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TIKRIT JOURNAL OF PURE SCIENCE

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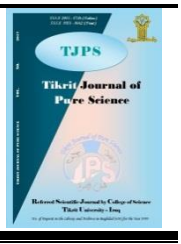

Using RAPD markers for genetic analysis in Three Species of Datura in Iraq

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ARTICLE INFO.

Article history:
-Received: 7 / 5 / 2018
-Accepted: 21 / 5 / 2018
-Available online: / / 2018

Keywords: Datura sp., RAPD, Polymorphic band, Main bind, unique bind.

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Abstract

The study amis to develop the evidence genetic of active substances for several kinds of the Datura plant (Datura sp.), So it was procedure steps to isolated the Genomic DNA from leaf of Datura specis it is (*Datura metel, Datura innoxia and Datura stramonium*).

Has been used 51 primers in the experiments of the RAPD markers, but did not show 17 primers including any amplified band while showed in genomic in Datura plants, and 34 primers show results Differentiated locations where all the primers gave a differentiated binds Polymorphic band These results have been invested to study genetic variability among the species involved in study.

Introduction

Datura is one of the most important Medicinal Plants in the world because it contains many biologically active chemical compounds such as Alkaloid Compounds, Phenolic Compounds, Terpenoid Compounds, Glycosides and others [1-4]. It is one of the plants of the family Solanaceae, which is characterized as a large plant families and includes important types of vegetables are tomatoes, eggplants, potatoes, green and red peppers [5,6], Datura is one of the most important plants of this family from the medical point of view on the presence of alkaloids Including Atropine, Hyoscine, Hyoscyamine and 7-Hydrohyosine [7-9].These alkaloids differ in quantity and quality depending on class and species. Datura are naturally growing in the fields and orchards of central and southern Iraq and bloom between August and September. The bioactive substances in roots, leaves and legs for plant causes fatal symptoms of humans, sheep, horses and cows [10,11]. In Iraq, three species of this plant was grown: *Datura Stramonuim* one of the most important species in the world, contains alkaloids higher than other species. It is spread out in the northern of Iraq. While the other two species are spread in central and south The other two types are *Datura metel* and *Datura innoxia*, In the central and southern regions as jungles and wild plants [12].

The Datura alkaloids alert the central nervous system (Nervous System). This alert is accompanied by a drop, minimization of most glandular secretions eg sweat, saliva, yogurt, and alkaloids extract in many analgesic drugs. The use of Datura has been used as an analgesic and as a base for the treatment of asthma. In South Africa, decoction is used as a treatment lotion and is used in the treatment of asthma. As well as the burning of their leaves or seeds to give the same result, and used boiled leaves for analgesia, colic and paralysis as well as material for hair loss and partial anesthesia and the use of Datura in ophthalmology Ophthalmology as Altropin expand pupil [13]. As a result of many studies, it was found that the drugs containing a proportion of Datura extracts in their composition succeeded in treating cases of mental disorders. In 1762 Stoerck was able to introduce the famous *Datura Stramonuim* in its toxicity in the synthesis of some Medical substances to treat certain diseases as a disease point, convulsions and mental disorders [9].

Materials and methods

The Three Speices of Datura sp. were collected Saladin province. The experts from the Faculty of Agriculture / University of Tikrit have been recruited to classify the three species, relying on diagnosis in

the form of leaves in the first place and on the general appearance of the plant.

DNA extraction and RAPD technique and PCR reaction:

The genetic material extracted from the young leaves of the three types by using CTAB and in the manner cited by[14] which established by[15]. RAPD interactions based on [16] on DNA samples of three different types of Datura plants were included in the study, Random Primers are provided by Operon Technologies, USA. Where RAPD interactions on DNA samples were carried out using 51 primer as shown in the Table (1-1)

Table (1-1) The RAPD marker primer

The primers used in the RAPD reaction					
T	Primer	(3'− 5') sequence	T	Primer	(3'− 5') sequence
1	OPJ_01	CCCGGCATAA	27	V_20 OP	CAGCATGGTC
2	OPL_17	AGCCTGAGCC	28	OPJ_05	CTCCATGGGG
3	OPB_10	CTGCTGGGAC	29	OPE_02	GGTGCGGGAA
4	OPR_06	GTCTACGGCA	30	OPW_08	GACTGCCTCT
5	OPN_16	AAGCGACCTG	31	OPY_10	CAAACGTGGG
6	OPP_04	GTGTCTCAGG	32	OPD_18	GAGAGCCAAC
7	OPJ_17	ACGCCAGTTC	33	OPK_13	GGTTGTACCC
8	OPH_19	TCAGGGAGGT	34	OPE_20	AACGGTGACC
9	OPN_01	CTCACGTTGG	35	OP Q-2	TCTGTCGGTC
10	OPL_08	AGCAGGTGGA	36	OPN_07	CAGCCCAGAG
11	RAPD 4546_041	CCCAGCAACTGATCGCACAC	37	OPY-09	AGCAGCGCAC
12	RAPD4546_0411	AATGTGGGCAAGCTGGTGGT	38	OPH_19	CTGACCAGCC
13	OPG_02	TGGACCGGTG	39	OPM_15	GACCTACCAC
14	OPL_20	TGGTGGACCA	40	OPO_19	GGTGCACGTT
15	OPA_18	AGGTGACCGT	41	OPP_06	GTGGGCTGAC
16	OPX_01	CTGGGCACGA	42	OPK_02	GTCTCCGCAA
17	G_05 OP	CTGAGACGGA	43	OPI_04	CCGCCTAGTC
18	Y_07 OP	AGAGCCGTCA	44	OPG_07	AAGCCTCGTC
19	O_11 OP	GACAGGAGGT	45	OPH_14	ACCAGGTTGG
20	M_01 OP	GTTGGTGGCT	46	OPY_03	ACAGCCTGCT
21	OPP_01	GTAGCACTCC	47	OPP_01	GTAGCACTCC
22	H_14 OP	ACCAGGTTGG	48	OPM_03	GGGGGATGAG
23	W_17 OP	GTCCTGGGTT	49	OPC_19	GTTGCCAGCC
24	Z_08 OP	GGGTGGGTAA	50	OPA_06	GGTCCCTGAC
25	U_12 OP	TCACCAGCCA	51	OPI_06	AAGGCGGCAG
26	R_10 OP	CCATTCCCCA			

The interaction mix consisted of Table (1-2):

Table (1-2) Ingredients of the main reaction mixture of PCR

Ingredients	Final Focus	The size of one sample is microliter
Distal Watar	-	16
Primer	Bicomol10	1
DNA	Ng / microliter25	1
Premix	-	2

On a special program as follows Table(1-3)

Table (1-3) PCR Program for RAPD

Steps	Temperature (c°)	Time (min)	Number of cycles
Initial denaturation	95	5	1
Denaturation	95	1	40
Annealing	36	1	
Extension	72	2	
Final exteusion	72	10	1

After the interaction has eneled, the samples were carried on the 2% agarose gel by running the Electrophoresis for 90 minutes. After that, the gel was dyed by ethidium bromide for 30 minute with the shaker and then exposed to ultraviolet light on the device Gel documentation system and gel imaging using a high resolution digital camera.

Results and discussion

used 51 primer in the experiments of the RAPD markers, but did not show 17 primers (OPP-06,OPQ-2, OPN-07, OPY-09, OPH-19, OPM-03, OPY-03, OPP-01, OPH-14, OPG-07, OPI-04, OPK-02, OPM-15, OPO-19, OPC-19, OPA-06, OPI-06) for not finding the primer of binding sites on the Datura genome, and the 34 primers showed results by different link locations as all primer gave a binds, All the primer gave a polymorphic band, the OPG-05 primer showed only one main band and 21 primers with unique bands, as well as (OPJ-01,OPL-17,OPR-06,OPN-16,OPD-18,OPW-08,OPH-19,OPR-10,OPU-12,OPJ-05,OPO-11,OPH-14,OPE-02,OPK-13 ,OPE-20)

These primer distinguished the first type from the second and the third species by a unique bind at a specific molecular size different from the primer used. The primer (OPG-02) produced a bind of Type III and (OPM-01),These primer The most visible in the first type from the second and the third species by a unique band at a specific molecular size, which

ISSN: 1813 – 1662 (Print)
E-ISSN: 2415 – 1726 (On Line)

differs according to the different primer used, The primer (OPG-02) produced a bind that distinguished the third type, Primer (OPM-01) distinguishes Type second from other species. and primers (OPL-08 ,OPY-07, OPL-20) produced distinctive binds for both Type first and second with different molecular sizes that distinguish each type from the other.

10 primers (OPP-10, OPP-04, RAPD 4546-041, RAPD 4546-0411, OPN-01,OPV-20,OPW-17,OPG-05,OPX-01,OPP-01) Showed a heterogeneous binds assortment of species but was unable to show distinct binds bundles, 3 primer did not show any result with a particular species, and showed a result combination with other species, Primers (OPY-10, OPZ-08) did not show any binds of type one .and primer (OPJ-17) Did not appear binds for the second type, and appeared binds for the rest of the species, I invested these results to study the genetic variance among the species involved in the study.

As for how to select the primer for this study, 51 were taken, some of which were selected on the basis of published scientific studies on the Datura and the other part was randomly entered among a large group of kits available and prepared by Operon company specialized in the production of such primer.

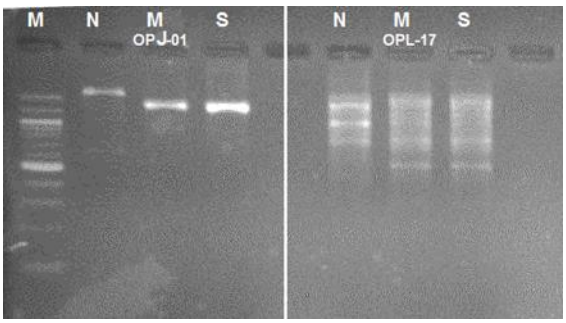

Figure (1-1) result of electrophoresis on 2% agarose gel for RAPD Marker both the primer OPP-01 and the primer OPL-17 for N-*Datura Innoxia*, M - *Datura Metels*, S - *Datura Stromonuim*

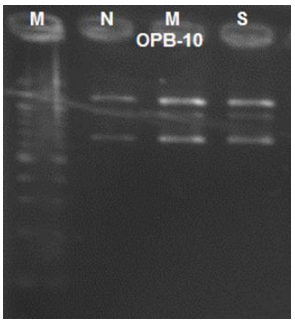

Figure (2-1) result of electrophoresis on 2% agarose gel for RAPD Marker the primer OBP-10 for N-*Datura Innoxia*, M- *Datura Metels*, S- *Datura Stromonuim*.

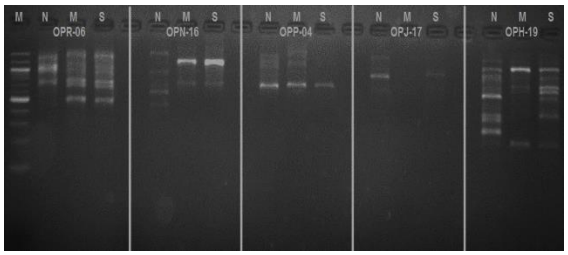

Figure (3-1) result of electrophoresis on 2% agarose gel for RAPD Marker the primer OPR-06 and primer, OPN-16 and primer OPP-04 and primer OPJ-17 and primer OPH-19, for N-*Datura Innoxia*, M- *Datura Metels*, S-*Datura Stromonuim*

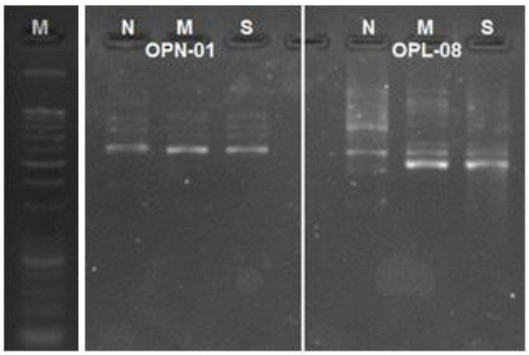

Figure (4-1) result of electrophoresis on 2% agarose gel for RAPD Marker the primer OPN-01 and OPL-08. for N-*Datura Innoxia*, M- *Datura Metels* , S - *Datura Stromonuim*. Primer

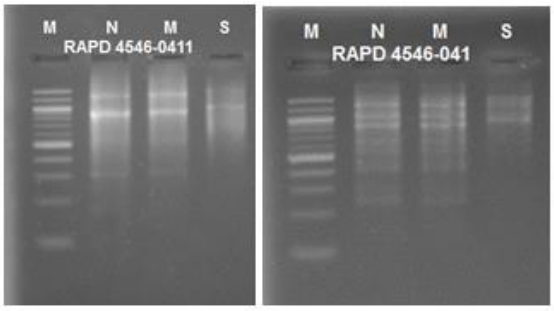

Figure (5-1) result of electrophoresis on 2% agarose gel for RAPD Marker the primer 4546-041, RAPD 4546-041, and RAPD 4541-0411 for N-*Datura Innoxia*, M- *Datura Metels*, S - *Datura Stromonuim*.

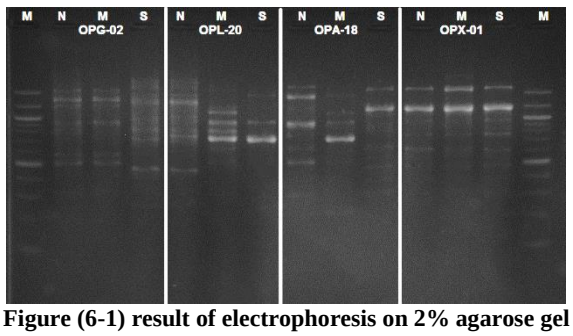

Figure (6-1) result of electrophoresis on 2% agarose gel for RAPD Marker the primer OPC-02 and primer OPL-20 and primer OPA-18 and primer OPX-01 for N-*Datura Innoxia*, M- *Datura Metels*, S-*Datura Stromonuim*.

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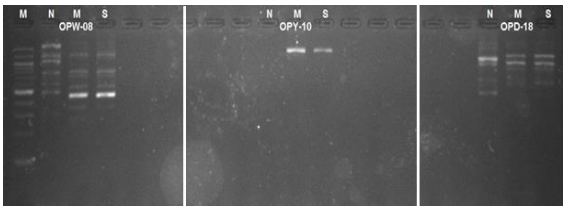


Figure (7-1) result of electrophoresis on 2% agarose gel for RAPD Marker the primer OPW-08.OP1-10 and OPD-18 for N-*Datura Innoxia* , M- *Datura Metels*, S- *Datura Stromonuim.*

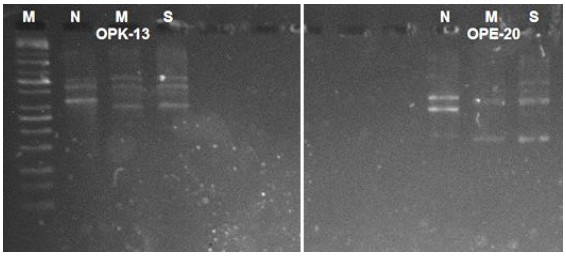
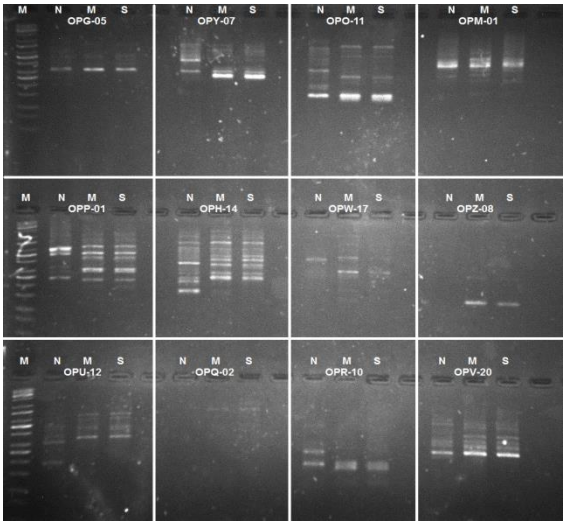


Figure (8-1) result of electrophoresis on 2% agarose gel for RAPD Marker the primer OPK-13 and OPE-20 for N-*Datura Innoxia*, M- *Datura Metels*, S - *Datura Stromonuim.*



Figure(9.1) result of electrophoresis on 2% agarose gel for RAPD Marker the primer OPG-05 and primer OPY-07, primer OPO-11,OPM-01 primer OPM-01, primer OPM-01, primer OPP-01 primer OPH-14, primer OPW-17, primer OPZ-08, primer OPU- 12, OPR-10 primer OPV-20,for . N-*Datura Innoxia*, M- *Datura Metels*, S - *Datura Stromonuim*

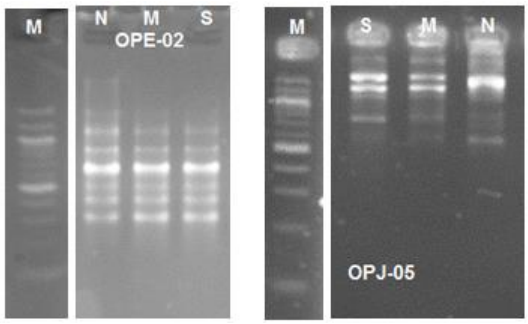


Figure (10.1) result of electrophoresis on 2% agarose gel for RAPD Marker the primer OPJ-05 and the primer OPE-02 for N-*Datura Innoxia*, M- *Datura Metels*, S - *Datura Stromonuim.*

The analysis of the results of the RAPD markers depends on the presence or absence of DNA binds and the difference in the number binds and molecular size of these [17].

The variation in the number of Binding sites with genomic DNA, and the distance between these sites primer distances produced either naturally through new recombinations during cell division, Either by mutation and both cause a lot of deletions or addition or replacement substitutions especially those that occur on the link sites, which leads to change the order of the rules complementary to the primer sequence, thus losing the chance of correlation, especially that it is affected by the change of only one base [16].

The absence of results for some of the primers used is one of the bases on which differences between individuals or groups are constructed [16 ,18].

The importance of the absence of specific sites on a particular object is so important The presence of those sites that will subsequently be firmly on the agarose gel is taken together [19,20].

This is what can be invested in this study through the first group of 17 primer that did not produce any bind on the agarose gel, which can be invested in the knowledge of any other type of Datura unknown classification and wants to know their affiliation or relationship with the types of Datura, Of these primers with RAPD reactions. If any bind appears on the agarose gel, this means that they are not the ones studied.

These the primers 17 that did not show any bind This is due to the fact that no binds has been found to find the primer on a complementary site on the DNA tape, resulting in its inability to bind and Duplication [21].

The 34 primer that were found to follow complementary sites on the genomic DNA of the Datura species were distinguished to the primers, that showed the same number and location of the Monomorphic Bands and the primates that showed a different combination of numbers and locations among the studied species Polymorphic Bands, It is divided into two categories: the first category is the primers that have shown unique bands, since the unique bands that appear at a certain size at a particular type without others are of great importance, It is a special marker, and its presence in the organism to differentiate it from its closest peers, so it becomes an important target for the researchers seeking genetic discrimination [22].

It is observed through these primers that the genotypes involved in the study have a clear genetic variation that can be relied upon to distinguish these genotypes. The appearance of these bundles in only one type indicates that these binds represent a marker, In the case of missing binds, it is an indication of a specific location defined by the primer in all studied species except for one sample. Is consistent with the results of many researchers [23-25].

The second category is the primers that showed a divergent binds between species but they were not able to show a distinctive bind .The absence of results for some of the primers used is one of the bases on which the differences between individuals or groups [16,18].

The importance of the absence of specific sites on a particular DNA is of particular importance so that it is taken along with the factor of the presence of those sites that will subsequently be firmly on the agaros gel

different combinations between species [19,20],This is what can be invested in this study through the first group of 17 primer that did not produce any bind on the agaros gel.

The results of the RAPD marker have shown another type of binds of importance. Many studies have focused on the main bands, which appeared unevenly between the primers and were of different molecular sizes. These binds are based on the identification of unknown or lost models of classification , As it is often a site shared by all members of the genus or type Species [26], It depends on a number of these binds, which are repeated and increase in value relatively large size to be a higher ability to distinguish, and some studies are extracted from the gel and the design of the primers or sensors thereof, as in the study of the classification of some of the genetic lines that have lost their classification of wheat [21,27]. or to identify sex in pistachios [28]. The absence of the general band of a band called the Absent band,[19] noted that this absence is a marker of that type, and the present study has shown many of these cases.

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استعمال مؤشرات ال RAPD لتحليل الوراثي لثلاثة انواع من نبات الداتورا في العراق

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الملخص

هدفت الدراسة الى تطوير مؤشر وراثي للمواد الفعالة في عدة انواع من نبات الداتورا .Datura sp لذا تم عزل الـ DNA المجيني من اوراق انواع الداتورا, وهي (*Datura metel* ,*Datura innoxia and Datura stramonium*), إذ تم استعمال 51 بادئاً لتجارب مؤشر (RAPD), لم يظهر 17 بادئاً ناتجاً وهي البادئات وذلك لعدم إيجاد البادئ لمواقع ارتباط على مجين نبات الداتورا , و ال 34 بادئاً أظهر نتائج باختلاف مواقع الارتباط إذ جميع البادئات أعطت حزمة متباينة Polymorphic band, ولقد استثمرت هذه النتائج لدراسة التنوع الوراثي بين الأنواع الداخلة في الدراسة.