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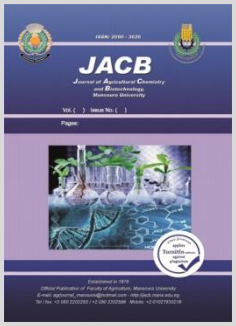
Using DNA Barcoding for Fingerprinting of Two Important Forage Crops Varieties (Alfalfa And Egyptian Clover)

Bondok, A. T.*

Department of Forage Crops Research. Field Crops Research Institute. Giza. Egypt



ABSTRACT

Study was executed to differentiate and discriminate **(***Medicago sativa* and *Trifolium alexandrinum***)** using DNA barcoding genes [rbcl] and Cox1 genes. Identification of (*Medicago sativa* (Rammah 1) was completed via rbcl and Cox1 genes and was identified as *Medicago sativa* voucher G00199095 ribulose1,5 bisphosphate carboxylase / oxygenase large subunit gene, partial cds; chloroplast Sequence ID: KJ204375.1 or *Medicago sativa* voucher Ahrendsen 23 for rbcl and Cox1 genes. Identity estimation were listed with 90% as alfalfa, Rammah 1 Genotype ribulose1 /5 bisphosphate carboxylase / oxygenase large subunit gene sequences ID: KJ206375.1] also, identity values of 91.24% were recorded with for alfalfa Rammah 1 Genotype, cytochrome c oxidase subunit I gene cox 1 sequences ID: KJ 204375.1). *Trifolium alexandrinum* Helaly genotype was identified as *Trifolium alexandrinum* (Sequence ID: HM850407.1) and *Trifolium alexandrinum* voucher K-016Hv (Sequence ID: KU234213.1) by rbcl and Cox1 genes respectively. Affiliation of genetic source was revealed for *Trifolium alexandrinum* with 100 % match with origin which indicate rising possibility for applying discrimination through comparing with *Medicago sativa* which reflect the lowest genetic likeness with the source. Moreover, we might detect from the available data that we can use DNA Bar-coding Technique in Discriminating the local Egyptian Clover Genotypes and Protect Them internationally. Also, DNA Bar-coding can be used to determine the genetic polymorphism in identifying superior genotypes as source of parental genotypes in Egyptian clover breeding program in future.

Keywords: DNA Barcoding; *rbcl*; Cox 1; *Trifolium alexandrinum*; *Medicago sativa*; NCBI blast

INTRODUCTION

Sequences of DNA to organisms have planned as an elder path than traditional of taxonomic purist [Blaxter 2004; Tautz *et al*., 2003]. Kress *et al*., (2005) have pretend the performance DNA bar-coding angiosperms using nrDNA and non-coding sequences of cpDNA. Within trifolium, massive germplasm set utmost wild collected species occur [Morris & Greene, 2001]. Trifolium is member belong to the broad clad of legumes which lacks chloroplast copy repeat, IRLC (Lavin *et al*., 1990 & Liston 1995). Phyllogenetic studies had specific a strongly supported "vicioid of clad" within the IRLC the tribes of (Trifolieae & Fabeae). Molecular polymorphism with casual magnifies DNA (RAPD) polymorphic and the inter simple sequence repeat [ISSR] were employed to mark taxonomic relevance among 25 samples representing nine species of Orobanche. (Orobanchaceae). Dendrogram generated by the evaluation of the molecular statistics (RAPD and ISSR) identify that structure by NJ dendrogram for the morphological distinction (Sahrawy and Karakishi 2015) estimated the use of two chloroplast regions, trnL and rpoC1, besides a nuclear internal transcriber region, ITS2, for their competence to barcode the master Mediterranean leguminous yields. Twenty-five legume species were deliberated. Species identification based the sequence uniformity tactic outright by the catalog of GenBank. DNA zone trnL & ITS2 positively 100% differentiate crop legume species in the Mediterranean region, whilst the rpoC.1 in particular around 72%. Likewise, the use of trnL zone vest the discrimination of even mightily concerning species, like *Phaseolus lunatus* also *P. coccineus* & *Vicia faba* subsp foremost marginal of *V. faba* sub sp which is neatly united smooth of N.C.B.I they were both point out like *Phaseolus vulgaris* & *V. faba*, correspondingly. trnL and ITS2 are effective DNA bar-coding intention regions so as to select leguminous crops in Mediterranean and impart credible the effective tool for agricultural sciences likewise the community in artificial (Madesis *et al*., 2012). Badr (2001) examined *Trifolium alexandrinum* utilize AFLP figures. In Syria and Egypt. The information backup adjacent connection *T. alexandrinum* accessions at *T. apertum* & *T. berytheum* or *T. salmoneum* strength of species to interbred blankly tick that is *T. salmoneum* & *T. berytheum* may be honor as the inicial ancestors from whom the familiar Egyptian clover in Syria has meantime artificial selection. Dependent domestication could have been brought into the production of rain-fed crops in Palestine and irrigated to Egypt. In this respect, Egyptian clover urbanization may be the same as other crops such as wheat and barley, which were also domesticated in the fertile crescent and grown in the Nile Valley.

This imitates the genetic enhancement of the plant in backward cultivation in Egypt and varieties that were sophist icated in Egypt were later extended throughout the world. Parsimony and Bayesian phyllogenetic analysis were conducted nuclear ribosomal of DNA inner spacer transcribed & chloroplast trnL intron acquired from 218 to ca. Number of 255 species of Trifolium envoy to 11 genera.

Incongruence via nrDNA and cpDNA tests demonstr ate six cases of manifested hybrid speciation and describe th

* **Corresponding author.**
E-mail address: haae73oa@yahoo.com
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e fantastic allopolyploid progenitors.A common herb, *T.dubi um*, and *T. Repens*, the most widely grown species of clover (Ellison *et al*., 2006). Provenance ancestry of Egyptian clover was tested via AFLP information. A relative relationship of *T. alexandrinum* accessions from Syria and Egypt of *T. apertum, T. berytheum, and T. salmoneum*. Whilst cross ability and regional distribution indicate that is *T. apertum* is out of the way ancestor. In disparity, [*T. salmoneum*] look alike to be utmost prospective progenitor for Egyptian clover's Syrian substance though, close relevance to [*T. berytheum*] was revealed.

Such species ' openly crossing potency points out th at [*T. Salmoneum* & *T. Berytheum*] may be known as the f irst ancestors from whom Egyptian clover was domesticate d by man over Syrian selection. Kergoat *et al*., (2004) reconstructed sequences in partial in three genes of mitochondrial [12S rRNA cytochrome. b and cytochrome c. oxidase subunit, I] phylogeny of seed beetles in Europe [Bruchidae] pertinence to Bruchus [Linnaeus & Bruchidius Schilsky]. In field the elder beetles were gained from larvae bred from seeds. Parsimony ultimate prospect and Bayesian reasoning were used to understand phylogenetic rapport between species. Genera [Bruchidius & Bruchus] formative monophyletic sets through whole analyses. Chloroplast-genome sequences encoded rbcl. *Medicago sativa cv*. gene Regen,S was confronted to pea. 94.1% of Alfalfa shares nucleotide sequence homology in pea 1721 bases cross beginning gene 213 bases upstream of the coding sequences over 83 bases into the 3′ flanking region ending at locus 1508. Sequences of Peas are extremely ramose alfalfa after that point. 94.3% of amino acid sequences are uniform to that of pea with about 56% (15/27) of the replacement non conservative (Aldrich *et al*., 1987). Also, DNA barcodes from extreme herbal products about (91%) were recovered and whole leaf taking (100%) with (95%) species resolution utilize a tiered tactic (*rbcl + ITS2*). Utmost (59%), of the products examined include DNA barcodes from plant species not bound on labels. Whilst we were fancy to notarize roughly half about (48%) of the products one-third of these also rein contaminants and or fillers not recorded on the label. Product replacement appear in (30/44) of the products examined and only (2/12) of corporations had products without any swapping contamination or fillers. Somewhat of the contaminants was formed constitute critical health hazards to consumers (Newmaster *et al*., 2013).

The aim of the present study was to:

- Use DNA barcoding (*rbcl* and *cox*) genes to identify and discriminate *Medeicago Sativa* and *Trifolum alexandrinum* genotypes as two important forage crops.

MATERIALS AND METHODS

The seeds of alfafa and Egyptian clover were obtained from the Forage Crops Research Department (ARC) (Medicago Sativa – alfalfa, Rammah 1 and *Trifolum alexandrium*, Egyptian clover Helaly) genotypes.

Methods:

Sequence Database for DNA Bar-coding:

It was applied for identification and comparing sequences under study was carried out at National Center for Biotechnology Information (NCBI) database.

Sampling Taxon and origin sequences.

Two Leguminosae samples (*Trifolium alexandrinum* and *Medicago sativa*) were studied including in reference database.

The extraction of DNA, amplification and sequencing

Samples in fresh stage collected and were stocked in silica prior to extraction. Extracted DNA by the Genomic of Gene JET purification kit following the protocol of manufacture. As shown in table (1), two plastid regions were amplified, [ribulose1, 5 bisphosphate carboxylase / oxygenase] large subunit gene (rbcl) and cytochrome c oxidase subunit 1 gene (Cox1) with specific primer according to [Kergoat *et al*., 2004 and Cai *et al*., 2008, Gurdon *et al*., 2014, Young *et al*., (2011)] into *Medicago sativa* and *Trifolium alexandrinum.* Magnify products were detached by gel electrophoresis (1.0%) Agarose. Obtained RT-PCR of products were purified from Agarose gel and quantities spectrophotometrically set for sequencing trial via ABI Prism 7000 instrument based on industrialist procedure

Nucleotide sequence accession numbers.

Nucleotide sequences of bar-coding genes (*rbcl and Cox1 genes*) were submitted to be identified through program of NCBI BLAST - www. ncbi. nlm. nin. Gov / BLAST just as a single sense strand close sequence to each of Rammah 1 as well as Helaly genotypes. Products PCR were immediately sequenced at 2 sides to every fragment through a massive Dye v3.1 terminator Cycle sequencing kit (PE Applied Biosystems, City Foster, CA, USA.) in ABI 3730 an automated sequencer [P.E Applied Biosystems.]. CLUSTAL W program for sequences were used.

Table 1. Specific Primer sequence under study.

Forge crop	Coding genes	Primer sequence	Length	Tm	GC%
Trifolium alexandrinum	*Rbcl*	CAAGGCTTTGCGTGCTCTAC	741	59.83	55.00
		TATCGCGGCAATAGTGAGCC		60.32	55.00
	Cox1	ATATTGCCCATAGAGGCCCTTC	289	59.69	50.00
		GCATAGTGATTGCTCCTGCT		58.04	50.00
Medicago sativa	*Rbcl*	CGGCTACCGATGGACTTACC	339	59.97	60.00
		GTTCCACCCTCTTCCAGACG		60.04	60.00
	Cox1	TATGGTTTGCCGGCGATGAT	759	60.18	50.00
		TTGTAATTGCCCCTGCCAGT		59.89	50.00

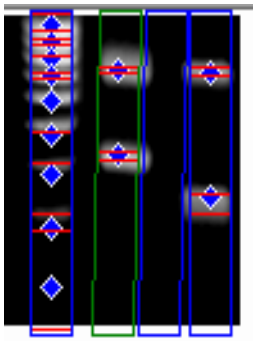
RESULTS AND DISSCUSION

Specific gene detection technique:

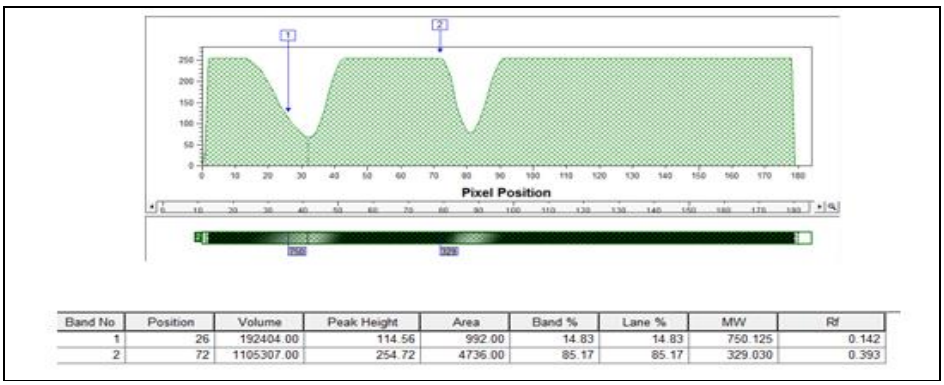
Main purpose of this investigation is identifying and evaluating *Medicago sativa* and *Trifolium alexandrinum* probability discrimination Genotypes. Thus, two bar-coding genes (*rbcl and Cox1 genes*) were employed for identification. Based on alignment data with reference genes, genetic similarity and possibility for discrimination were evaluated for *Medicago sativa* and *Trifolium alexandrinum.*

Photograph (1 and 2) show molecular weight parameters. Thus, specific fragments lengths were detected for each of *Medicago sativa and Trifolium alexandrinum*.

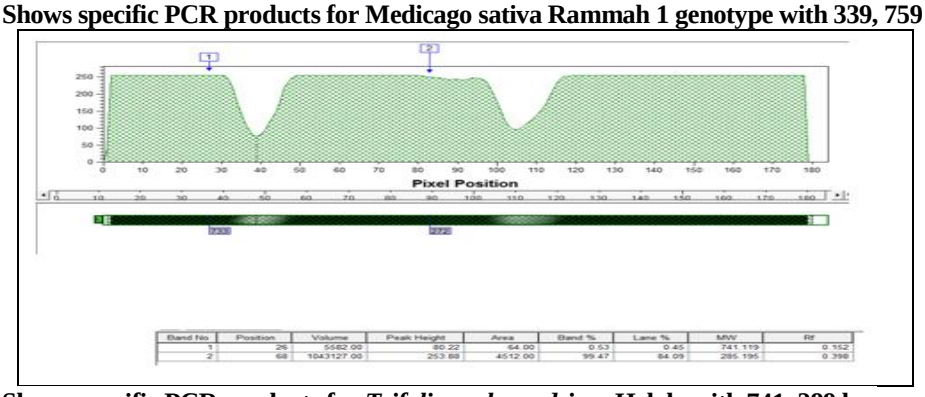
Photograph 1. shows specific PCR products for 1. *Medicago sativa* Rammah 1 genotype and *2. Trifolium alexandrinum* Helaly genotype with 339, 759 bp and 741, 289 bp for *rbcl* marker gene plus cytochrome c oxidase subunit (1) gene respectively



Photograph 2. Detection of specific PCR products for *Medicago sativa* Rammah 1 genotype *and Trifolium alexandrinum* Helaly with 339, 759 bp and 741, 289 bp for *rbcl* marker gene in addition cytochrome c oxidase subunit (1) gene respectively



Photograph 3. Shows specific PCR products for Medicago sativa Rammah 1 genotype with 339, 759 bp.



Photograph 4. Shows specific PCR products for *Trifolium alexandrines* Helaly with 741, 289 bp.

Identification of *Medicago* sativa Rammah 1 genotype was performed through rbcl and genes Cox1. Figure (1) shows *rbcl* marker gene for Medicago indicating it as *Medicago sativa* voucher G00199095 ribulose 1, 5 bisphosphate carboxylase / oxygenase large subunit gene (rbcl) partial cds; chloroplast sequence ID; KJ204375. 1.

To evaluate genetic similarity for *Medicago sativa* sample, *rbcl* marker gene for *Medicago sativa a*nd *rbcl* original sequence were compared. Interestingly, comparison of data showed, 90 % of genetic similarity which was detected between *rbcl* marker gene for *Medicago sativa and rbcl* reference sequence (Fig. 2).

For further confirmation the cytochrome c. oxidase subunit 1 gene (Cox1) marker gene were applied to identification of *Medicago* sativa Rammah 1 genotype (Fig.3) *and* indicated it as *Medicago sativa* voucher Ahrendsen_23 cytochrome c. oxidase subunit 1 gene complete cds; mitochondrial.

For further confirmation the cytochrome c. oxidase subunit 1 gene Cox l. marker gene was applied to identification of Medicago sativa Rammah 1 Genotype (Fig.3) and indicated it as Medicago sativa voucher Ahrendsen_23 cytochrome c. oxidase subunit (1) gene complete cds; mitochondria.

Highly genetic similarity (91.24) was found between the cytochrome c oxidase subunit 1 gene (Cox1.) for *Medicago sativa* and c oxidase subunit (1) gene (Cox1) for reference sequence and estimated with 91.24% (Fig.4).

Figure 1. Comparison alignments between *rbcl* marker gene for *Medicago sativa* Rammah 1 genotype and *rbcl* reference sequence.

```
>EMBOSS_M-Rbcl
     cgcaacctgg agttccggct gaagaagcag gtgcagcggt agctgccgaa cgagctttct        60
     ggacatggac ggcatcggct accgatggac ttaccagtct tgatcgttat aaaggacgct       120
     gctaccacat cgaacctgtt gctggagaag agactcaatt tattgcttat gtagcttatc       180
     ccttagacct ttttgaagaa ggttctgtta ctaacatgtt tacctccatt gtaggtaatg       240
     aacgctttct caaggccttg cgtgctctac gtctggaaga gggtggaac  cccgttgctt       300
     atgttaaaac tttccaaggt gaggtctctt gaatccaagt                             340
//
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Figure 2. Shows *rbcl* marker gene sequence for *Medicago sativa* Rammah 1 genotype. (DNA Barcoding of alafalfa Rammah I Genotype (*rbcl*) gene).

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>EMBOSS_M-Cox
     TCAAATTCTT GGTGGGAATC ATCAACTTTA TAATGTTTTA ATAACGGCTC ACGCTTTTTT        60
     AATTCTCTTC TTTATGGTT TGCCGGCGAT GATAGGTGGA TCTGGTAATT  GGTCTGTTCC       120
     GATTCTTATA GGTTTTGAA  ACATGGCATT TCCACGATTA AATAATATTT CATTCTGGTT       180
     GTTGCCACCA AGTCTCTTGC TCCTATTAAG CTCAGCCTTA GTAGAGGTGG GTAGCGGCAC       240
     TGGGTGGACG GTCTATCCGC CCTTAAGTGG TATTACCAGC ACCTATTTTC GAGCAGTTGA       300
     TTCAGCAATT TCTAGTCTTC ATCGTTTCAT CCATTTTAGG TTCTATCAAT TTTATAACAA       360
     CTATCTCCAA CATGCGTGGA TTTTACACAT CTATGCATAG ATCACCCCTA TTTGTGTGGT       420
     CCGTTCCAGT AACAGCATTC CCACTTTTAT TATCACTTCC GGTACTGGCA GGGGCAATTA       480
     CAATGTTATT AACCGATCGA AACTTTAATA CAACCTTTTC TGATCCCGCA CCCATTACCT       540
     GGACTATCTG ATACCAGCAT CTCTTTCGGT TCTTCGGTCA TCCAGAGGTG TATATTCCAA       600
     TTCTGCCTGG ATCCGGTATC ACGGCATTTC TCGTTTCGAC TTTTTCGGGA AAACCGGTCT       660
     TCGGGTATCT GGGAGGGGA  TATGCCATGA TCAGTATAGG TGTTCTTGGA TTAGGGGCTT       720
     GGGCTCATCA TATGTTTACT GTGGGCTTAG ACGTTGATAC CC
```

Figure 3. Shows cytochrome c oxidase subunit 1 gene (Cox1) marker gene sequence for *Medicago sativa* Rammah 1 genotype.

(DNA Barcoding of alfalfa Rammah 1 Genotype (Cox 1) gene).

Figure 4. Comparing alignments between the cytochrome c oxidase subunit (1) gene to *Medicago sativa* Rammah 1 genotype and cox 1 reference sequence.

Suspected *Trifolium alexandrinum* Helaly genotype was identified based [ribulose 1, 5 bisphosphate carboxylase / oxygenase large subunit (*rbcl*) gene] *Trifolium* sample was identified as *Trifolium alexandrinum* [ribulose 1, 5 bisphosphate carboxylase / oxygenase large subunit (*rbcl*) gene] partial cds: chloroplast. [Sequence ID: HM850407.1] with 100% of genetic identity (fig.5).

To estimate genetic relationship between *Trifolium alexandrinum* and genetic origin of *Trifolium alexandrinum*, alignment results were analyzed between [ribulose 1, 5 bisphosphate carboxylase / oxygenase large subunit (*rbcl*)] gene for *Trifolium alexandrinum.* Helaly genotype in addition [ribulose 1, 5 bisphosphate carboxylase / oxygenase large subunit (*rbcl*)] reference of

gene. Thus, 95.92 % of genetic similarity was recorded (fig.6).

In the light of *rbcl* marker identification gene, comparing the cytochrome c. oxidase subunit 1. gene Cox1 gene marker to *Trifolium alexandrinum* indicate uniformity as *Trifolium alexandrinum* voucher K-016Hv cytochrome c. oxidase (C.O.I) gene, partially CDS; mitochondrial (Sequence of ID: KU234213.1) with 100 % at genetic similarity (figure 7).

```
>EMBOSS_Tri-Rbcl
     ACCACATCGA GCCGGTTGCT GGAGAAGAAA CTCAATTTAT TGCTTATGTA GCTTATCCCT        60
     TAGACCTTTT TGAAGAAGGT TCTGTTACTA ACATGTTTAC CTCCATTGTA GGTAATGTAT       120
     TTGGGTTCAA GGCTTTGCGT GCTCTACGCC TGGAAGATTT GCGAATCCCC GTTGCTTATG       180
     TTAAAACTTT CCAAGGTCCT CCTCACGGAA TCCAAGTTGA GAGAGATAAA TTGAACAAGT       240
     ATGGACGTCC CCTATTGGGA TGTACTATTA AACCTAAATT GGGTTTATCC GCTAAGAATT       300
     ACGGTAGAGC AGTTTATGAA TGTCTACGCG GTGGACTTGA TTTTACAAAA GATGATGAAA       360
     ATGTGAACTC CCAACCATTT ATGCGTTGGA GAGACCGTTT CTTATTTTGT GCCGAAGCTA       420
     TTTATAAATC ACAGGCCGAA ACGGGTGNNN TCACGGAATT NNNNNNNNNN NNNNNNNNNN       480
     NNNTTCCGGT GCGGTTGTTT GGCTGTATTT GCAAGAGAAT TGGGCGTTCC TATAGGCCAC       540
     TAATGCAGGA CTACCTAACA GGCGGATTCA CTGCAAATAC TACCCTGGCT CACTATTGCC       600
     GCGATAATGG TCTACTTCTT CATATCCACC GTGCAATGCA TGCAGTTATC GATAGACAGA       660
     AAAATCATGG TATGCACTTT CGTGTATTAG CTAAAGCGTT ACGTTTGTCT GGTGGAGATC       720
     ATATTCACGC CGGTACTGTA G                                                741
//
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Figure 5. Shows the [ribulose 1,5-bisphosphate carboxylase/oxygenase large subunit (*rbcl*)] marker gene sequence for [*Trifolium alexandrinum*] Helaly Genotype.
(DNA Barcoding of Egyptian clover Helaly Genotype (*rbcl*) gene).

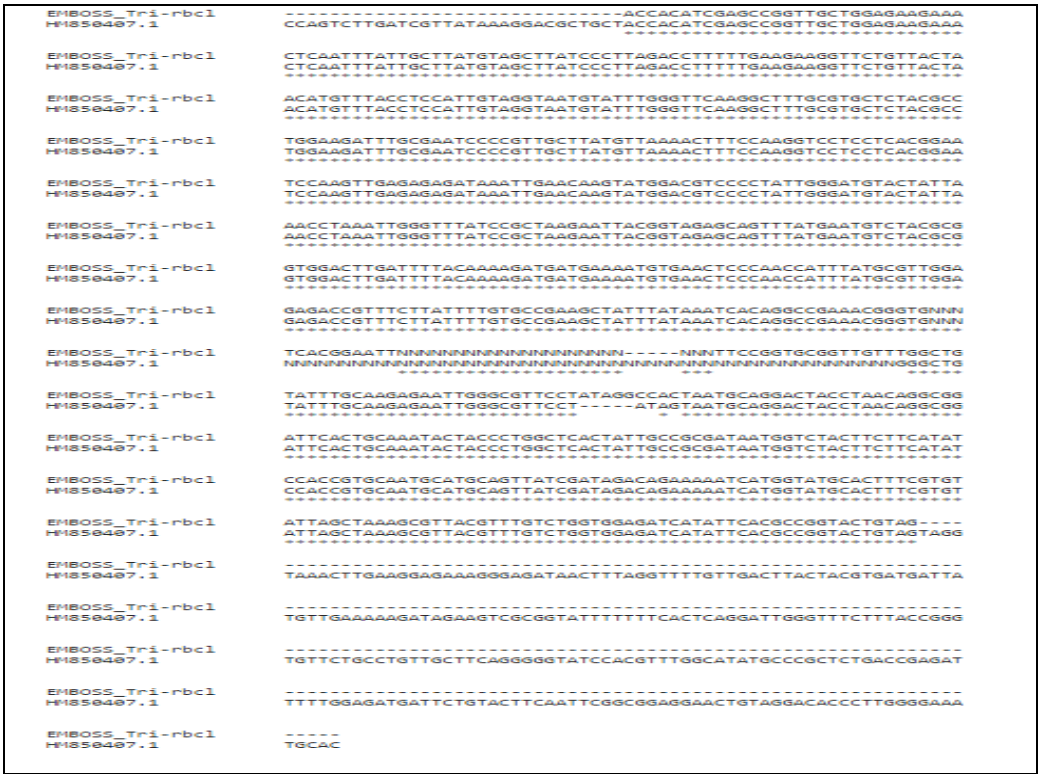

Figure 6. Comparison alignments between *rbcl* marker gene for *Trifolium alexandrinum* Helaly genotype and *rbcl* reference sequence

```
>EMBOSS_Tri-Cox
     TCTTTCAGCT AATATTGCCC ATAGAGGCCC TTCTGTTGAT TTAGCTATTT TTAGATTACA        60
     TTTAGCTGGT GTATCATCAA TTTTAGGAGC AATTAATTTT ATTACTACCA TGATTAATAT       120
     ACGACCTATT GGTATACAAT TAGATAAACT TCCTTTATTT GCTTGGTCAG TTTTAATTAC       180
     TGCTATTTTA CTTCTGCTTT CCCTCCCTGT ATTAGCAGGA GCAATCACTA TGCTTTTAAC       240
     AGATCGAAAT ATTAATACTT CATTTTTTGA CCCTGCAGGA GGTGGGGAT                   289
```

Figure 7. Shows the cytochrome c. oxidase subunit (1) gene (Cox1) marker gene sequence to *Trifolium alexandrinum* Helaly genotype.

(DNA Barcoding of Egyptian clover Helaly Genotype (Cox 1) gene).

Preserve the originality was detected (fig.8) through comparing the cytochrome c. oxidase (C.O.I) gene CDS: sequence of mitochondrial with cytochrome c oxidase (COI) gene partial cds: reference of mitochondrial sequence and showed completely identical similarity with 100 % of genetic similarity.

Figure 8. Comparison of alignments between cytochrome c oxidase subunit 1 gene for *Trifolium alexandrinum* Helaly genotype and cox 1 reference sequence

It is significant to consider that DNAbased classification by Trifolium would challenging without any accessibility of universal comprehensive monograph & biological data to utmost genus (Gillett and Taylor. 2001).

The results obtained in this study for identification and evaluation of Similarity with the original genetic base are in agreements with the results of Ganopoulos *et al*., (2012). Who applied barcode of DNA high resolution melting
system employ the global nuclear power plant DNA barcodi ng area ITS2 for meadow species uniformity, quantification and deception reveals Medicago lupulina deceit as low as (1: 100) in Trifolium pretense seeds. Their results indicated that [Bar-HRM] analyses could be a faster with extreme resolution and cost-effective replacement method to back up forage and meadow species and quantitatively disclose the purity of their seeds and their feed products. More light was added to our findings as obtained by Gillett and Taylor, (2001). They applied DNA-based identification in *Trifolium* and reported that its potential confronts without availability of an overall international monograph, (Zohary and Heller 1984) biological noticed of ultimate genus.

Efficacy of various genes (cox1. *Rbcl*. 18. S and I.T.S. rDNA.) were assessed for recognize species of cryptic in the morphospecies model Cox1. divergence was usually much greater than *rbcl* variance and always extremely variable than 18S rDNA. I.T.S. rDNA sequences were significant variable than cox1, but well-known problems with regard to variability of intragenomic caution against its use in identification. More information and less sequencing effort mean that is the cox1. can benefit aid in identification diatom. Advantages of cox1. for crucial phylogenetic relationships between tree topologies were very identical, even though back up values were generally decrease for cox1 (Evans *et al*.,2007). With agreements to our findings, Hawkins *et al*., (2015) metabarcoding DNA plus melissopalynological fitted to most numerous floral honey compounds and plant Taxa. there were 92% harmony for taxa had plentiful over 20%.

Whereby, when all taxa were comparable, the rate of classification decreased from 2245 in addition there was little agreement among the relative abundance of taxa found using the two techniques. DNA of meta-barcoding given more repeatability 64% taxa compared to 28% melisspalynology.

Altschul *et al.*, (1990) introduced BLAST tool for finding sequence similarity (Basic Local Alignment Tool). BLAST border constancy that optimize a measure of local identification the maximal segment pair score. Such a border may be concept of as decrease the evolutionary distance or maximizing the uniformity between the two sequences compared. BLAST employs a magnitude based on well – defined mutation scores to compare two sequences, whether DNA or amino acid sequences to discover sequence homology. Pairwise alignment is deciding if a pair of sequences is evolutionary related or not. Pairwise uniformity mark for the sequences that be fed into a cluster analyses, or program of tree calculating. The tree program is calculated to place uniformity pairs of sequences closer altogether on the tree than sequences that are less identical.

CONCLUSION

This work aims at discrimination and identifying *Medicago sativa* and *Trifolium alexandrinum* (two important Forage crops) via two DNA bar-coding genes (*rbcl and Cox1 genes*).

Identification of *Medicago sativa* Rammah 1 genotype was performed through rbcl and Cox1 genes identified it as *Medicago sativa* voucher G00199095 ribulose1, 5 bisphosphate carboxylase / oxygenase large subunit gene. (rbcl) CDS; chloroplast. Sequence ID: KJ204375.1 and *Medicago sativa* voucher Ahrendsen 23_ for rbcl and Cox1 genes respectively. Moreover, *Trifolium alexandrinum* Helaly genotype was identified as *Trifolium alexandrinum* (Sequence ID: HM850407.1) and *Trifolium alexandrinum* voucher K-016Hv (Sequence ID: KU234213.1) as rbcl and Cox1 genes respectively.

Trifolium alexandrinum showed more success for genetic similarity comparing with [*Medicago sativa*] as output of genetic similitude eminence with origin sequences.

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استخدام تقنية DNA Barcoding في عمل البصمة الوراثية لنوعين من محاصيل العلف

عبد العزيز طلعت بندق

قسم بحوث محاصيل العلف ، معهد بحوث المحاصيل الحقلية ، مركز البحوث الزراعية ، الجيزة ، مصر

في هذه الدراسة تم عمل شفرة كودية مميزة لكلاً من البرسيم المسقاوي صنف الهلالي والبرسيم الحجازي صنف رماح 1 وكذلك تم تمييز وتعريف كل من البرسيم المسقاوي صنف الهلالي والبرسيم الحجازي صنف رماح 1 باستخدام تقنية الباركود DNA وهذا عن طريق جينين يستخدمان في إعطاء شفرة كودية مميزة لكل منهما وهما (*rbcl* *and Cox1 genes*). ولقد أظهرت النتائج أن استخدام جين (*rbcl*) فى تعريف البرسيم الحجازي صنف رماح 1 بأنه *Medicago sativa* voucher G00199095 ribulose 1,5 bisphosphate carboxylase/oxygenase large subunit (*rbcl*)gene (Sequence ID: KJ204375.1) وأيضاً أمكن تعريف البرسيم الحجازي صنف رماح 1 باستخدام جين (Cox 1) وأوضحت النتائج أنه Medicago Sativa Voucher Ahrendsen-23 وأعطى نسبة تشابه بلغت 91,24% بالمقارنة بالأصل الوراثي (Sequence ID : KJ 204375.1) أما بالنسبة للبرسيم المسقاوي صنف الهلالي فقد أظهرت النتائج تعريفه باستخدام جين (*rbcl*) بأنه *Trifolum Alexandrinum* (Sequence ID : HM850407.1) وأعطى نسبة تشابه بلغت 95,92% بالمقارنة بالأصل الوراثي (Sequence ID : HM850407.1) وأيضاً أظهرت النتائج تعريفه باستخدام (Cox 1 Gene) بأنه *Trifolum alexandrinum* voucher K-016 HV (Sequence ID : KU234213.1) وأعطى نسبة تشابه 100% بالمقارنة بالأصل الوراثي (Sequence ID : HM850407.1) وفي ضوء النتائج المتحصل عليها تم استخدام التشابه الوراثي مع الأصل الوراثي كمقياس للتماثل الوراثي وأوضحت النتائج التماثل الوراثي العالي للبرسيم المسقاوي صنف الهلالي كنتيجة لتطابقه بنسبة 97,9% مع الأصل الوراثي بالمقارنة بالبرسيم الحجازي رماح 1 الذي بلغت نسبة تطابقه مع الأصل الوراثي 90,6%.وبناء على النتائج المتحصل عليها يصبح من الممكن استخدام تقنية DNA Barcoding في التمييز بين الأصناف والأنواع والأجناس للبرسيم المصري وكذا حمايتها دولياً، وتخلص الدراسة أيضاً إلى أهمية استخدام DNA Barcoding في تحديد الاختلافات الوراثية ودرجات التماثل الوراثي التي تستخدم كأساس في برامج التحسين الوراثي مستقبلاً.