SJIF IMPACT FACTOR (2015): 5.79 CRDEEP Journals
International Journal of Life Sciences Heba S.A. Taha Vol. 5 No. 4 ISSN: 2277-193x
International Journal of Life Sciences Vol. 5. No. 4. 2016. Pp. 234-239

Full Length Research Paper

Evaluation of Start Codon Targeted Polymorphism among Seven Egyptian Mango Cultivars

Heba S.A. Taha
Genetics Department, Faculty of Agriculture, Zagazig University, Egypt.

Article history
Received: 15-12-2016
Revised: 18-12-2016
Accepted: 25-12-2016

Corresponding Author:
Heba S.A. Taha
Genetics Department, Faculty of Agriculture, Zagazig University, Egypt.

Abstract
The objective of this study was to obtain molecular genetic identification of some mango varieties in Egypt, in order to clarify the genetic distribution among different mango populations and examine the efficiency of start codon targeted primers (SCoT) to evaluate genetic diversity. Fresh young leaves were collected from seven Egyptian mango cultivars (Ewais, Senara, Sukary, Zebda, Alfons, Sedikka and Hindi). In this respect, six start codon targeted primers amplified polymorphic DNA fragments and scored specific marker bands with different molecular weights for each cultivar. Dendrogram and similarity index were performed showing that the highest differences were recorded between Alfons and all studied cultivars, followed by the diversity between Sukary and both Senara and Ewais. On the other hand, the highest similarity was noticed between Sedikka and Hindi cultivars, followed by Ewais and Senara then Ewais and both Hindi and Senara. The present study explained that start codon targeted markers were able to determine high levels of genetic diversity in plant tissues specifically mango cultivars in Egypt which originate at different geographical locations. This confirms the usefulness of SCoT marker system in genetic diversity studies and analysis of the population structure.

Key words: *Genetic diversity, genetic similarity, SCoT-PCR technique, mango.*

Introduction

Mangifera indica L. or Mango fruits are belonging to the family *Anacardiaceae*, the most commercial famous countries in mango production are India, China and Thailand, (Sivakumar *et al.,* 2011). Egypt and some African countries are not considered to belong to the approved geographical areas of mango production but are seeking to increase mango production to compete in export markets (Tharanathan *et al.,* 2006).

Mango is particularly popular in terms of nutritional properties and active vitality and its important nutrients such as different percentages of glucose, fructose and sucrose depending on the species and stage of maturity, It also contains many important acids such as Oxalic, lemons, malaysic, suxine, pyruvic, adipic, galacturonic and glucuronic (Poovarodom *et al.,* 2010). Mango plants are highly cross-pollinated; most breeding methods depend on natural selection of the desired traits depending on the morphology of the fruit seedlings (Karihaloo *et al.,* 2003). Determine the types of mango through morphological characteristics are considered imprecise and ineffective because the morphological characteristics are highly affected by the environment (He *et al.,* 2007a and Rahman *et al.,* 2007).

Souza *et al.,* (2011) mentioned that classification based on the visual characteristics of the plant is already a waste of time because it requires that the plant be grown to a suitable stage of development to determine its characteristics. So it is a prompt need for specific types of markers based on molecular characteristics. The use of molecular marker analysis introduced new insights for breeders that known as Molecular assisted selection (MAS) (Mansour *et al.,* 2008).

In 2009 (Collard and Mackill) innovate a new simple DNA targeted molecular marker technique termed (SCoT) start codon targeted, it targets sequences that flanking translation initiation codon ATG in genome. SCoT maker is 18-mer dominant primer as well as RAPD and ISSR, but it is preferable for its higher polymorphism, better resolvability and its close relation to the gene function (Luo *et al.,* 2014). SCoT molecular technique has been widely used to estimate genetic diversity and relationships in plant species as peanut (Xiong, *et al.,* 2011), tomato (Shahlaei *et al.,* 2014), date palm (Al-Qurainy *et al.,* 2015), ramie (Satya *et al.,* 2015) and Jojoba (Heikrujam *et al.,* 2015).The objective of this study was to obtain molecular genetic identification of some mango varieties in Egypt and to clarify the genetic distribution among different mango populations, as well as examination of efficiency of SCoT markers to evaluate suitable level of genetic diversity.

Materials and methods

This study was carried out at Department of genetics, Faculty of Agriculture, Zagazig University, Zagazig, Egypt. Seven mango cultivars in Egypt used in this study, their characteristics and origin are shown in Table 1. Fresh young leaves were collected from two different farms in Ismailia and Sharkia governorates, Egypt.

Genomic DNA extraction:

1 gm of fresh young leaves used to extract genomic DNA using DNA Extraction kit (EZ10/ Bio Basic Genomic DNA extraction kit, Canada), extracted DNA concentration was examined and electrophoresed at 1% agarose gel stained with Ethidium bromide.

SCoT-PCR analysis:

15 SCoT were used in this study, only nine primers were able to amplify DNA fragments. Six primers scored genetic polymorphism (Sc 2, Sc 4, Sc 7, Sc 11, Sc 12, Sc 15). The six primers and their sequences are listed in Table 2.

PCR reaction for each primer was performed in a 20 µl final volume Distributed as follows:

Master mix	1 µl
Primer	1 µl
DNA template	1 µl
Distilled water	Up to 20 µl

Table 1: Characteristics and origin of seven mango cultivars in Egypt.

Cultivar	Origin	Tree size	Fruit skin	Time of ripening
Ewais	Selected seedy clone in Egypt	big	yellow	September
Senara	India	medium	Greenish yellow	Late July to August
Sukary	Selected seedy clone in Egypt	big	yellow	July to August
Zebda	Selected seedy clone in Egypt	big	Dark green	Late September to October
Alfons	India	medium	Yellow with red spots	August to first October
Sedikka	Selected seedy clone in Egypt	Big to medium	Green with red spots	August
Hindi	Selected seedy clone in Egypt	medium	Green	Late August toSeptember

Table 2: Sequences, molecular weights and GC percentage of six start codon targeted primers (SCoT)

Primer	Sequences	MW	GC%
P1 (Sc 1)	5' CAA CAA TGG CTA CCA CCC- 3'	5397.6	55.56
P2 (Sc 2)	5' ACC ATG GCT ACC ACC GGC-3'	5429.6	66.67
P3 (SC 3)	5' CAA CAA TGG CTA CCA CGC-3'	5437.6	55.56
P4 (Sc 4)	5' CAA CAA TGG CTA CCA CCG-3'	5437.6	55.56
P5 (Sc 5)	5' ACG ACA TGG CGA CCA CGC-3'	5478.6	66.67
P6 (Sc 6)	5' CCA TGG CTA CCA CCG CAG-3'	5429.6	66.67

Amplifications conditions:

1) initial denaturation step at 94°C for 5 min, 2) 40 cycles consisting of 45 s of denaturation at 94°C, 3) 1 min at 50°C , 4)3 min of elongation at 72°C, 5) final elongation step at 72°C for 5 min. 1.5% agarose gel stained with ethidium bromide was used to separate amplification products and visualized under ultraviolet light in gel documentation system (bio rad) .

Statistical analysis:

PCR products were scored across the lanes as variables. The presence of a band of amplified DNA was scored as "1" and absence as "0". The obtained data were used to calculate similarity index and dendrogram by SPSS 14.0 evaluation version.

Results and discussion

This is the first Implementation of SCoT marker in Egyptian mango cultivars. It is important to confirm genetic relationships on the basis of recorded pedigrees and assess the utility of SCoT markers for the management of germplasm. This information will be useful in identifying and preserving mango varieties as well as the development of existing varieties. Six SCoT markers scored 36 bands in total, their sizes ranged from 415 bp to 2850 bp, the number of bands contributed from 3 (SCoT P1) to 8 (SCoT P6) with average 6 bands /primer. 36 bands distributed as 17 polymorphic bands (47.2%) that varied from 1(SCoT P1) to 4 with SCoT P6 with average of

2.8 polymorphic band/primer. 10 positive unique bands (27.8%) were also detected, ranged from 0 with SCoT P5 to 3 with SCoT P6 and SCoT P2 with average 1.7. Three unique negative bands were also observed, they were recorded as 2 absent bands with the (SCoT P2) and one other band with (SCoT P5). 9 monomorphic bands were distinguished ranged from 0(SCoT P2) to 3 (SCoT p3) it constitutes 25% with average 1.5 band/primer, (Fig. 1)

The highest polymorphic primer was (SCoT P2) with 100% percentage, while the lowest polymorphism was obtained with primer (SCoT P3) 57.14%. Primers succeeded in distinguishing specific unique positive and negative bands in most of studied samples in all studied cultivars except Senara. It is clear that SCoT markers were able to detect high polymorphism among mango cultivars in Egypt (Table 3).

SCoT specific markers analysis:
Comparing obtained data of 6 SCoT primers among seven mango cultivars, novel markers could be obtained to distinguish different mango cultivars. The SCoT specific marker produced by different primers is shown in (Table 4 and Fig 1). Ewais and Zebda were characterized by the presence of one positive unique band for each using the same primer (P6) with 534.5 bp and 765 bp respectively. Sukary was identified by the appearance of one positive unique band with 660 bp molecular weight using P4 primer and also by the absence of one band weighted 844.5 bp using P5 as negative unique band. Alfons was characterized by the presence of one positive unique band using primer P3 with molecular weight of 1572 bp. On the other hand, Sedikka was the most distinguishable cultivar; as four positive unique bands were appeared, three with P2 with molecular weights of 1222 bp, 650 bp and 416 bp. Sedikka also could be characterized by the positive unique band obtained with P6 having molecular weight of 2850 bp. It is worth noting that two negative unique bands were absent, they had molecular weights of 587 bp and 525 pb. Hindi was characterized by the presence of one positive unique band with primer P1 that has molecular weight of 1806.8 bp.

Unfortunately, the studied primers could not allocate a specific marker for the Senara cultivar. Table 5 demonstrates the specific genetic SCoT markers for the seven studied mango cultivars using SCoT-PCR analysis. Based on SCoT markers results the similarity index values were used to construct a dendrogram using SCoT- PCR data among the seven mango cultivars (Fig 2).

Similarity index and dendrogram:
Similarity index and dendrogram shows the diversity and relationship among the seven studied mango cultivars using strat codon targeted primers (Fig 2). The results showed that the largest diversity was recorded between Alfons and all the rest cultivars, followed by the diversity between Sukary and each of Senara, Ewais, Hindi and Sedikka. However the highest similarity was noticed between Sedikka and Hindi cultivars, followed by Ewais and Senara then Ewais and both Hindi and Senara. These results reflect the genetic material they share. Comparative similarity matrix data showed high genetic similarity between the seven studied mango cultivars ranged from 0.889 between Sedikka and Hindi followed by 0.833 between Ewais and Senara. While the lowest similarities were recorded between Alfons and most other varieties. The most notable being 0.444, 0.500 and 0. 583 with Sukary, Zebda and Sedikka, respectively. High genetic similarity between Sedikka, Hindi and between Ewais, Senara Indicate that plants may be genetically closely related. The hierarchical cluster analysis method was used to construct a dendrogram based on the presence and absence of scale bands. Cluster analysis showed significant genetic variation between studied mango cultivars.

Table 3: Number of total, monomorphic, polymorphic and unique bands obtained using six SCoT primers in seven mango cultivars in Egypt.

Primer	Total no. bands	Monomorphic bands	Polymorphic bands	Unique bands		%Polymorphism
				Positive Unique	Negative Unique	
SCoT P1	3	1	1	1	-	66.67%
SCoT P2	7	-	4	3	2	100%
SCoT P3	7	3	2	2	-	57.14%
SCoT P4	6	2	3	1	-	66.77%
SCoT P5	5	2	3	-	1	60.00%
SCoT P6	8	1	4	3	-	87.50%
Total	36	9	17	10		75.0%

Table 4: Molecular weights (bp) of specific unique SCoT positive and negative markers scored using six start codon targeted primers with seven Egyptian mango cultivars.

Primer	Ewais	Senara	Sukary	Zebda	Alfons	Sedikka	Hindi
SCoT P1							1806.8
SCoT P2						1222 650 416	

				587.8* 525*
SCoT P3			1572	
SCoT P4		660		
SCoT P5		844.8*		
SCoT P6	534.5		765	2850

(*)Negative specific bands

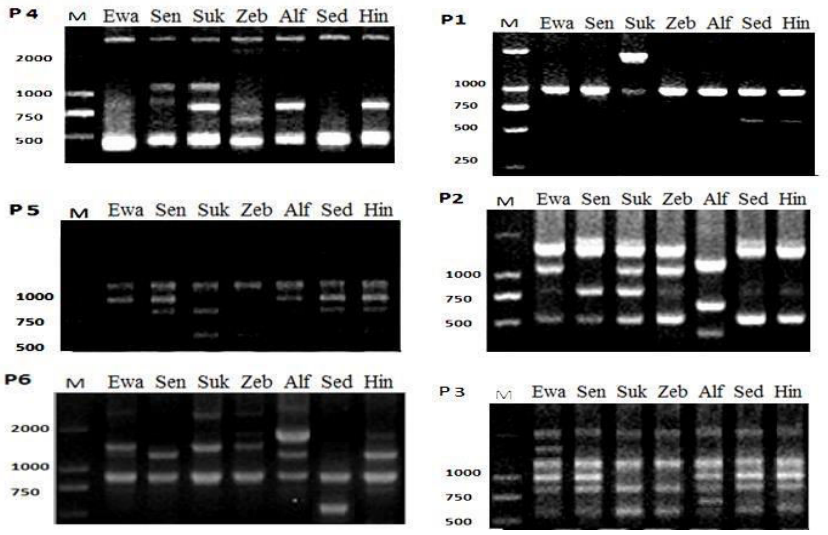

Fig 1: SCoT –PCR fragments produced by using six SCoT primers with seven Egyptian mango cultivars.

Table 5: Similarity index among the seven studied mango cultivars based on SCoT –PCR analysis

Cultivars	Ewais	Senara	Sukary	Zebda	Alfons	Sedikka
Ewais						
Senara	0.833					
Sukary	0.722	0.722				
Zebda	0.722	0.611	0.778			
Alfons	0.611	0.667	0.444	0.500		
Sedikka	0.806	0.806	0.694	0.694	0.583	
Hindi	0.750	0.806	0.694	0.639	0.639	0.889

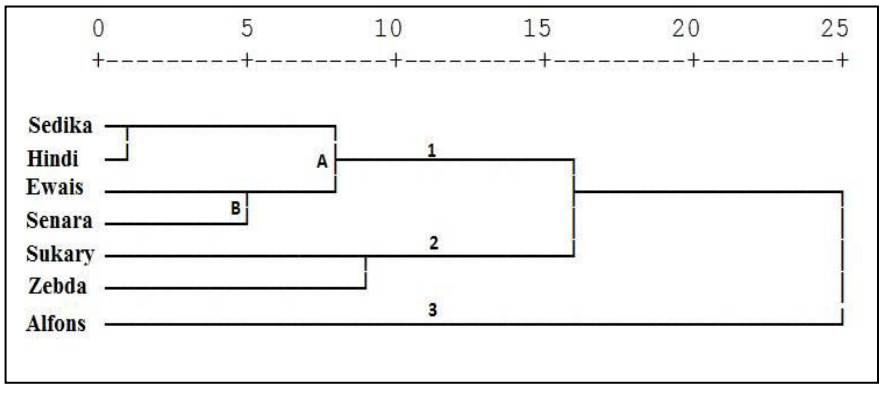

Fig 2: Dendrogram presentation based on SCoT –PCR data among the seven mango cultivars.

The clustering algorithm from SCoT marker analysis grouped the 7 mango cultivars into three main clusters (Fig. 2).The cluster 1, consisted of two sub-clusters (A, B); sub-cluster A have very related two cultivars (Sedikka and Hindi). While sub-cluster B, contained cultivars (Ewais and Senara), this result indicate that these cultivars are genetically related. Cultivars (Sukary and Zebda) represented the second major cluster 2. The third cluster 3 consists only of the cultivar (Alfons) indicating large diversity between Alfons and all other studied cultivars. The genetic diversity observed in the studied cultivars may be explained by the difference in the origin of the cultivars (Qiang *et al.,* 2008). Also Mangoes are cross- pollinated fruits, genetic diversity is expected to be high.

Traditional breeding programs can reduce genetic variation in mango cultivars, so there is a need to conserve and characterize mango genetic resources (Terzopoulos and Bebeli, 2008).

Genetic diversity is very important for maintaining plant species in their natural environment because they protect plants from various environmental stresses. The impact of these environmental stresses on the same or different plant species may vary depending on the morphological and physiological characteristics of the plant (Manners *et al.,* 2013). Molecular markers are a promising tool to differentiate genotypes in populations as well as the estimation of genetic diversity. It is therefore important to discover new markers that allow breeders to assess mango germplasm, especially in breeding programs. SCoT markers are used in many applications including genetic diversity studies and bulked segregation analysis, even in QTL mapping (Collard and Mackill, 2009). The high polymorphism obtained by SCoT markers in mangoes would be more effective in DNA fingerprints, analyzing population structure and effective management of genetic resources.

In this study, the estimated genetic polymorphism using SCoT markers in Egyptian mango cultivars was (75.0%). This result coincided with the results of (Luo *et al*., 2012) the study of the genetic diversity of mango varieties in China, which was estimated at 73.82%. Calculated genetic polymorphism using molecular SCoT marker technique with other plants such as potato was 36.14% (Gorji *et al*., 2011), Cicer 97.32% (Amirmoradi *et al.,* 2012) who showed that this marker is an effective tool in estimating genetic variations. SCoT markers could be used for tracing domesticated genotypes depending on the genetic polymorphism generated and reconstruction of breeding history (Satya *et al.*, 2015) as well as obtaining new specific clustering (Xiong *et al.*, 2011)

Conclusion

The present study explained that start codon targeted markers were able to determine high levels of genetic diversity in plant tissues specifically mango cultivars in Egypt which originate at different geographical locations. This confirms the usefulness of SCoT marker system in genetic diversity studies and analysis of the population structure.

References

Al-qurainy, F., Khan S., Nadeem M. And Tarroum M. (2015). SCoT marker for the assessment of genetic diversity in Saudi Arabian date palm cultivars. Pak. J. Bot., 47(2): 637-643, 2015.

Amirmoradi, B., Talebi, R. and Karami, E. (2012). Comparison of genetic variation and differentiation among annual Cicer species using start codon targeted (SCoT) polymorphism, DAMD-PCR, and ISSR markers. Plant Systematics and Evolution. 298(9), 1679-1689.

Collard, B, and Mackill, D. (2009). Start Codon Targeted (SCoT) polymorphism: a simple, novel DNA marker technique for generating gene-targeted markers in plants. Plant Mol. Biol. Rep., 27(1):86–93.

Gorji, A., Poczai, P., Polgar, Z. and Taller, J. (2011). Efficiency of Arbitrarily Amplified Dominant Markers (SCOT, ISSR and RAPD) for Diagnostic Fingerprinting in Tetraploid Potato. Am. J. Pot Res, 88(3), 226-237.

He, X., Guo, Y., Li, Y. and Ou J. (2007a). Assessment of the genetic relationship and diversity of mango and its relatives by cp lSSR marker. Agricultural Sciences in China, 6(2):137-142.

Heikrujam, M., Kumar, J. and Agrawal V. (2015) Genetic diversity analysis among male and female Jojoba genotypes employing gene targeted molecular markers, start codon targeted (SCoT) polymorphism and CAAT box-derived polymorphism (CBDP) markers. Meta Gene, 16(5):90-97.

Karihaloo, J., Dwivedi, Y., Archak, S. and Gaikwad, A. (2003). Analysis of genetic diversity of Indian mango cultivars using RAPD markers. J Hortic Sci Biotechnol 78(3):285– 289.

Luo, C., Hua, H., Hu, C., Hu, Y. and Shi-Jin, O. (2012). Genetic relationship and diversity of Mangifera indica L.: revealed through SCoT analysis, Genet Resour Crop Evol: 59(4):1505–1515.

Luo, C., He X., Y. Hu, H., Yu, O. and Fang Z. (2014). Oligo-dT anchored cDNA–SCoT: a novel differential display method for analyzing differential gene expression in response to several stress treatments in mango (*Mangifera indica L.)* Gene, 548(2): 182-189.

Manners, V., Kumaria, S. and Tandon, P. (2013). SPAR methods revealed high genetic diversity within populations and high gene flow of Vanda coerulea Griff ex Lindl (*Blue Vanda*), an endangered orchid species. Gene, 519(1): 91–97.

Mansour A, Ismail, O., Solliman, M. and Mohei EL-Din, M. (2008). Diversity Assessment among Mango (*Mangifera indica L.*) Cultivars in Egypt using ISSR and Three-Primer Based RAPD Fingerprints. The African Journal of Plant Science and Biotechnology 2 (2): 87-92

Poovarodom, S., Haruenkit, R., Vearasilp, S., Namiesnik, J., Cvikrova, M., Martinincova,´ O., Ezra, A., Suhaj, M., Ruamsuke, P. and Gorinstein, S. (2010) Comparative characterization of durian, mango and avocado. Food Sci Technol 45(5):921–929.

Qiang, l., Qing-chang, L., Hong, Z., Dai-fu, M., Xin, W., Xue-qin, l. and Yu-ping, W. (2008). Genetic diversity in main parents of sweetpotato in china as revealed by ISSR markers. Acta agronomica sinica, 34(6): 972-977.

Rahman, M., Rabbani, M., Siddique, M., Rahman, M., Garvey, E.J., Rahaman, E. (2007). Molecular characterization of 28 mango germplasm using RAPD. Plant Tissue Cult. Biotech., 17(1): 71–77.

Satya, P., Karan, M., Sourav, J., Sabyasachi, A., Karmakar, P. and Ray, D. (2015). Start codon targeted (SCoT) polymorphism reveals genetic diversity in wild and domesticated populations of ramie (*Boehmeria nivea L. Gaudich*.), a premium textile fiber producing species. Meta Gene, 20 (3): 62–70.

Shahlaei, A., Torab,i S. and Khosroshahli, M. (2014). Efficacy of SCoT and ISSR markers in assessment of tomato (*Lycopersicum esculentum Mill*.) genetic diversity. International journal of biological sciences, 5(2):14-22.
Sivakumar, D., Jiang, Y., Yahia, E.M., (2011). Maintaining mango (*Mangifera indica L.*) fruit quality during the export chain. Food Res. Int. 44(5): 1254–1263.
Souza, I. G. B., Valente, S. E. S., Britto, F. B., de souza, V. B., and Lima, P. C. (2011). RAPD analysis of genetic diversity of mango (*Mangifera indica*) germplasm in Brazil. Genetics and Molecular Research, vol. 10(4): 3080-3089.
Terzopoulos PJ, Bebeli PJ. (2008). DNA and morphological diversity of selected Greek tomato (*Solanum lycopersicum L.*) landraces. Scientia Horticulturae. 116, 354–361.
Tharanathan, R.N., Yashoda, H.M., Prabha, T.N., (2006). Mango *(Mangifera indica L.*), ‘‘The King of Fruits”: an overview. Food Rev. Int, 22(2):95–123.
Xiong, F., Zhong, R., Han, Z., Jiang, J., He, L., Zhuang, W. and Tang, R. (2011). Start codon targeted polymorphism for evaluation of functional genetic variation and relationships in cultivated peanut (*Arachis hypogaea L.*) genotypes. Mol. Biol. Rep., 38(5): 3487–3494.