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Morphological, Chemical and Molecular Variations Among Some Jackfruit Landraces

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Abstract

Assessment of genetic diversity and relationship among six landraces of jackfruit (*Artocarpus heterophyllus*) grown in Aswan Botanical Garden (Aswan, Egypt) was preformed using morphological, chemical and molecular evaluations. A wide range of variability was observed among the tested landraces, reflected by highly significant differences in all evaluated traits. Among which, fruit length, seed weight, fruit peel weight, total phenolics, non-reducing sugars, protein, calcium, phosphorus and potassium content showed higher influence than others, the way that affected the relationship among landraces and their grouping in dendrogram. These traits are highly recommended to be included in successive evaluation studies of jackfruit. Cluster analysis based on the phenotypic data allowed the grouping of the landraces according to their shared features of higher or lower values of morphological and chemical traits, which could be helpful in the subsequent crop improvement efforts. On the other hand, molecular analysis using inter simple sequence repeats (ISSR) markers clearly confirmed the degree of variability among jackfruit landraces by showing a high percentage of polymorphism (up to 71.43%) and providing a good cluster relationship in the ISSR-based-dendrogram. Furthermore, combining phenotypic and molecular data to perform cluster analysis, gave an insight into the landraces' relationships. High similarity was observed between molecular- and combined-based dendrograms unlike phenotypic-based one. Results herein can benefit the breeding programs of jackfruit.

Keywords: *Artocarpus heterophyllus, Cluster analysis, Genetic variability, Molecular markers, Morphology, Moraceae.*

Introduction

Jackfruit (*Artocarpus heterophyllus* Lam., family Moraceae) is cultivated widely in many parts of the world, including Southeast Asia (Rahman *et al.,* 1999), the evergreen forest zone of West Africa (Burkill, 1997), and northern Australia (Azad *et al.,* 2007). Jackfruit is an evergreen tree with a medium size (8 to 25 m height), producing heavier yields, and has the largest known edible fruit (up to 35 kg). The plant has valuable importance among fruit crops in tropical and subtropical regions. Its fruit has a high nutritional value, as a rich source in carbohydrates, potassium, calcium and vitamins (Samaddar, 1985). The seeds also are eaten after boiling or roasting. Fruit extract exhibited antimicrobial activities (Ragasa *et al*., 2004) and rich antioxidant, phenolic and flavonoids contents (Shanmugapriya *et al.,* 2011). In

addition, medicinal properties have been reported for many parts of the plant including the bark, roots, leaves and fruits (Arung *et al.,* 2006).

Plant breeding and improvement programs rely on the evaluation of genetic diversity, and the selection of proper genotypes with higher diversity and better performance (Etminan *et al*., 2016). Phenotypic evaluation of genetic diversity is frequently being used in many plant breeding approaches, however, when combined with molecular evaluation, better information could be obtained rather than using a single assessment (Youssef and Ibrahim, 2016). Many types of molecular markers exist with varying basis and the regions of the genome target. The inter simple sequence repeats (ISSR) belong to one of the most common molecular markers used in the assessment of plant genetic diversity; they rely on the amplification of DNA regions flanked by microsatellites (Gupta *et al.,* 1994). ISSR markers have been used to assess genetic diversity in many plant species, including jackfruit (Hai *et al*., 2009), guava (Youssef and Ibrahim, 2016), date palm (Purayil *et al.,* 2018), banana (Babu *et al.,* 2018) and doum palm (Khalil *et al.,* 2019). Moreover, other molecular markers have been used in the assessment of genetic variability in jackfruit, including AFLP (Schnella *et al*., 2001; Shyamalamma *et al*., 2008; Li *et al*., 2010), RAPD (Simon *et al.,* 2007; Gopalsamy *et al.,* 2012; Prasad *et al.,* 2014; Krishnan *et al*., 2015) and SSR (Nakintu *et al*., 2019).

The objective of this study is to assess the genetic diversity in six accessions of jackfruit grown in Aswan Botanical Garden, (Aswan, Egypt) using phenotypic and molecular evaluations.

Materials and Methods

Plant materials

Six mature trees of jackfruit (*Artocarpus heterophyllus* Lam.) grown in Aswan botanical garden, Aswan, Egypt (24°05`37"N, 32°53`13"E) were selected and used in this study to assess their genetic diversity at phenotypic and molecular levels.

Phenotypic evaluation

Seven vegetal and fourteen chemical traits of fruit were screened among the tested jackfruit landraces after fruit ripening during July in two seasons (2016 and 2017). Assessment of each studied parameter was carried out in five replicates, ten samples per each replicate were used as a bulk to assess the phenotypic traits using randomized complete block design (RCBD).

Vegetal traits

Vegetal traits included fruit (FW, g), seed (SW, g), fruit peel (FPW) and flesh (FLW, g) weight, fruit length (FL, cm) and diameter (FD, cm) and shape index (SI) as calculated by division of fruit length by fruit diameter.

Chemical traits

A mixture of pulp fruit samples was used to measure some chemical traits as described by AOAC (1995 and 2000), including, total soluble solids (TSS%), total titratable acidity (AC%), moisture (M%), reduced sugars (RS%), non-reduced sugars (NRS%), total carbohydrates (TC%), crude protein (P%), crude fibers (CF%) and crude oil (O%). In addi-

tion, modified Folin-Ciocâlteu colorimetric method (Singleton *et al.,* 1999) was used to determine total phenolics (TP) as milligram of gallic acid equivalents/100-gram sample (mg GA/100 g DW). Some minerals contents were detected as well, including sodium (Na), calcium (Ca), phosphorus (P) and potassium (K), measured as mg/100 g pulp.

Phenotypic data analysis

The combined analysis of genotypes over years was performed by Mstat-C software (Nissen 1984) for the analysis of variance. Averages were compared using revised least significant difference (LSD`) at 5% significance. Phenotypic-based cluster analysis using on the Euclidean dissimilarity coefficient was carried out using NTsysPC v.2.21q package, and the unweighted pair-group method with arithmetic averages (UPGMA) dendrogram was made.

Molecular evaluation

Young fresh leaves were collected separately from each jackfruit landrace and transferred in an ice tank to the molecular biology lab and stored at -80 °C until used. Genomic DNA was extracted from young leaves samples of each tree following the enhanced protocol for plant DNA extraction described by Youssef *et al.* (2015). Concentration and quality of extracted DNA were determined spectrophotometrically (Stulnig and Amberger 1994).

ISSR assay

Ten primers of the inter simple sequence repeats (ISSR) marker were used for the molecular evaluation (Table 1). PCR mixture and program conditions for ISSR were identical with those described by Gupta *et al.* (1994). ISSR-PCR products were separated on agarose gel (2%) and visualized by ethidium bromide stain observed under the UV-light.

Table 1. ISSR primers codes and sequences used in molecular analysis.

Code	Sequence (5` - 3`)
UBC-807	AGAGAGAGAGAGAGAGT
UBC-808	AGAGAGAGAGAGAGAGC
UBC-810	GAGAGAGAGAGAGAGAT
UBC-811	GAGAGAGAGAGAGAGAC
UBC-812	GAGAGAGAGAGAGAGAA
UBC-815	CTCTCTCTCTCTCTCTG
UBC-826	ACACACACACACACACC
UBC-834	GAGAGAGAGAGAGAGAGAGAT
UBC-840	GAGAGAGAGAGAGAGATT
UBC-846	CACACACACACACACAAT

Molecular data analysis

A binary matrix of presence (1) or absence (0) of bands was made from clear and strong detected ISSR amplicons. Cluster analysis based on ISSR data was performed using NTsys software, and the UPGMA-dendrogram was made using Jaccard's (1908) similarity coefficient. Moreover, some diversity parameters were calculated from the molecular data, including percentage of polymorphism (%Pm), polymorphism information content (PIC) (Roldan-Ruiz *et al.,* 2000), primer resolving power (Rp) (Prevost and Wilkinson, 1999), diversity index (DI) (Nei, 1987) and marker index (MI) as a multiplication of the number of polymorphic bands by PIC for each primer. In addition, averages of similarities revealed by molecular markers and dissimilarity of phenotypic data were calculated and used for combined cluster analysis.

Results and Discussion

Selection of proper genotypes with superior characteristic is a prerequisite for breeding programs

which could be achieved mainly by the assessment of genetic diversity, as one of the most important tasks in breeding programs (Etminan *et al.,* 2016). In the present study, the genetic diversity of six jackfruit landraces was assessed using phenotypic and molecular evaluations. Both vegetal and chemical traits of fruit showed high level of variability among the tested landraces (Table 2, Figs. 1 and 2). Moreover, significant differences among jackfruit landraces in all phenotypic traits were revealed by analysis of variance. In this regard, results of the present study agree with those of Jagadeesh *et al.* (2007) who analyzed 24 clones of jackfruit for variability assessment. They found a wide range of variability among genotypes in several chemical traits measured in fruit pulps including TSS, acidity, sugar, starch and carotenoid contents. Also, some landraces in the present study showed their exclusivity by displaying the highest or the lowest values of certain traits, which can be leveraged in the subsequent breeding programs (Table 2, Figs. 1 and 2). Furthermore, some traits (i.e. FL, SW, SPW, TP, NRS, PR, Ca, P and K) showed a higher influence than the others on the grouping of landraces on the phenotypic-based-dendrogram. These findings are supported by previous results of Wangchu *et al.* (2013) who reported that most of the above-mentioned traits are recommended as selection criteria for development of effective and productive plant types in jackfruit.

Morphological and chemical data of the present study were used to perform the cluster analysis based on Euclidian dissimilarity coefficient. UPGMA-Dendrogram of the phenotypic data discriminated the jackfruit landraces into three main clusters (I: JC-01 and JC-06; II: JC-02 and JC-04III: JC-03 and JC-05; Figure 3(a)). The scale of dissimilarity values of Euclidian coefficient ranged between -5.97 to 3.33 which reflected the degree of variability among the landraces (Fig. 3(a)). Some observations were noticed as affecting the grouping of landraces based on phenotypic data into the dendrogram. In this regard, landraces JC-01 and JC-06 shared lower value of FL and SPW, whereas landraces JC-02 and JC-04 shared higher values of estimated minerals (Ca, P and K) and lower values of NRS and TP. In addition, landraces JC-03 and JC-05 exhibited higher values of SPW, SW and TP and lower expression of SHI, PR% and P (Table 2 and Figs. 2 and 3(a)).

Table 2. Average values of some chemical traits evaluated within six jackfruit landraces over two seasons (2016 and 2017).

Landraces	TSS (%)	AC (%)	M (%)	RS (%)	NRS (%)	PR (%)	TC (%)
JC-01	29.05±0.81	0.12±0.01	76.29±0.02	21.32±0.08	4.08±0.09	7.4±0.01	86.08±0.07
JC-02	30.82±0.94	0.22±0.02	76.88±0.01	14.09±0.16	2.63±0.09	7.27±0.01	84.49±0.02
JC-03	39.66±3.65	0.15±0.02	77.37±0.03	20.39±0.25	3.83±0.11	6.86±0.02	84.82±0.01
JC-04	29.29±0.77	0.20±0.01	76.39±0.04	18.45±0.05	3.05±0.13	7.55±0.02	85.16±0.02
JC-05	24.02±0.54	0.20±0.01	76.64±0.04	17.13±0.11	3.12±0.11	6.92±0.05	84.72±0.03
JC-06	24.85±0.36	0.17±0.01	76.49±0.05	19.05±0.01	3.29±0.06	7.24±0.03	84.08±0.01
Mean	29.61	0.18	76.68	18.40	3.33	7.20	84.89
LSD`	6.00	0.04	0.12	0.43	0.34	0.31	0.09
Landraces	**CF (%)**	**O (%)**	**TP**	**Na**	**Ca**	**P**	**K**
JC-01	2.32±0.01	0.74±0.02	1542.63±1.53	9.47±0.06	88.31±0.06	157.61±0.19	1285.13±0.21
JC-02	2.64±0.04	1.08±0.03	1479.31±2.34	8.84±0.02	92.85±0.07	163.38±0.21	1327.63±0.11
JC-03	2.68±0.01	1.04±0.02	1545.02±12.66	8.46±0.02	89.44±0.12	153.68±0.09	1294.82±0.07
JC-04	2.84±0.02	0.81±0.01	1507.29±2.04	9.17±0.03	93.64±0.13	161.58±0.09	1300.35±0.18
JC-05	2.61±0.01	1.00±0.02	1543.68±2.71	7.51±0.07	86.74±0.07	151.26±0.16	1292.71±0.12
JC-06	2.85±0.02	1.11±0.03	1518.95±1.93	8.02±0.01	85.61±0.06	155.22±0.15	1299.31±0.20
Mean	2.65	0.96	1522.81	8.58	89.43	157.12	1299.99
LSD`	0.06	0.04	18.3	0.10	0.25	0.31	4.73

Values represent as means ± standard error (n=3), TSS: total soluble solids, AC: total acidity, M: moisture, RS: reduced sugars, NRS: non-reduced sugars, PR: protein, TC: total carbohydrates, CF: crude fibers, O: oil, TP: total phenolics, Na: sodium, Ca: calcium, P: phosphorus and K: potassium. Max: maximum value, Min: minimum value, LSD`: revised least statistical difference, α=0.05. All chemical traits were determined as g/100g dry weight except total phenolics which was determined as mg GAE/100g dry weight. Minerals were determined as mg/100g pulp.

On the other hand, the ISSR markers were used to determine the molecular diversity among the jackfruit landraces. Out of the ten ISSR primers used, six produced polymorphic bands among the tested landraces; these were then selected and used for analysis (Fig. 4). Of the total of 52 amplified bands, 27 (52%) were polymorphic. The average number of amplified bands ranged from 6 to 12 per primer. Several parameters were calculated from the molecular data to assess the level of diversity among the tested landraces (Table 3). The percentage of polymorphism (%Pm) ranged from 33.33 (UBC-811) to 71.43% (UBC-815). In addition, the primer UBC-808 generated the highest values of PIC (0.075), RP (6.00), DI (2.08) and MI (0.45). Comparatively, the primer UBC-811 scored the lowest values of all calculated parameters (Table 3). Previous studies reported lower values of %Pm than those observed in the current study, depending on the type of molecular marker used and genotypes/species analyzed. In this regard, %Pm of (49.2, 22.0 and 20.3%) using AFLP was reported by Schnell *et al.* (2001), Shyamalamma *et al.* (2008) and Li *et al.* (2010), respectively, and using RAPD (21.87) reported by Prasad *et al.* (2012). Contrastingly, other studies reported higher values of %P than those reported in this study, e.g., Simon *et al.* (2007) and Krishnan *et al.* (2015) used RAPD markers and reported 67.3 and 66.27% polymorphism, respectively.

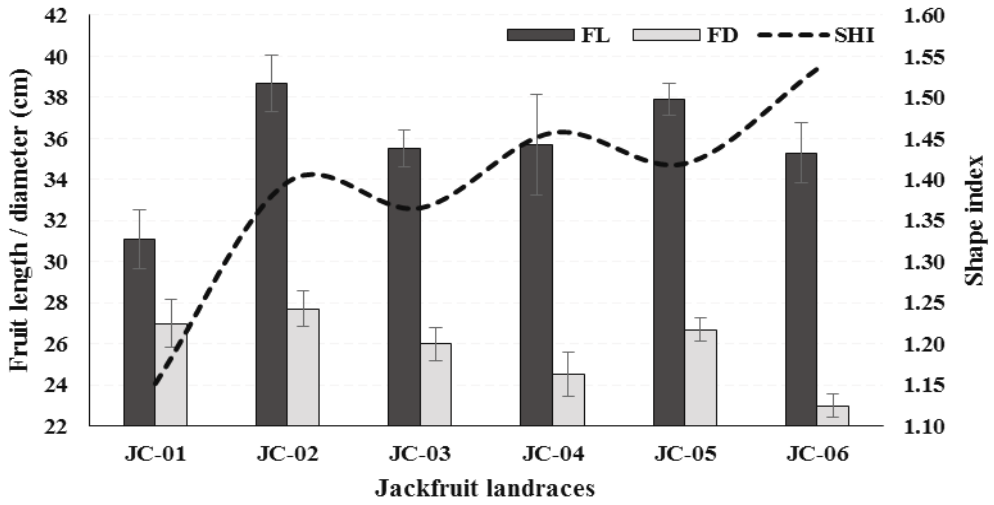

Figure (1): Averages of fruit length (FL), fruit diameter (FD) and shape index (SI=FL/FD; on the secondary Y axis) of six jackfruit landraces evaluated over two seasons (2016 and 2017). LSD` for FL=6.64, for FD=3.67 and for SI=0.34, $p \leq 0.05$. *Bars* indicate standard error (n=5).

Molecular data of ISSR were used for cluster analysis using Jaccard's coefficient. UPGMA-dendrogram based on ISSR data differentiated the tested landraces into two main clusters (I: JC-01, JC-03 and JC-06; II: JC-02, JC-04 and JC-05). The two closest landraces in the ISSR-based dendrogram were JC-02 and JC-05 at 0.98 similarity (Fig. 3(b)). Molecular analysis performed in the current study confirmed the degree of variability among the tested landraces revealed by morpho-chemical evaluation. ISSR markers showed a reasonable degree of polymorphism among the tested landraces reflecting their relationship.

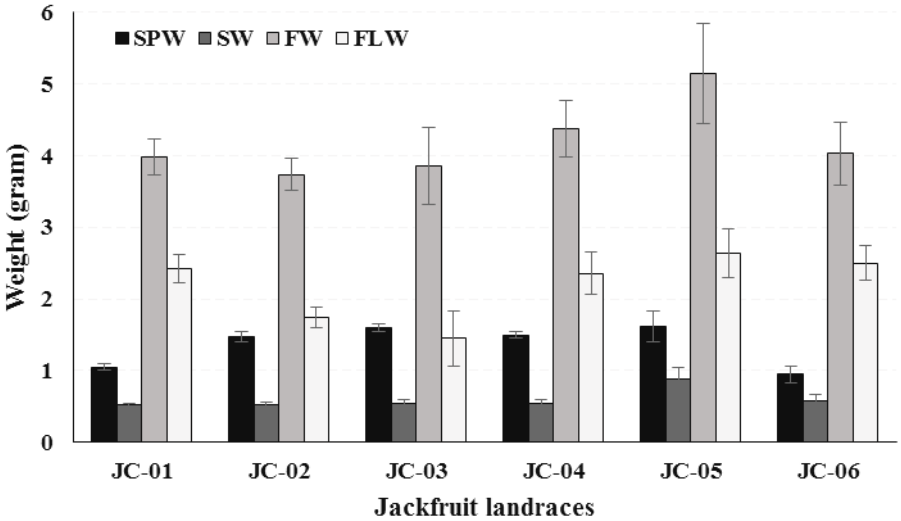

Figure (2): Averages of fruit peel (FPW), seed (SW), fruit (FW), and flesh (FLW) weight (g) of six jackfruit landraces evaluated over two seasons (2016 and 2017). LSD` for FPW=0.33, for SW=0.24, for FW=1.19 and for FLW=0.63, $p \leq 0.05$. *Bars* indicate standard errors (n=5).

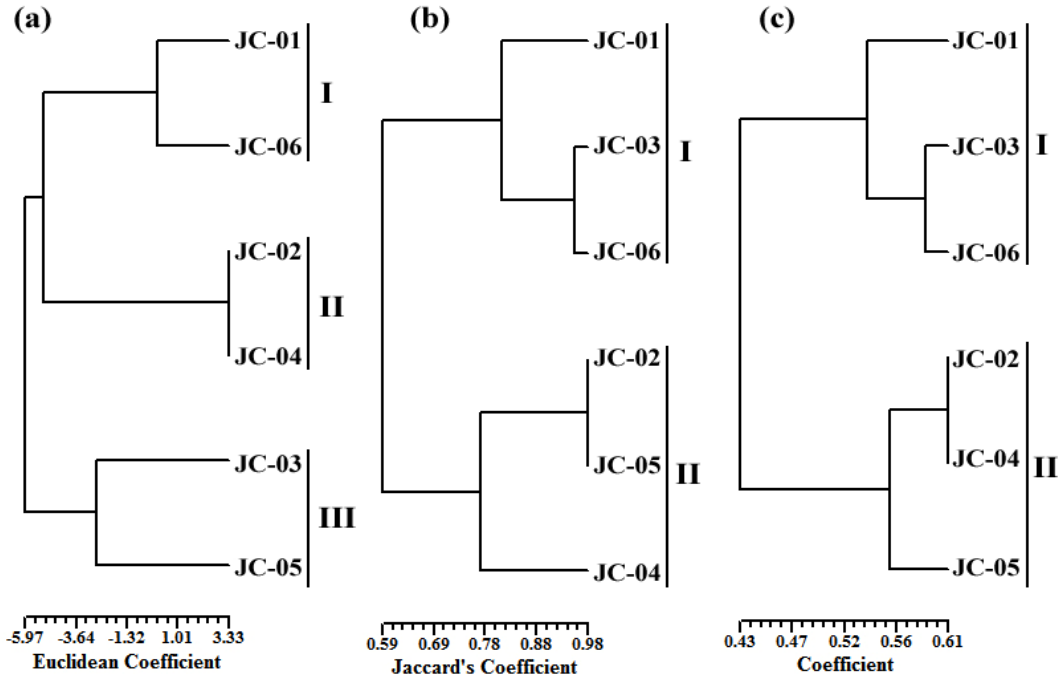

Figure (3): UPGMA-dendrograms revealing the dissimilarity (a) and similarities (b, c) among the six jackfruit landraces based on: a) phenotypic characteristics, b) ISSR and c) combination of phenotypic and molecular data.

Table 3. A survey of total number of bands, number of polymorphic bands, %Pm, PIC, Rp, DI and MI determined per primer used for the amplification of ISSR molecular markers among six jackfruit landraces.

Primer	TNB	NPB	%Pm	PIC	RP	DI	MI
UBC-807	8	4	50.00	0.040	2.33	0.96	0.16
UBC-808	12	6	50.00	0.075	6.00	2.08	0.45
UBC-811	6	2	33.33	0.022	1.33	0.54	0.04
UBC-812	9	5	55.56	0.057	3.67	1.81	0.28
UBC-815	7	5	71.43	0.056	4.00	1.56	0.28
UBC-846	10	5	50.00	0.054	3.67	1.53	0.27
All	52	27	51.92	0.051	3.50	1.41	0.25

TNB: total number of bands, NPB: number of polymorphic bands, %Pm: percentage of polymorphism, PIC: polymorphism information content, Rp: resolving power, DI: diversity index, MI: marker index.

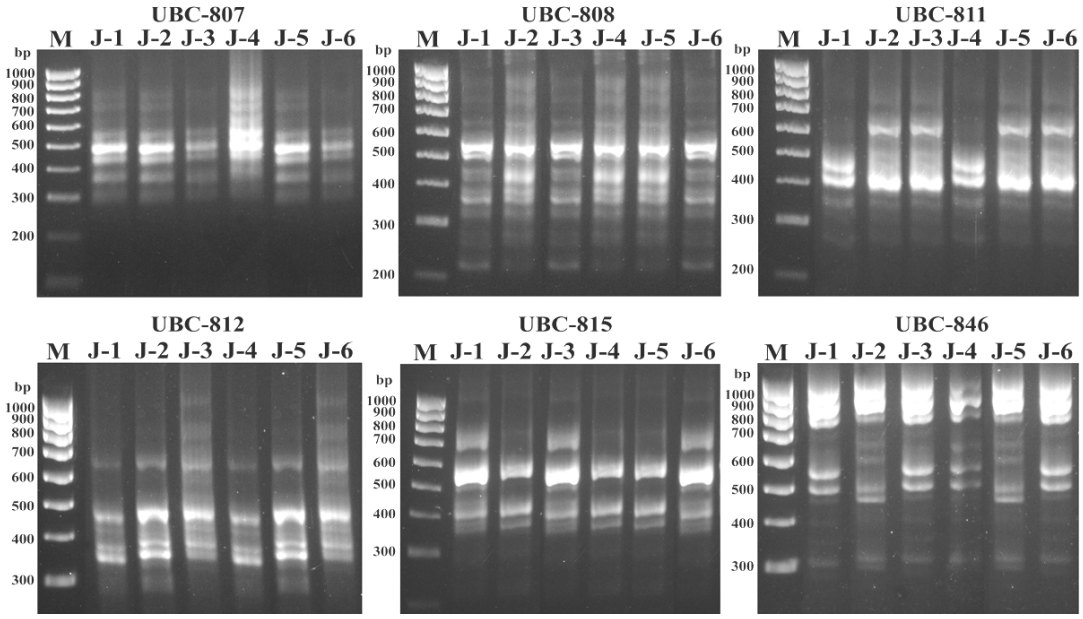

Figure (4): ISSR pattern of six primers exposed the molecular variations among the six jackfruit landraces.

Combining molecular and morphological markers in the assessment of genetic diversity would provide an excellent overview, unlike a single-approach evaluation (Youssef *et al.,* 2019). In the present study, the cluster analysis based on the combined phenotypic and molecular data provided an excellent outline of the genetic relationship among the jackfruit accessions, better than using one system of evaluation. In this regard, UPGMA-dendrogram based on the combined data of morphological and molecular analyses was able to discriminate the jackfruit landraces into two main clusters (I: JC-01, JC-03 and JC-06; II: JC-02, JC-04 and JC-05). Although this grouping is more similar to that based on ISSR data, some differences exist including grouping within the second main cluster and the level of similarity reflected by dendrogram scale (Fig. 5). In addition, likeness between dendrograms of molecular and combined data gave ISSR more power than morphological analysis in grouping the landraces under study.

Conclusion

Phenotypic and molecular analyses were used in this study to assess the genetic diversity among six jackfruit landraces. Several phenotypic traits showed high influence in drawing the relationships among the tested accessions and agreed with previous reports. These traits are recommended to be involved in successive studies dealing with genetic diversity in jackfruit. ISSR markers used in the present study underlined their efficiency in discriminating the accessions. Although both phenotypic and molecular analyses resulted in varying placement among the tested accessions displayed on dendrograms, the combined data-based cluster analysis revealed comparatively stronger influence of the molecular dataset. Information of this study is valuable for the future jackfruit improvement and breeding approaches.

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الاختلافات المورفولوجية والكيمائية والجزيئية بين بعض سلالات الجاك فروت

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الملخص

تم تقدير التنوع الوراثي والعلاقة الوراثية بين ست سلالات من الجاك فروت المنزرعة في حديقة النباتات بأسوان على مستوى الشكل الظاهري والكيميائي والجزيئي. شوهد مدى واسع من الاختلافات بين السلالات المختبرة منعكساً في صورة فروق معنوية جداً في كل الصفات التي تم دراستها. وبين تلك الصفات، أوضح كلاً من صفة طول الثمرة ووزن البذور ووزن غلاف الثمرة والفينولات الكلية والسكريات غير المختزلة والبروتين ومحتوى الكالسيوم والفسفور والبوتاسيوم فروق معنوية عن باقي الصفات، مما أثر في شكل العلاقة بين السلالات وطريقة ارتباطهم في التحليل العنقودي. ومن الموصى به أن تستخدم هذه الصفات في دراسات مستقبلية لدراسة التنوع في الجاك فروت. كما أظهر التحليل العنقودي بناء على الصفات المظهرية ارتباط السلالات الست بناءً على الصفات المشتركة بينهم والتي تميز كل مجموعة عن الأخرى، سواء كانت قيم عالية أو منخفضة في الصفات المورفولوجية والكيميائية. ويمكن الاستناد على هذا التحليل في برامج التحسين للجاك فروت. أما بالنسبة للتحليل الجزيئي، فقد تم استخدام الواسم الجزيئي ISSR، حيث تمكن من تأكيد درجة التنوع بين السلالات المتحصل عليها على المستوى الظاهري. حيث أظهر التحليل الجزيئي نسبة تعدد أشكال بلغت ٧١,٤٣% مما أدى تحديد علاقة واضحة بين السلالات على المستوى الجزيئي باستخدام التحليل العنقودي. بالإضافة لذلك، فإن ضم البيانات المورفولوجية والكيميائية مع البيانات الجزيئية لإجراء التحليل العنقودي المشترك أدى إلى توضيح العلاقة والفروق بين السلالات بشكل فعال. ولوحظ وجود تشابه بين نتيجة التحليل العنقودي بالبيانات المشتركة مع نتيجة التحليل بالبيانات الجزيئية بعكس البيانات المورفولوجية. ويمكن الاستفادة من النتائج المتحصل عليها في هذه الدراسة في برامج تربية الجاك فروت.