

## The Role Extract of (*Citrullus Colocynthis*) Fruits in the Stimulation of the Genetic Variations of Varsity wheat

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**Abstract:** An experiment conducted at the University of Baghdad, College of Agriculture during 2014-2015 by Germination of seeds of wheat in the stimulation of genetic variations in three-variety wheat *abo greab*, *ebaa*, *Iraq*. The variables were the extract of (*Citrullus Colocynthis*) fruits at concentrations (0.0, 50.0, 100.0, 150.0, and 200.0) mL.L<sup>-1</sup>. PCR-RAPD test and detection PCR-RAPD test in differences presence in band number and weights in the extract of (*Citrullus Colocynthis*) fruits treatment and control treatment in light of the results can be concluded that the extract of (*Citrullus Colocynthis*) fruits the ability to make a genetic variation in wheat and recommends isolating the substance responsible for causing these variations and diagnosis.

**Keywords:** The extract of (*Citrullus Colocynthis*) fruits, PCR-RAPD.

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### I. Introduction

Wheat play an important role in human nutrition since ancient times. A favorite food of all grain in most countries of the world, dates grown to the pre-Christmas, and is likely to be the kinds of wheat cultivated originated from wild species by natural hybridization and mutation election and believed that the wheat originated in the area Middle East and West Asia (6). The wheat is of the most important grain crops and the most widespread in the world. Especially in European and American countries as the total area of cultivated globally about 230 million hectares of productivity 343 million tonnes (13). Due to the adoption of methods of plant breeding to genetic mutations and hybridization and selection and the fact that genes are the factors responsible for the emergence of genetic traits. The mutagens may alter the genetic makeup of an organism or part of it resulting in a new recipe were not present previously and that spontaneously occurring mutations in nature, very few can give rise industrially using physical mutagens like chemical rays such as Ethyl methane sulphonate (EMS) and Sodium azide (SA) (22). The natural extracts include a range of plant extracts or a part of it. *Citrullus Colocynthis* extract gained special significance through research and studies in the field of biotechnologies because of its medical importance. Many of the studies on the viability of phenolic compounds on genetic mutation. Nash et al. (1994) indicate that some flavonoids scalability genetic mutation bacteria (21) attributed the ability flavonoids on genetic mutation to the presence of compounds hydrocarbon annular polycyclic aromatic hydrocarbons in the composition (26). Delazar et al. (2006) found in his study of phenolic compounds in *Citrullus Colocynthis* that the first chemical examination between the presence of large amounts of phenols and Flavonoids scattered in all parts of the plant (9). Mehni and Shahdadi (2014) showed the importance of phenols in *Citrullus Colocynthis* as antioxidants and free radicals found that the highest concentration has reached 4.104 mg per 1 g of dry weight and lower her concentration was 3.696 mg per 1 g of dry weight (18). Abbas et al. (2012) showed in his study on Genetic mutation and counter the effect of mutagenesis of the alcoholic extract of the seeds of *Citrullus Colocynthis* is that the seed extract to induce mutations of resistance to two anti-vital streptomycin and rifampicin as indicators of genetics especially when concentration of 125 µg.mL<sup>-1</sup> has attributed this ability to contain the seeds of *Citrullus Colocynthis* on phenolic compounds (1). The technology interaction polymerase chain sequential (PCR) of molecular technologies that are of great importance in the field of molecular biology. There are a number of indicators used in technology of PCR for knowing the DNA of which (RAPD). This technology need mainly to the starters with a random sequence containing more than 50% of the rules Cytosine and Guanine (27). Abd-El-Haleem and others (2009) found when conducting genetic analysis of five varieties of wheat that genetic variation accounted for 70.13% and it is testing for RAPD using 8 random starters as it resulted in a 129bp (2). Soliman and Hendawy (2013) found when testing six varieties of wheat cultivated histologically to drought stress that the items have lost their ability to multiply and give copies of the tape of the DNA when grown on high concentrations of PEG using RAPD index and four random starters (24). Khavarinejad et al. (2013) showed when studying the genetic improvement ten varieties of wheat using index RAPD and six starters Random resulted in 33bp and SSR and five random starters resulted in a 17bp, as RAPD index detect genetic variation ratio, which amounted to 50 and

53% of the variety UBC350 and UBC109 sequentially, with SSR index detect genetic variation ratio, which amounted to 5 and 57% for the variety Xgwm469-6b and Xgwm120-2b sequentially (17). Shende and Walunj (2013) found when studying the genetic traits of the seventeen varieties of wheat using RAPD index and 25 random initially, the five starters only revealed the existence of the proportion of genetic variation ranged between 23-57% (23). Moghaieb et al. (2011) found when studying the nine varieties of Egyptian wheat tolerant to salinity number of bands reached the molecular weight of 82-118 bp and contrast Legacy of 69.5% when using RAPD index, while the SSR index revealed a number of bands reached the molecular weight of 42-59 bp and contrast Legacy of 71% (19). Bhutta and Hanif (2013) used RAPD and 240 initially random index in the detection of some varieties tolerant to salinity, as it gave the primer GLE-14 package molecular weight 970 bp found in varieties tolerant to salinity (6). El-Ameen (2013) showed in his study of the six varieties of wheat that the items non-tolerant to drought has failed to give the bands, while the primer B-02 seven bands in the category stoic drought revealed (11). Tahir (2008) found when his studies the genetic differences between varieties of soft wheat and coarse using index RAPD which gave bands amounted to a molecular weight of 70 -75bp, as it revealed the proportion of genetic variation were 40% in the soft wheat and 37.5% in coarse wheat (25). Grewal et al. (2007) showed when studying the genetic variation of the twentieth class of Indian wheat using RAPD index and 25 initially random gave 372 package contrast Legacy of 86.8% (15). Bibi et al (2009) indicated when studying the genetic improvement of the twelve varieties of wheat resulting from program hybridization using RAPD index and 14 initially random, it ranged molecular weight of bands resulting 42bp-5.3kb and contrast Legacy of 89.2% (7). Yagdi showed when studying the genetic improvement of the 16 varieties of wheat using RAPD index and 45 initially random, reaching the resulting number of bands 142 package ranged molecular weight have 300-2800 bp (28). In the study of genetic and phenotypic variations, Ahmad et al. (2013) found 41 cultivars of saline-tolerant wheat at seed germination stage using RAPD. Genetic similarity was 38-95%. The OPA-02 initiator gave a package with a molecular weight of 1000 bp, while the OPF- 13 bands weighing 1200 and 1400 bp in the tolerant varieties (3).

## II. Experimental And Method

In this study, three varieties of wheat Abu Ghraib, fathers, and Iraq of shoots produced from the seed germination of wheat seed laboratory and soaked with different concentrations of the fruits of *Citrullus Colocynthis* extract followed way (14). In the preparation of the extract, the fruits of *Citrullus Colocynthis* with milled fruits of dry *Citrullus Colocynthis* in crucible, and weight of 100 grams of *Citrullus Colocynthis* powder, add 250 ml of distilled water, and let it steep for one day. The next day was filtrated and then distributed in the centrifuge and quickly centrifuged 3000 cycle. Minutes<sup>-1</sup> for 10 minutes, and used concentrations (0.0, 50.0, 100.0, 150.0, 200.0) mL.L<sup>-1</sup> and added to the seeds in the early stage of germination to drink well, isolating the genetic material DNA of shoots soft transactions fruits of *Citrullus Colocynthis* extract (0.0, 50.0, 100.0, 150.0, 200.0) mL.L<sup>-1</sup> according to the method (10). I use the Wizard Genomic DNA Purification Kit to extract DNA. Four randomized starters obtained from the US Promega Corporation of origin each initially composed of ten random rules nucleotides and sequencing basement used as follows:

Primer code	Sequence(5' - 3')
OPA-02	TGCCGAGCTG
OPA-03	AGTCAGCCAC
OPG-05	CCCAGTCACT
OPB-09	TGGGGGACTC

Comprised combination DNA interaction (PCRPreMix) for one sample on the following:

Tris-HCl	10 Mm
KCl	30 Mm
dNtps(Datp,Dctp,dGTP,dTTP)	µM250
MgCl2	mM1.5
Taq DNA Polymerase	1 U = 1 µM

Equipped from Canadian BioNeer company origin, added to 2 µL of the primer and 2 µL of DNA sample and 11 µL of sterile distilled water, were separated beams using gel agarose concentration 2% and voltage 90 volts for two hours, and by comparing the multiplying bands of samples studied resulting from the interaction of RAPD with standard DNA bands and estimate molecular sizes were obtained outputs interactions

RAPD's DNA cultivars studied transactions according to the primer of the user type. All experiments carried out in a complete randomized design CRD, and global experiences and analyzed the results using statistical program genestate, and compared to the averages, according to test teams less moral (LSD) and at the level of 5% probability (12).

### III. Results And Discussion

#### The number of bands resulting molecular weight estimated using random primers varieties treated with different concentrations of the fruits of *Citrullus Colocynthis* extract

The figures (1) to (3) show the number of bands resulting weights molecular estimated samples DNA isolated from the extract transactions fruits of *Citrullus Colocynthis* is used in different concentrations (0.0, 50.0, 100.0, 150.0, 200.0) mL.L<sup>-1</sup> as well as the treatment of comparison using prefixes random OPA-02, OPG-05.

#### The primer OPA-02

Figure (1) indicates the number of bands resulting molecular estimated samples DNA isolated and weights. The from the use of the primer OPA-02 in concentration 50 mL.L<sup>-1</sup> to extract the fruits of *Citrullus Colocynthis* is equipped for food amid an implanted the seeds germinated for class fathers wheat two bands molecular weights ranged from (800- 1000 bp). 100 The concentration mL.L<sup>-1</sup> gave two bands molecular weight reached (650- 1000 bp). 150 The concentration mL.L<sup>-1</sup> gave three bands molecular weight reached (800-900 bp). The concentration 200 mL.L<sup>-1</sup> of the fruits of *Citrullus Colocynthis* soaked extract the seeds of class fathers wheat gave three bands molecular weight reached (600-1000 bp). The given comparative treatment of the first same one package molecular weight reached (800bp) the seeds germinated and soaked in fruit extract *Citrullus Colocynthis*.

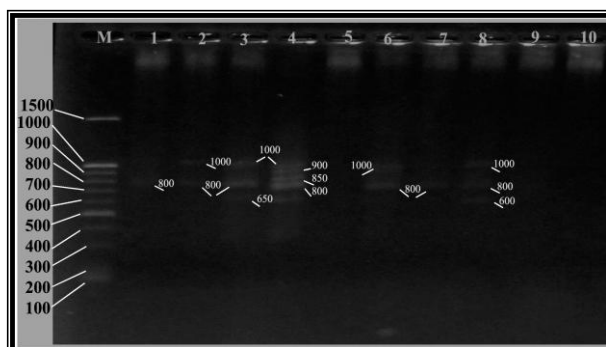


Figure (1): The results of the analysis RAPD- PCR using the primer 02- OPA in the category ofIbaa.

M: (DNA Ladder).

1. Treatment comparison.
2. 50 mL.L<sup>-1</sup> fruits of *Citrullus Colocynthis* extract.
3. 100 mL.L<sup>-1</sup> fruits of *Citrullus Colocynthis* extract.
4. 150 mL.L<sup>-1</sup> fruits of *Citrullus Colocynthis* extract.
5. 200 mL.L<sup>-1</sup> fruits of *Citrullus Colocynthis* extract.
6. Treatment comparison
7. 50 mL.L<sup>-1</sup> fruits of *Citrullus Colocynthis* extract.
8. 100 mL.L<sup>-1</sup> fruits of *Citrullus Colocynthis* extract.
9. 150 mL.L<sup>-1</sup> fruits of *Citrullus Colocynthis* extract.
10. 200 mL.L<sup>-1</sup> fruits of *Citrullus Colocynthis* extract.

This result from the use of the primer OPA-02 in concentration 100 mL.L<sup>-1</sup> to extract the fruits of *Citrullus Colocynthis* is equipped for food amid an implanted the seeds germinated for the variety Abu Ghraib of wheat 4 bands molecular weights ranged from (400- 1000 bp), the concentration 150 mL.L<sup>-1</sup> gave 6 bands molecular weight reached (100- 1200 bp), 200 the concentration mL.L<sup>-1</sup> gave three bands molecular weight reached (550-1000 bp), the concentration mL.L<sup>-1</sup> 50 of the fruits of *Citrullus Colocynthis* extract the seeds soaked product was Abu Ghraib gave 3 bands molecular weight reached (600-1000 bp), and gave and gave a comparative treatment of the first same one package molecular weight reached (550-1000bp) the seeds germinated and soaked in the fruits of *Citrullus Colocynthis* extract.

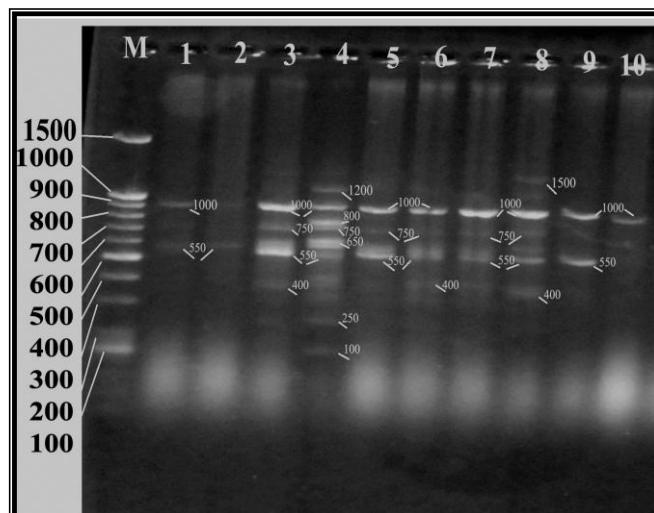


Figure (2):The results of the analysis RAPD- PCR using the primer 02- OPA in the category of Abu Ghraib. M: (DNA Ladder).

1. Treatment comparison.
2. 50 mL.L<sup>-1</sup> fruits of *Citrullus Colocynthis* extract.
3. 100 mL.L<sup>-1</sup> fruits of *Citrullus Colocynthis* extract.
4. 150 mL.L<sup>-1</sup> fruits of *Citrullus Colocynthis* extract.
5. 200 mL.L<sup>-1</sup> fruits of *Citrullus Colocynthis* extract.
6. Treatment comparison.
7. 50 mL.L<sup>-1</sup> fruits of *Citrullus Colocynthis* extract.
8. 100 mL.L<sup>-1</sup> fruits of *Citrullus Colocynthis* extract.
9. 150 mL.L<sup>-1</sup> fruits of *Citrullus Colocynthis* extract.
10. 200 mL.L<sup>-1</sup> fruits of *Citrullus Colocynthis* extract.

**The primer OPG-05**

The result from the use of the primer OPG-05 in concentration 200 mL.L<sup>-1</sup> of the fruits of *Citrullus Colocynthis* extract processed food grown amid the germinated seeds of the variety of wheat Iraq three bands ranged between molecular weights (200-480 bp) the concentration has 50 mL.L<sup>-1</sup> gave oneband molecular weight reached (100 bp). 100 the concentration mL.L<sup>-1</sup> gave nobands molecular weight reached (150-450 bp). and given the comparative treatment of the first seven bands the same molecular weight reached (100-450) in germinated seeds and soaked in the fruits of *Citrullus Colocynthis* extract.

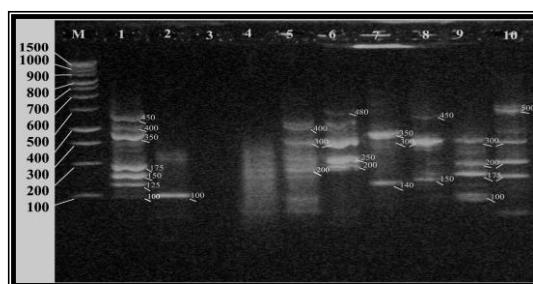


Figure (3): The results of the analysis RAPD- PCR using the initiator 05- OPG in the category of Iraq M: (DNA Ladder).

1. Treatment comparison.
2. 50 mL.L<sup>-1</sup> fruits of *Citrullus Colocynthis* extract.
3. 100 mL.L<sup>-1</sup> fruits of *Citrullus Colocynthis* extract.
4. 150 mL.L<sup>-1</sup> fruits of *Citrullus Colocynthis* extract.
5. 200 mL.L<sup>-1</sup> fruits of *Citrullus Colocynthis* extract.
6. Treatment comparison.
7. 50 mL.L<sup>-1</sup> fruits of *Citrullus Colocynthis* extract.
8. 100 mL.L<sup>-1</sup> fruits of *Citrullus Colocynthis* extract.
9. 150 mL.L<sup>-1</sup> fruits of *Citrullus Colocynthis* extract.
10. 200 mL.L<sup>-1</sup> fruits of *Citrullus Colocynthis* extract.

The non-appearance of the bands in certain transactions due to the absence of complementary sites for starters on the DNA samples, and this is in line with the specificity of interactions RAPD, as the lack of outputs doubled indicating a lack of complementary sites for starters on DNA transactions wheat studied (5). The bands lost or difference in their positions as a result of changing the complementary positioning of the first on the DNA studied transactions samples indicates that the impact extract the fruits of *Citrullus Colocynthis* to the induction of mutations, this assured (16), and the cut DNA disparate may result from differences in the correlation prefixes DNA plant or delete or add base or the number of rules nitrogenous components of the bar DNA of these processes are working on changes in the number of bands and molecular weights that appear after the separation bands process depending on the molecular weight in the agarose gel using electric paging device, and this would be able to activate certain genes or silencing of other genes and thus a change in one or more of the qualities found in the plant, and thus can identify the genetic differences between varieties and transactions and (4,20) happens. The genetic differences that emerged in the fruits of *Citrullus Colocynthis* extract transactions for varieties of wheat, may be due to a change in the sequence of nucleotides as a result of addition, deletion or re-arrangement of the nucleotides in DNA transactions cells varieties studied such as changes in body chromosomal or point mutations correlation, transit or the presence of what is known as jumping genes or change in the DNA organelles (8). Seen from the shapes (1-3) The RAPD index is efficient indicators to identify the genetic variation between species and plant varieties and even transactions, is its simplicity the possibility of using more of the first to learn about the different sequences and similarities in DNA bar as the simplicity of the electrical relay device it makes it easier to isolate the cut DNA, depending on the molecular weight, and this is in line with many of the researchers (17,19,23,24) who indicated the efficiency of PCR-RAPD technology to identify genetic variations.

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