.

MATERIALS AND METHODS

Source of Banana Varieties:

A number of seven banana cultivars collected from Upper and Lower Egypt were used. These varieties are Grand Naine, Williams, Basri, Baladi, Hindi, Maghrabi and Edward Cavendish. These cultivars were gathered by Fatma Salah Abdel Razek, Department of Agricultural Microbiology (Virology Laboratory), Faculty of Agriculture, Ain Shams University, between 2021 and 2022, and kept under calcium chloride hydration conditions until use.

DNA Extraction:

The total DNA extracts were prepared and purified according to the method of Abdel Razek *et al*. (2022). The concentration of genomic DNA was estimated by NanoDrop to adjust for PCR amplification (Desjardins and Conklin 2010).

PCR Amplification:

The amplification reaction was conducted in 25 µL reaction volume containing, 10X PCR buffer, 2.0 mM $MgCl_2$, 0.2 mM dNTPs, 25 pmol/primer, 1.0 U *Taq* DNA polymerase and 80 ng template DNA, and dsH_2O). Six primers are: P01: 5'-AGA GAG AGA GAG AGA GTC-3'; P02: 5'-AGA GAG AGA GAG AGA GAG-3', P03: 5'-ACA CAC ACA CAC ACA CAT-3', P04: 5'-GTG TGT GTG TGT GTG TAG-3', P05: 5'-CGC GAT AGA TAG ATA GAT-3' and P06: 5'-ACA CAC ACA CAC ACA CTA-3' were used.

PCR Program and Analysis:

Using a Perkin-Elmer/GeneAmp® PCR System 9700, the PCR amplification was conducted. The machine was programmed as follows: initial denaturation cycle for 5 min at 94ºC, 35 cycles consisting of 94ºC for 45s, 46ºC for 50s, and 72ºC for 1 min. The final cycle was extended for 7 min at 72ºC. The PCR products were resolved by electrophoresis in a 1.5% agarose gel containing ethidium bromide (0.5 µg/mL) in 1X TAE buffer at 95 volts. The DNA fragments were scored as 1 (Present) or 0 (Absent), and the similarity coefficient (F) between banana cultivars was determined using the formula of Nei and Li (1979). Using the distance by un-weighted paired-group method (Rohlf 1990) a dendrogram was drawn.

RESULTS AND DISCUSSION

This study was designed to determine the genetic variation among seven banana cultivars of Egypt grown in different climate conditions using the total DNA extracts of seven banana cultivars as templates in PCR of ISSR assay in the presence of six primers.

ISSR was successfully used in several investigations to differentiate between the banana cultivars in different regions worldwide (Lakshmanan *et al.*,

2007, Swain *et al.*, 2016, Poerba *et al.*, 2019, Borborah *et al.*, 2020).

Results in Table (1), and illustrated by Figure (1), showed the scoring of DNA polymorphisms of ISSR-PCR of seven banana cultivars grown in Egypt using the six used primers, which generated 51 DNA fragments containing 50 polymorphic fragments and one monomorphic fragment. The ISSR-PCR primers were varied in their amplified DNA fragments which ranged between 5 to 11 bands. In other means, 5, 6, 10, 11, 11 and 8 fragments were amplified using the primers P01, P02, P03, P04, P05, and P06, respectively. The 50 DNA polymorphic fragments representing 98.04% differentiated between the seven banana cultivars under investigation which belong to different banana groups. The banana cultivars (Grand Naine, Williams, Basri, Baladi, Hindi, Maghrabi and Edward Cavendish) showed 28, 28, 30, 34, 33, 29 and 30 DNA fragments, respectively (Table 2).

Data in Table (3), showed the similarities between these cultivars which ranged from 27.57 to 80.18%, and proved the genetic variations between these cultivars and also confirmed the morphological and horticultural differences between them. The highest similarity of 80.18% was found between Williams and Baladi (Unknown) cultivars, while the lowest similarity was between Maghrabi and Baladi banana cultivars. A dendrogram of phylogenetic relationships confirmed the genetic and morphological diversity between the seven banana cultivars (Fig. 2).

The experimental results were in harmony with that of Swain *et al*. (2016) who assessed the molecular variations between 22 local dessert banana genotypes of Odisha using ISSR markers. In that study, the primers were generated 76 DNA fragments which classified as 36 (47.4%) polymorphic and 39 (51.3%) monomorphic fragments. The percentages of genetic similarities were ranged from 71 to 90%.

The results of this study agree with the investigation of Singh *et al*. (2021) who reported that molecular variability of banana germplasm based on DNA markers including ISSR was always reliable. They determined the genetic diversity of 191 banana genotypes using 10 ISSR primers, which generated a total of 37 polymorphic DNA fragments with an average of 3.7 fragments per primer. They also revealed that the genotypes which showed higher similarity were found in same the cluster.

Table 1. Scoring of DNA polymorphisms of ISSR-PCR of some banana cultivars grown in Egypt.

Primers used	**Banana cultivars**							**Type of DNA fragments**
	Grand Naine	Williams	Basri	Baladi	Hindi	Maghrabi	Edward Cavendish	
P01-01	1	1	1	1	1	0	1	Polymorphic
P01-02	0	0	1	1	1	1	1	Polymorphic
P01-03	1	0	1	1	1	1	1	Polymorphic
P01-04	1	1	1	0	0	0	1	Polymorphic
P01-05	1	1	1	1	1	0	0	Polymorphic
P02-01	0	1	0	1	1	1	1	Polymorphic
P02-02	0	1	1	0	1	0	0	Polymorphic
P02-03	1	1	1	1	1	1	1	Monomorphic
P02-04	0	0	0	1	1	0	0	Polymorphic
P02-05	0	1	1	1	1	1	0	Polymorphic
P02-06	0	0	0	1	1	1	1	Polymorphic
P03-01	0	0	0	0	0	0	1	Polymorphic
P03-02	0	0	0	1	0	1	1	Polymorphic
P03-03	0	1	1	0	0	1	0	Polymorphic
P03-04	0	1	0	1	0	1	1	Polymorphic
P03-05	0	1	0	1	1	0	1	Polymorphic
P03-06	1	1	1	1	1	0	1	Polymorphic
P03-07	1	1	1	1	0	1	1	Polymorphic
P03-08	1	0	1	0	0	1	1	Polymorphic
P03-09	1	0	1	0	1	0	1	Polymorphic
P03-10	1	0	1	0	1	1	1	Polymorphic
P04-01	0	0	1	1	1	1	1	Polymorphic
P04-02	0	1	0	0	1	0	0	Polymorphic
P04-03	1	0	1	1	1	1	1	Polymorphic
P04-04	0	0	1	1	0	1	1	Polymorphic
P04-05	0	1	0	0	1	0	0	Polymorphic
P04-06	1	1	1	0	1	0	0	Polymorphic
P04-07	1	1	1	1	0	0	0	Polymorphic
P04-08	1	1	1	1	1	1	1	Polymorphic
P04-09	1	0	1	1	1	1	1	Polymorphic
P04-10	1	1	0	0	0	1	1	Polymorphic
P04-11	1	1	0	0	1	0	0	Polymorphic
P05-01	1	0	0	0	1	1	1	Polymorphic
P05-02	0	1	0	1	1	0	0	Polymorphic
P05-03	0	0	0	1	0	0	0	Polymorphic
P05-04	0	1	0	1	1	0	0	Polymorphic
P05-05	1	1	0	1	1	1	1	Polymorphic
P05-06	0	1	1	1	1	1	0	Polymorphic
P05-07	0	0	1	1	0	1	1	Polymorphic
P05-08	1	0	1	1	0	1	1	Polymorphic
P05-09	1	1	1	0	1	1	1	Polymorphic
P05-10	0	1	0	1	1	1	0	Polymorphic
P05-11	1	1	0	1	1	0	0	Polymorphic
P06-01	1	0	1	1	0	0	0	Polymorphic
P06-02	1	0	1	1	1	1	0	Polymorphic
P06-03	0	1	0	0	0	1	0	Polymorphic
P06-04	1	0	1	1	0	0	1	Polymorphic
P06-05	1	0	1	1	1	1	1	Polymorphic
P06-06	0	1	0	0	1	1	1	Polymorphic
P06-07	1	0	0	0	0	0	0	Polymorphic
P06-08	1	0	1	1	0	0	0	Polymorphic
Total	28	28	30	34	33	29	30	1(MF)+50(PF)

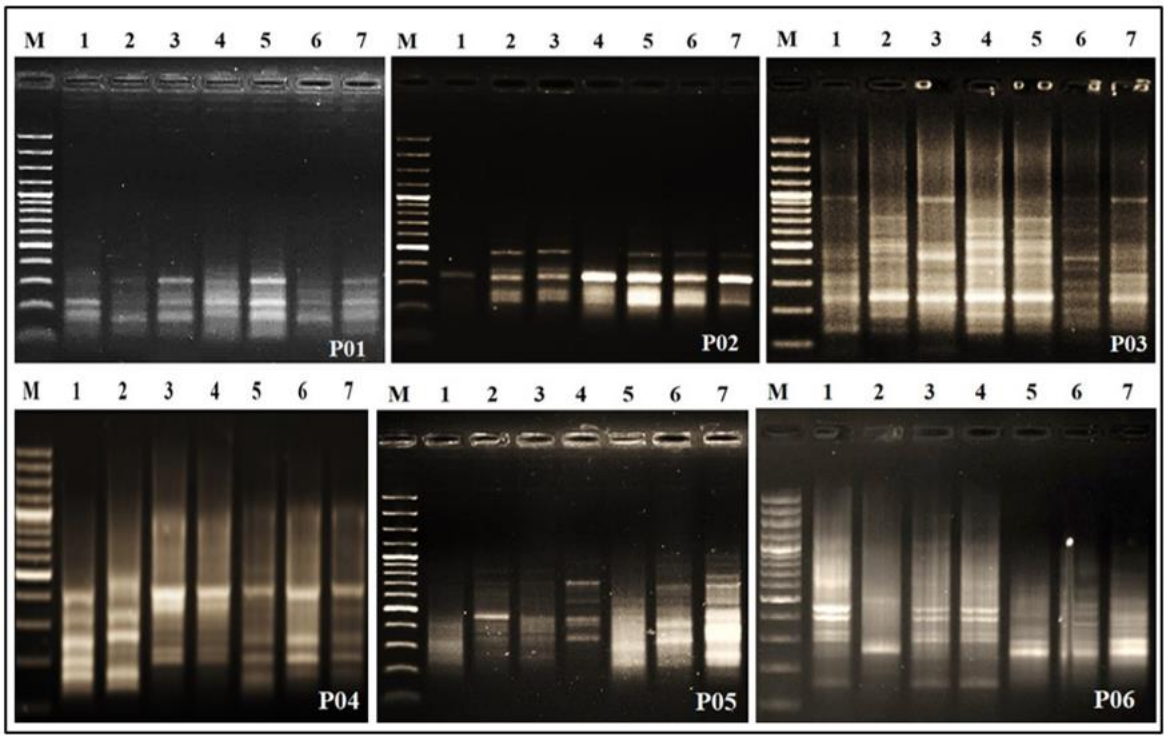

Fig 1. Agarose gel (1.5%) stained with ethidium bromide shows the DNA polymorphisms generated by different ISSR-PCR primers and DNA extracts of seven banana cultivars (Grand Naine (1), Williams (2), Basri (3), Baladi (4), Hindi (5), Maghrabi (6), and Edward Cavendish (7)) grown in Egypt. M: 100 bp Plus DNA Ladder (3000, 2500, 2000, 1500, 1000, 900, 800, 700, 600, 500, 400, 300, 200, 100 bp).

Table 2. Total amplified fragments (TAFs) of ISSR-PCR of seven banana cultivars grown in Egypt.

ISSR primers	**TAFs**	**Banana cultivars**						
		Grand Naine	Williams	Basri	Baladi	Hindi	Maghrabi	Edward Cavendish
P01	5	4	3	5	4	4	2	4
P02	6	1	4	3	5	6	4	3
P03	10	5	5	6	5	4	6	9
P04	11	7	7	7	6	8	6	6
P05	11	5	7	4	9	8	7	5
P06	8	6	2	5	5	3	4	3
Total	51	28	28	30	34	33	29	30

Table 3. Similarities between DNA polymorphisms of fragments of ISSR-PCR generated for seven banana cultivars grown in Egypt**.**

Banana cultivars	**Banana cultivars**						
	Grand Naine	Williams	Basri	Baladi	Hindi	Maghrabi	Edward Cavendish
Grand Naine	100						
Williams	69.31	100					
Basri	27.57	72.76	100				
Maghrabi	53.89	59.61	37.27	100			
Hindi	52.40	36.98	50.44	37.59	100		
Edward Cavendish	62.42	69.31	42.23	43.36	46.99	100	
Baladi	42.23	80.18	40.55	41.93	50.44	27.57	100

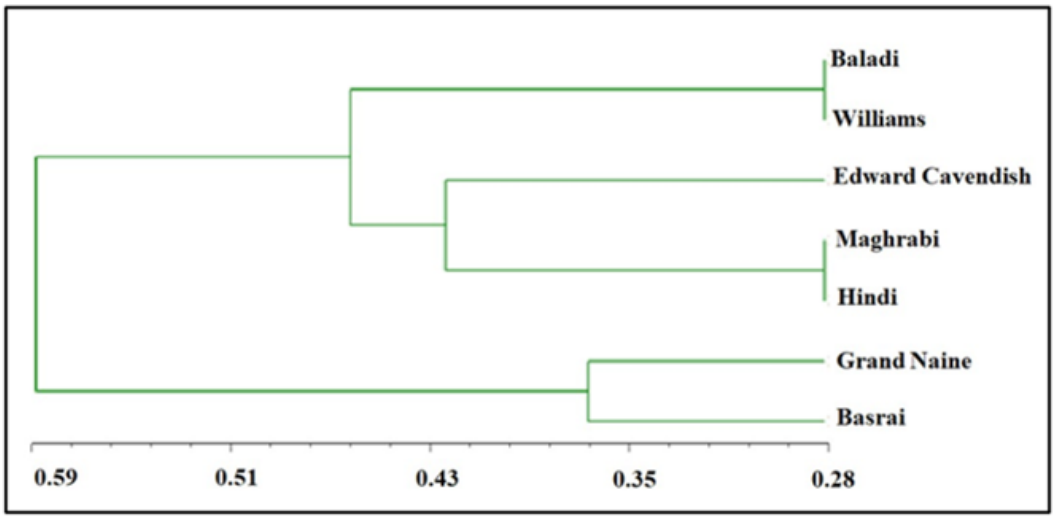

Fig 2. Dendrogram shows the genetic relationship between the seven banana cultivars based on the DNA polymorphisms generated by ISSR markers.

Conclusion

ISSR markers were successfully differentiated between the seven banana cultivars and genus. The cultivars belonging to the banana groups appeared with higher similarities and were allocated in the same cluster. The DNA fragments which amplified using the ISSR primers generated a high percentage of DNA polymorphic fragments which distinguished between the banana cultivars of this investigation. Another important differentiation between bananas is that it can be used to produce new varieties of bananas with good characteristics using tissue culture.

Declarations:

Ethical Approval: It is not applicable.

Conflicts of Interest: The authors declare that they have no conflict of interest.

Authors Contributions: I hereby verify that all authors mentioned on the title page have made substantial contributions to the conception and design of the study, have thoroughly reviewed the manuscript, confirm the accuracy and authenticity of the data and its interpretation, and consent to its submission.

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Availability of Data and Materials: All datasets analysed and described during the present study are available from the corresponding author upon reasonable request.

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