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Assessment of Genetic Diversity and Polymorphism of Pyruvate Kinase in some Commercial Tomato Cultivars

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ABSTRACT

Tomato or (*Solanum lycopersicum*) is the most prevalent vegetable all over the world; it is an important source of minerals, vitamins and amino acids. This study was designed to estimate genetic diversity in LOC101 gene that is coding for pyruvate kinase enzyme in 14 commercial tomato cultivars. Pyruvate kinase is serious enzyme of glycolytic pathway as it stimulates the final step of glycolysis. Genetic diversity was estimated using two marker systems; first pyruvate kinase start codon surrounding regions using characterized marker (SCoT5). Second; specific molecular marker for LOC101 gene. The results showed that the genetic diversity related to Pyruvate kinase start codon using SCoT5 primer was 75%. The 14 tomato cultivars were divided into 5 clusters in relation with genetic diversity. Similarity matrix and dendrogram revealed that the highest diversity was between cultivars Advantage 2 with cultivars; Floradade and Vt 916 hybrid followed by the diversity between (Floradade and Super queen, Peto 86 and Super strain b) and Super queen with Advantage 2, Uc $_{97\text{-}3}$, Vt 916 hybrid, Edkawy and Sahrawy 1 hybrid. also high diversity was recorded between (Vt 916 hybrid and Peto 86) and each of Advantage 2, Edkawy, Sahrawy 1 hybrid and Uc $_{97\text{-}3}$ with Super marmand, Rio pilcomojo, Safa 2033 and Radwa f1. The Amplification of LOC101 gene showed the existence of one common fragment of nearly 725 bp in the entire examined cultivars. These results present good sources of genetic diversity that can help breeders to estimate genetic diversity and relationships in substantial biological processes such as glycolysis.

Keywords: *Solanum lycopersicum*, Pyruvate kinase, gene polymorphism, Scot markers.

INTRODUCTION

Tomato or (*Solanum lycopersicum*) is the most prevalent vegetable all over the world. Actually tomatoes are fruits, belong to family Solanaceae. Tomatoes are favorable for their health benefits; it is an important source of minerals, vitamins and amino acids as glutamic, ascorbic and Aspartic acids. Tomato contains bioavailable iron due to the presence of ascorbic acid that keeps iron in reduced form (Nasir *et al*., 2015). Also tomatoes are rich in potassium, magnesium and phosphorus. Tomatoes provide many important vitamins such as A, B and C. Tomatoes contain a high level of lycopene that important for healthy skin. They can prevent many kinds of cancer; as well as they present plentiful amount of calcium and Vitamin K that is essential for strong bone. Tomatoes provide us with antioxidants and Fytosterols that reduce both of cholesterol levels and blood pressure (khan *et al*., 2017). Utilizing tomatoes in daily diet reduce the risk of kidney stones. They also, improve vision and prevent night blindness development. They are so helpful for diabetics because of chromium mineral that preserve low level of blood sugar (Freeman and Reimers 2010).

Appreciation of genetic diversity is a need for improving genetic markers, which assists in the genetic refinement of different plant species (khan *et al*., 2017).The estimation of genetic diversity at DNA level was an important tool in improving molecular markers to help in genetic improvement in species breeding Programs; moreover the evaluated strength of gene pool (Maria *et al*. 2011).

Start Codon Targeted (SCoT) polymorphism is an unprecedented functional marker procedure targeting functional genes (Collard and Mackill, 2009). SCoT primers were developed to detect short conserved region that surrounded the ATG start codon in plant genes (Xiong *et al*., 2011).

SCoT procedure is a kind of specific molecular marker that targets ATG region as a part of a functional gene, markers created from SCoT technique are often associated to functional genes and their corresponding traits (Collard and Mackill, 2009, Xiong *et al*., 2011 and khan *et al*., 2017).

Luo *et al*., (2012) discussed many SCoT markers that associated with functional genes such as SCoT5 (CAACAATGGCTACCACGA) related to pyruvate kinase protein sequence, SCoT26 (ACCATGGCTACCACCGTC) associated with Glycolate oxidase and Harpin like protein and SCoT20 (ACCATGGCTACCACCGCG) that targeted the Heat shock protein. It is worth mentioning that this marker use single primer as the forward and reverse primer (Collard and Mackill 2009 and Etminan *et al*., 2016).

Pyruvate kinase is serious enzyme of glycolytic pathway as it stimulates the final step of glycolysis.

It stimulate the transfer of a phosphate group from phosphoenolpyruvate (PEP) to adenosine diphosphate (ADP), producing one molecule of pyruvate and one molecule of ATP. Also it is responsible for the saving of carbon skeleton for fatty acid biosynthesis. Pyruvate kinases in plants contain two sections: cytosolic and plastidic (Ambasht and Kayastha 2002).

SCoT Primers were destined from series sequences Inferred from the studies by Sawant *et al*. (1999), Primer was designed as follows, the ATG codon take place in (1, 2, and 3), while in (4,7,8 and 9); Located G, A, C and C respectively as fixed form (Table 1).

Table 1. Designing of SCoT primer and primer SCoT5 sequence.

series sequences	-5	-4	-3	-2	-1	1	2	3	4	5	6	7	8	9	10	11	12	13
1	C	A/C	A/G	C/A	C	A	T	G	G	C	G	A	C	C	A/G	C	C	G
2*	A/C	A	A	C	A	A	T	G	G	C	T	A	C	C	T/A	C	A	T
SCoT5**	C	A	A	C	A	A	T	G	G	C	T	A	C	C	A	C	G	A

*** Example of series sequences flanking the ATG start codon in highly expressed genes explained (Sawant *et al*. 1999).**
**** SCoT5 primer sequence that related to pyruvate kinase used in this study.**

This study was designed to estimate genetic diversity in pyruvate kinase coding gene (LOC101) as a functional diversity among different commercial tomato cultivars using sequence characterized marker (SCoT5) that related to pyruvate kinase start codon surrounding region and to examine these genotypes for existence and diversity of LOC101 gene.

MATERIALS AND METHODS

Materials:

Seeds of 14 tomato cultivars (Table 2) were planted in cell seeding pots (5 in 5 lines replicates in 5 lines for each cultivar). Randomly, fresh green leaves were collected and squashed in liquid nitrogen to extract bulk DNA. Studied cultivars and their sources are listed in Table (2).

Methods:

The genomic DNA was extracted from the studied samples using (DNA easy plant Biovision Kit) according to kit instructions. Extracted DNA samples were stored at -20^{o}C until PCR analysis.

Specific-SCoT-PCR analysis:

SCoT primers use single primer as the forward and reverse primer (Collard and Mackill 2009).The primer used in this study is specially-related to pyruvate kinase gene sequence (Luo *et al*., 2014 and Xiong *et al*., 2011). The primer's sequence and design is shown in Table (1).

PCR reaction components was: 1 µl of each master mix, DNA template and the primer then up to 20µl with distilled water as final volume. PCR reaction conditions were performed as follows: initial denaturation at 94 °C for 4 min followed by 35 cycles of denaturation step at 94 °C (1 min), Annealing at 50 -55°C (for 1 min) and Elongation step at 72°C (1 min) then Final elongation was done at 72 °C for 5 min. All reactions were repeated to verify the results validity.

PCR products were electrophoresed at agarose (1.5%)

C. Amplification of LOC101 gene:

The LOC101 gene of the studied tomato cultivars was amplified using specific primers: forward PCR primer 5'- GCCAAGCGCATTCAACTAAT-3' and reverse PCR primer 5'- TGACCCCTTCTTGGTTTTCA-3 (GenBank, www.ncbi.nlm.nih.gov/ gene/). The PCR conditions were done as follows: 94° C for 10 min (denaturation), followed by 35 cycles of denaturation step for 30 s at 94° C, 30 s at 60° C, and 1 min at 72° C and the final extension at 72° C for 5 min. PCR amplification products were separated into two samples; first sample was examined utilizing agarose gel (1.5%), stained with ethidium bromide and visualized under UV light. To insure molecular weights validity of amplified fragments; Second sample was separated using polyacrylamide gel electrophoresis (10%). Obtained bands were visualized by means of ethidium bromide staining and subsequent UV light visualization.

Statistical analysis:

Obtained bands were registered as 1 for existence and 0 for obscurity. Similarity matrix and dendrogram was done using Average Linkage (Between Groups) using SPSS 14.0 evaluation version.

Table 2. Source and origin of 14 tomato cultivars used in this study.

	Cultivar name	Source & Origin
1	Floradade	USA (commercial)
2	Super queen	USA (commercial)
3	Peto 86	USA (commercial)
4	Advantage 2	Egypt (Sam trade Co.)
5	Red star	USA (commercial)
6	Uc 97-3	USA (commercial)
7	Vt 916 hybrid	Egyapt (techno green Co.)
8	Super strain b	USA (commercial)
9	Edkawy	Egypt (Agricultural Research Center)
10	Sahrawy 1 hybrid	Egypt (Agricultural Research Center)
11	Super marmand	Holland (commercial)
12	Rio pilcomojo	Mexico (commercial)
13	Safa 2033	Egypt (International Co. For Agricultural & Animal Production Supplies)
14	Radwa f1	Egypt (Ismailia seeds Co.)

RESULTS AND DISCUSSION

Specific-SCoT analysis:

Using targeted-SCoT marker to compare the diversity among 14 tomato cultivar at the level of pyruvate kinase start codon sequences, was resulted in 12 bands in total, mean of band frequency was (0.476). Bands were distributed as; firstly: 3 monomorphic (common) bands with molecular weights of 753.55 bp, 650.29 bp and 326.47 bp, secondly: 9 polymorphic bands including 3 unique bands (1361.42, 984.03 and 711.262) with cultivars (Floradade, Super queen and Advantage 2). The total average of polymorphism was appreciated as (75.0%) Fig (1) and Tables (3&4).

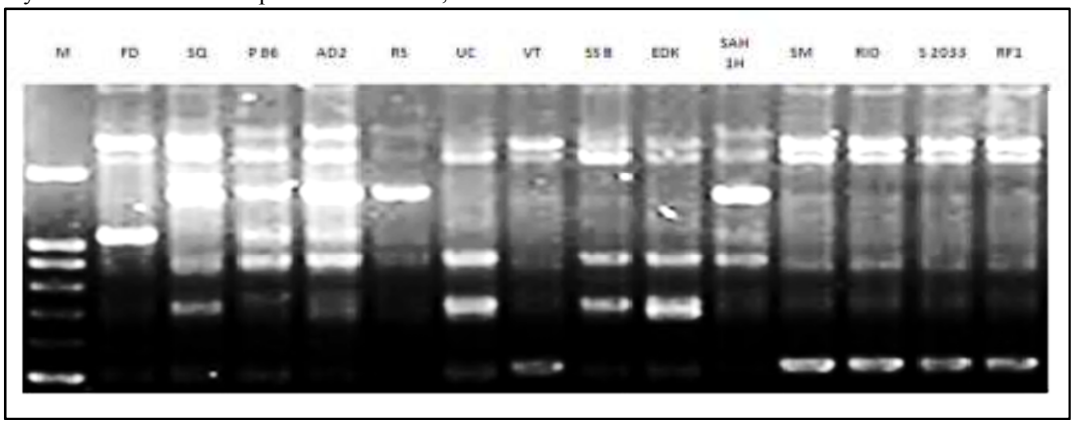

Figure 1. SCoT marker gel profile was obtained using the SCoT5 primer.

Table 3. No. Monomorphic, Polymorphic bands and (%) polymorphism obtained using SCoT5 primer with 14 tomato cultivars.

Monomorphic bands	3
Polymorphic (without Unique)	6
Unique bands	3
Polymorphic (with Unique)	9
Total number of bands	12
Polymorphism (%)	75.000%
Mean of band frequency	0.476

Excluding the monomorphic and unique bands; the 1154.0 bp band was common in eight cultivars (Super marmand, Rio pilcomojo, Safa 2033, Radwa f1, Super strain b, Super queen, Peto 86, Advantage 2). While seven cultivars (Floradade, Super queen, Vt 916 hybrid, Super marmand, Rio pilcomojo, Safa 2033, Radwa f1) were resembled by the presence of band weighted (1262.19 bp). The band (579.35 bp) was existed in each of the following six cultivars; (Peto 86, Advantage 2, Uc 97-3, Super strain b, Edkawy and Sahrawy 1 hybrid).

For the band (896.10) it was found in five cultivars (Super queen, Peto 86, Advantage 2, Red star and Sahrawy 1 hybrid). On the other hand, the band (282.86 bp) was distinguished in five cultivars (Super marmand, Rio pilcomojo, Safa 2033, Radwa f1 and Vt 916 hybrid). Finally; the four cultivars (Super queen, Uc 97-3, Super strain b and Edkawy) were Participants in the presence of band weighted (421.26 bp).

Table 4. Molecular weights of obtained bands using SCoT5 primer with 14 tomato cultivars.

MW bp cultivar	1361.42	1262.19	1154.0	984.03	896.10	753.55	711.26	650.29	579.35	421.26	326.47	282.86
Floradade	-	+	-	-	-	+	+*	+	-	-	+	-
Super queen	-	+	+	+*	+	+	-	+	-	+	+	-
Peto 86	-	-	+	-	+	+	-	+	+	-	+	-
Advantage 2	+*	-	+	-	+	+	-	+	+	-	+	-
Red star	-	-	-	-	+	+	-	+	-	-	+	-
Uc 97-3	-	-	-	-	-	+	-	+	+	+	+	-
Vt 916 hybrid	-	+	-	-	-	+	-	+	-	-	+	+
Super strain b	-	-	+	-	-	+	-	+	+	+	+	-
Edkawy	-	-	-	-	-	+	-	+	+	+	+	-
Sahrawy1 hybrid	-	-	-	-	+	+	-	+	+	-	+	-
Super marmand	-	+	+	-	-	+	-	+	-	-	+	+
Rio pilcomojo	-	+	+	-	-	+	-	+	-	-	+	+
Safa 2033	-	+	+	-	-	+	-	+	-	-	+	+
Radwa f1	-	+	+	-	-	+	-	+	-	-	+	+
polymorphism	Uni	Poly	Poly	Uni	Poly	Mono	Uni	Mono	Poly	Poly	Mono	Poly

Shaded cells refer to monomorphic (common) bands, * unique positive bands.

Similarity matrix and dendrogram:

It could be concluded from (Table 5 and Fig 2) that the gene of pyruvate kinase (PK) was most diverse in cultivar Advantage 2 which showed the lowest similarity with value of (0.500) with cultivars Floradade and Vt 916 hybrid followed by the similarity (0.583) between (Floradade and Super queen, Peto 86 and Super strain b) and Super queen with Advantage 2, Uc $_{97-3}$, Vt 916 hybrid, Edkawy and Sahrawy 1 hybrid. The same value (0.583) was recorded between (Vt 916 hybrid and Peto 86) and between each of Advantage 2, Edkawy, Sahrawy 1 hybrid and Uc $_{97-3\ and}$ with Super marmand, Rio pilcomojo, Safa 2033 and Radwa f1 these results indicating different genetic origins.

Table 5. Similarity matrix among 14 tomato cultivars using SCoT5 primer.

	Floradade	Super queen	Peto 86	Advantage 2	Red star	Uc $_{97-3}$	Vt 916 hybrid	Super strain b	Edkawy	Sahrawy 1 hybrid	Super marmand	Rio pilcomojo	Safa 2033	Radwa f1
Floradade	1.000	0.583	0.583	0.500	0.750	0.667	0.833	0.583	0.667	0.667	0.750	0.750	0.750	0.750
Super queen		1.000	0.667	0.583	0.667	0.583	0.583	0.667	0.583	0.583	0.667	0.667	0.667	0.667
Peto 86			1.000	0.917	0.833	0.750	0.583	0.833	0.750	0.917	0.667	0.667	0.667	0.667
Advantage 2				1.000	0.750	0.667	0.500	0.750	0.667	0.833	0.583	0.583	0.583	0.583
Red star					1.000	0.750	0.750	0.667	0.750	0.917	0.667	0.667	0.667	0.667
Uc 97-3						1.000	0.667	0.917	1.000	0.833	0.583	0.583	0.583	0.583
Vt 916 hybrid							1.000	0.583	0.667	0.667	0.917	0.917	0.917	0.917
Super strain b								1.000	0.917	0.750	0.667	0.667	0.667	0.667
Edkawy									1.000	0.833	0.583	0.583	0.583	0.583
Sahrawy 1 hybrid										1.000	0.583	0.583	0.583	0.583
Super marmand											1.000	1.000	1.000	1.000
Rio pilcomojo												1.000	1.000	1.000
Safa 2033													1.000	1.000
Radwa f1														1.000

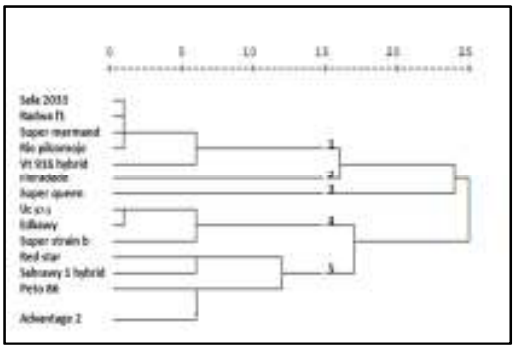

Figure 2. Dendrogram showing relationships of 14 tomato genotypes as scored by SCoT5 marker.

On the other hand, the most related cultivars were Radwa f1 with Safa 2033, Rio pilcomojo and Super marmand, and Safa 2033 with both Rio pilcomojo and Super marmand with full similarity (1.00) in relation with pyruvate kinase polymorphism, followed by(0.917) between Advantage 2, Peto 86 and Sahrawy 1 hybrid, and the cultivar Vt 916 hybrid with Super marmand, Rio pilcomojo, Safa 2033 and Radwa f1. The same value (0.917) also obtained between Uc $_{97\text{-}3}$ and Super strain b.

Data obtained from the dendrogram, showed that the diversity in studied PK gene distributed the 14 tomato cultivars in to 5 clusters. First cluster (1) include cultivars (Radwa f1, Safa 2033, Rio pilcomojo, Vt 916 hybrid and Super marmand), second and third clusters include only one cultivar for each, Floradade and Super queen respectively. Fourth cluster include 3 cultivars (Uc $_{97\text{-}3}$, Edkawy and Super strain b), while the fifth cluster include four cultivars (Red star, Sahrawy 1 hybrid, Peto 86 and Advantage 2).

Amplification of LOC101 gene

LOC101 is the pyruvate kinase gene located in chromosome (4) and has 5 exon regions (GenBank, www.ncbi.nlm.nih.gov/gene/). It is serious enzyme of glycolytic pathway as it stimulates the last step of glycolysis.

It stimulate the transfer of a phosphate group from phosphoenolpyruvate (PEP) to adenosine diphosphate (ADP) , producing one molecule of pyruvate and one molecule of ATP. Also it is responsible for the saving of carbon skeleton for fatty acid biosynthesis. Pyruvate kinases in plants contain two sections: cytosolic and plastidic enzyme (Ambasht and Kayastha 2002).

Agarose gel electrophoresis of PCR products showed the existence of one common fragment of nearly 725 bp in the entire examined tomato cultivars Fig (3).

Furthermore, polyacrylamide gel electrophoresis of PCR products revealed that the obtained bands; their weight ranged from 698 bp to 720 bp in studied cultivars Fig (4).

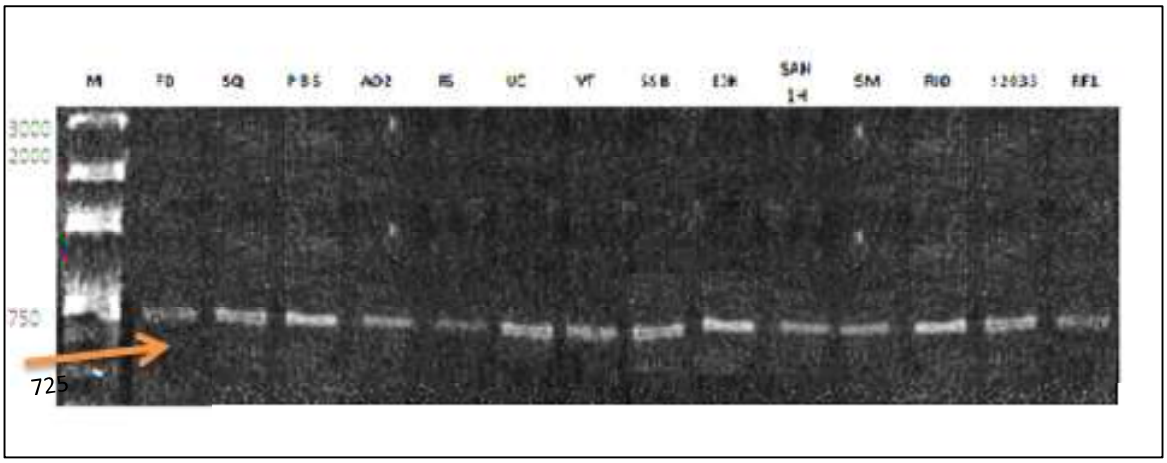

Figure 3. PCR Amplification of LOC101 gene in 14 tomato cultivars using agarose gel.

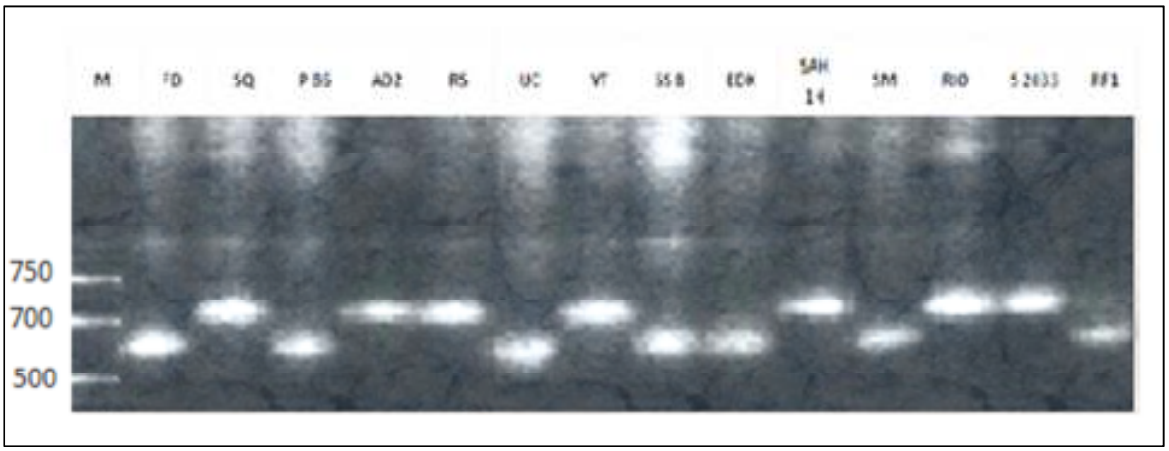

Figure 4. PCR Amplification of LOC101 gene in 14 tomato cultivars using polyacrylamide gel.

Further studies are required to estimate the polymorphisms of this gene at the level of nucleotide sequences in different populations and to evaluate the expression level of this gene under different environmental stress in tomato genotypes.

SCoT markers have known as dominant and reproducible markers, they have been efficiently utilized in genetic relationship analysis and species fingerprinting (Xiong *et al*., 2011 and Aliki *et al*., 2015). Also it has been used in numerous investigations to detect the percentage of polymorphism in different cultivars as date palm 52% (Al-qurainy *et al*., 2015) and Wheat 59% (Abdel-Lateif and Hewedy, 2018).

Recently, the great improvement of DNA sequencing facilities resulted in an abundant rise of DNA sequence data, leading to major expansion of functional markers existed in or near important genes (Andersen and Lubberstedt, 2003). Start codon (ATG) and surrounding sequences are highly conserved in the plant genes. SCoT marker is based on the conserved regions which is surrounded the start codon ATG (Collard and Mackill 2009, Xiong *et al*., 2011 and Aswathy *et al*,. 2016). SCoT methodology is a kind of targeted molecular marker that attacks the region containing the start codon ATG, as a fraction of functional genes (khan *et al*., 2017).

SCoT marker was deduced from the transcribed regions that related to the candidate genes and their available trait (Wu *et al*., 2013, Luo *et al*., 2014 and Aswathy *et al*,. 2016). SCoT utilizes single primer as the forward and reverse primer that targeting regions scattered in the genome, it is likely to a single primer to anneal at same reaction at two sites of both DNA strands in a directed orientation; so it amplifies only the functional genes (Collard and Mackill 2009 and Kochert *et al*., 2012).

Utilizing gene targeted markers is more beneficial than random pattern markers (RAPD and ISSR) as they estimate genetic diversity related to coding genes and consequently detect functional diversity exist in the studied samples (Seyedimoradi *et al*., 2015).

SCoT marker is considered a sequence characterized marker or specific marker, because these markers have weakly prospect to anneal between marker loci and objective loci; wherefore they could be used for trait-association analysis and marker-assisted selection (Aswathy *et al*., 2016)

CONCLUSION

SCoT markers are functional markers existed in or near important genes, they estimated the genetic diversity in tomato cultivars in relation with the coding region (LOC101 gene) for pyruvate kinase enzyme. LOC101 gene was existed in all studied cultivars but with different molecular weights. The dendrogram and the similarity matrix explained the genetic relationship among studied cultivars in accordance with their genetic origin or source. The conclusion of this study will be beneficial for the management of tomato germplasm to improve the current breeding protocols, and conservation of the genetic resources of distinct cultivars.

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تقييم التنوع الوراثي و التباين الجيني لجين البيروفات كيناز في بعض الأصناف التجارية للطماطم

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الطماطم هي أكثر الخضروات انتشارا في جميع أنحاء العالم. وهى مصدر مهم للمعادن والفيتامينات والأحماض الأمينية. صُممت هذه الدراسة لتقدير التنوع الجيني في الجين LOC101 الذي يشفر لإنزيم بيروفات كيناز في 14 صنفا من الطماطم التجارية. إنزيم بيروفات كيناز هو إنزيم أساسى فى دورة التحلل الجليكولى حيث انه يحفز الخطوة النهائية . تم تقدير التنوع الجيني باستخدام نظامين من البادئات الجزيئية؛ الأول : على مستوى المناطق المحيطة بمنطقة start codon باستخدام البادئ المتخصص (SCoT5). ثانيا؛ بادئ متخصص محدد لجين LOC101. أوضحت النتائج أن التنوع الوراثي المرتبط بكود البداية لجين الـ Pyruvate kinase الذي تم حسابه باستخدام SCoT5 كان 75٪. تم تقسيم 14 صنفا من الطماطم فى الدراسة إلى 5 مجموعات متعلقة بالتنوع الجيني. أوضح تحليل similarity matrix و dendrogram أن أعلى تنوع كان بين الأصناف Advantage2 مع الأصناف Floradade و hybrid Vt_{916} متبوعان بالتنوع بين (Floradade و Super queen، Peto $_{86}$ و(super strain b Super queen مع Advantage2 ، و Uc_{97-3} ، و hybrid Vt_{916} ، و Edkawy و Sahrawy 1 hybrid. كما تم تسجيل تنوع كبير بين (hybrid Vt_{916} و Peto 86) و كل من Advantage 2 و Edkawy و Sahrawy 1 hybrid و Uc 97-3 مع Super marmand و Rio pilcomojo و Safa 2033 و Radwa f1. أظهر تحليل جين LOC101 وجود حزمه واحدة مشتركة بين جميع الأصناف المدروسة حجمها 725 زوج قواعد تقريبًا. تقدم هذه الدراسة مصدراً جيداً للتنوع الجيني والذى يمكن أن يساعد المربين على تقدير التنوع الجيني والعلاقات الوراثية في العمليات البيولوجية الهامة مثل دورة التحلل الجليكولى.