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IN VITRO GENETIC VARIATIONS OF TWO RICE VARIETIES SEEDS USING (*CITRULLUS COLOCYNTHIS*) FRUITS EXTRACT

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Abstract

Two rice varieties seeds (Furata-1 and Yasamin) were laboratory geminated in (0.0, 100.0, 200.0 and 300.0) $mL.L^{-1}$ of (*Citrullus colocynthis*) fruits extract; the buds then dried and grinded. The DNA samples were extracted by electrophoresis, PCR-RAPD marker using the primers OPA-12,OPB-11 and OPC-13.PCR results depending on RAPD and electrophoresis for DNA samples which isolated from buds to two varieties and subjected to various concentrations of (*Citrullus colocynthis*) fruit extract the concentrations 200 and 300 $mL.L^{-1}$ showed differences in amplified bands, molecular weights by using primers OPA-12, OPB-11 and OPC-13, while PCR results depending on RAPD and electrophoresis for DNA samples, which isolated from buds to two varieties did not show differences in amplified bands and molecular weights using concentration 100 $mL.L^{-1}$ of (*Citrullus colocynthis*) fruit extract by using primers OPB-11 and OPC-13.

Key words : The extract of *Citrullus colocynthis* fruits, rice, PCR-RAPD.

Introduction

Rice (*Oryza sativa* L.) is one of the major food crops on which half of the world's population depends on feeding and important cereal crops in the world (Khush, 2005). Rice is considered a source of energy and carbohydrates to increase production systems for body (FAO, 2014). The rice planted over 114 countries but 90 percent of world's rice is produced in Asia. Genetic variations and Mutationsare the necessary and reliable basis for conducting selection and hybridization programs to obtain genetically modified varieties; a lot of genes are responsible for the appearance of genetic traits. Genetic mutations may occur in the organism or part of it; these mutations occur naturally or artificially such as chemical mutations caused by ethyl methane sulphonate (EMS) and sodium azide (SA) andphysical mutations caused by gamma rays (Patade and Suprasanna, 2009). Plant extracts contain many effective biochemical compounds used in many medical and therapeutic fields. These extracts may be taken from the entire plant or part of it. Many researchers have found that it (*Citrullus colocynthis*) extract contains compounds, including phenolic compounds, which are capable to stimulate genetic mutations. Nash *et al*. (1994) induced genetic mutations in a type of bacteria when treated with some flavonoids, the cause of genetic mutations in bacteria has been attributed to the presence of multiple hydrocarbon compounds, including flavonoids (Tomas-Barbera and Robins, 1997). Delazar *et al*. (2006) found in his study of phenolic compounds in (*Citrullus colocynthis*), he demonstrated the presence of large amounts of phenolic and flavonoid compounds found in all parts of the plant when conducting chemical tests on the plant. Mehni and Shahdadi (2014) demonstrated the importance of phenolic compounds in *Citrullus colocynthis* as antioxidants and free radicals, the highest concentration was 4.104 mg per 1 g dry weight and the lowest concentration was 3.696 mg/g dry weight. Abbas *et al*. (2012) demonstrated the ability of phenolic compounds in the *Citrullus colocynthis* seed extract to induce two-streptomycin-resistant and rifampicin-resistant mutations as genetic markers at the concentration of 125 $\mu g.mL^{-1}$. The technique of polymerase chain reaction (PCR) of important molecular techniques in the field of molecular biology. There are many markers that are used in the polymerase chain reaction technology to learn about DNA-RAPD marker. This technology need mainly to the primers with a random sequence containing more than 50% of the bases Cytosine and Guanine (Williams *et al*., 1990). Sadia *et al*. (2012)

found 35 varieties of rice using RABD and 14 that the cultivars had a high level of genetic relationship, there was a similarity between the rice varieties used in this study, which is expected to be due to their own predecessors and the selection of properties. Similar, rapid analysis is a simple and quick way to evaluate variance between different classes (Sadia *et al*., 2012). Dikshith *et al*. (2016) found in his evaluation of the relationship between rice varieties using ten RAPDs, two SRS and two STS markers of rice genotypes of 56 varieties of improved varieties and plant varieties collected from different parts of India the aggregation of these cultivars according to their genetic patterns as well as the apparent patterns of possible association or multi-phenotypic effects of genomic areas associated with some grain quality practices have been revealed (Disshith *et al*., 2016). Rajani *et al*. (2012) analyzed randomized DNA (RABD) to assess genetic diversity in ten selected rice varieties using 30 random randomized primers of the 30, 25 primers, RABD detects polymorphism while the remaining 5 primers do not show any reaction. Mahmoud *et al*. (2005) concluded that in studying the genetic variation of seven varieties of rice were established by using eight RAPD primers, six SSR primer pairs, eight AFLP primer combinations to DNA analysis could determine the different genetic patterns of rice and some genotypes of Egyptian rice the research probably originated from closely related ancestors and It was possessed a high degree of genetic similarity. Mehfuz and Raihan (2014) in the study of genetic variations among 25 species of rice from Bangladesh using amplified polymorphic DNA (RAPD) primers, of the first 60 samples, three were selected and used in a comparative analysis of different varieties of original Bangladeshi rice, this study provided a fast and reliable method for estimating the variability between different varieties that breeders could use to further improve varieties. Rajani *et al*. (2013) revealed a randomized DNA (RABD) analysis to assess genetic diversity in 10 selected rice varieties using decamer random primers out of 30, 25 RAPD primers revealed polymorphism while the remaining 5 primers showed no reaction. Nawroz (2014) used to study the variability and genetic diversity of ten varieties of Iraqi rice by using 20 decamer random primers and SDS remaining. Out of 22, 20 random amplified polymorphic DNA (RAPD) primers revealed polymorphism, while the remaining 2 primers showed no reaction and pointed out the usefulness of the genetic diversity results obtained in the selection of parents to develop rice varieties in the future. Malik (2008) used to analyze the genetic diversity of 10 traditional cultivars and 28 improved Pakistani rice using RABD markers and noted that RABD markers are a useful tool for assessing genetic differences between cultivars. Nantawan *et al*. (2011) used the RAPD and SRS markers to analyze the genetic diversity of saline tolerant rice varieties. While, Ravindra *et al*. (2012) using simple sequence repeats (SSR) markers for evaluation of genetic diversity in rice. Krupa *et al*. (2017) detection when studying molecular marker based genetic diversity analysis in rice genotypes using SSR markers, he pointed to the importance of studies of genetic variation in the selection of parents for hybridization because the improvement of healthy crops depends on the size of variance in the basic population. Hamza and Ali (2017) when stimulating the hereditary changes using the extract of *Citrullus colocynthis* fruits for embryos of the two genotypes of the two genotypes, indicated a difference in the number and molecular size of the multiplication bands. In this work, we used the extract of *Citrullus colocynthis* fruits to induce the genetic variations into two rice varieties seeds.

Experimental and Methods

Mature seeds of two genotypes of rice (Furata-1 and Yasamin) were soaked in distilled water for 24 hours. The seeds then were sterilized with 70% alcohol for 30 seconds, washed with distilled water three times, sterilized with ($HgCl_2$) 0.1% for 10 minutes and washed with sterilized distilled water three times and were placed on filter paper in petri dishes containing different concentrations of fruit (*Citrullus colocynthis*) extract (0.0, 100.0, 200.0 and 300.0) $mL.L^{-1}$ prepared from the solution 200 g (*Citrullus colocynthis*) fruits in 1000 mL distilled water and the extract was filtered, centrifuged over 3000 $cycle.min^{-1}$ for 10 min (Fleming, 2000) and diluted to the volumes above. The solutions prepared were added to the seeds in the early stage of germination. Isolation of DNA from buds resulting from seeds germination treated with fruit of (*Citrullus colocynthis*) extract concentrations (0.0, 100.0, 200.0 and 300.0) $mL.L^{-1}$ according to the method (Dellaporta *et al*., 1983) by using Genomic DNA Purification Kit to extract DNA. Five randomized primers obtained from the US Promega Corporation of origin each initially composed of ten random bases nucleotides and sequencing basement used as follows :

Primer code	Sequence (5´-3´)
OPA-02	TGCCGAGCTG
OPA-12	TCGGCGATAG
OPB-11	GTAGACCCGT

OPC-13	AAGCCTCGTC
OPC-15	**GACGGATCAG**

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

10 mM	Tris-HCl
30 mM	KCl
250µM	dNTPs(dATP,dCTP,dGTP,dTTP)
1.5mM	$MgCl_2$
1 U=1 µM	Taq DNA Polymerase

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. The molecular size of the DNA fragments was determined by the location of the beams of known molecular sizes derived from DNA fragments of the standard volumetric index (Zaid *et al*., 1999). Y-axis represents the molecular size values of the volumetric guide and X-axis represents the distances values that distance these beams from the loading holes inside the gel, the distance each band (double piece) were measured from the specimens of the studied samples and cast a column of that distance on the curve from this point of intersection, another column was dropped on the y-axis to represent the size of the multiplier (Sambrook *et al*., 1989). By comparing the multiplying bands of the studied samples from the RAPD reaction with standard DNA bands and estimating their molecular size, the RAPD reactions of DNA obtained the coefficients of the studied species and the type of initiator used.

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 figs. 1 to 3 shows 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, 100.0, 200.0 and 300.0) $mL.L^{-1}$ as well as the treatment of comparison using bands random OPA-12, OPB-11 and OPC-13.

The primer OPA-12

Fig. 1 indicates the number of bands resulting molecular estimated samples DNA isolated and weights. The primer OPA-12 showed when using the concentration 100 $mL.L^{-1}$ of *Citrullus colocynthis* fruits extract that added to the seeds in laboratory germination state for varietyrice of Furata-1 three bands molecular weights ranged from (500-750 bp). The concentration 200m $L.L^{-1}$ gave three bands molecular weight reached (500- 700 bp). The concentration 300 $mL.L^{-1}$ gave four bands molecular weight reached (500-850 bp). The given treatment comparison of gave two bands molecular weight reached (500 and 600 bp).

The primer OPB-11

The primer OPB-11 showed when using the concentration 100 $mL.L^{-1}$ of *Citrullus colocynthis* fruits extract that added to the seeds in laboratory germination state for variety rice of Furata-1 seven bands molecular weights ranged from 200- 800 bp, the concentration 200 $mL.L^{-1}$ gave eight bands molecular weight reached (200-850 bp), the concentration 300 $mL.L^{-1}$ gave nine bands molecular weight reached (200-900bp). The given treatment comparison of seven bands molecular weight reached (200, 800 bp), which did not differ from concentration 100 $mL.L^{-1}$ this is shown in Fig. 2.

The primer OPC-13

The primer OPC-13 showed when using the concentration 100 $mL.L^{-1}$ of *Citrullus colocynthis* fruits extract that added to the seeds in laboratory germination state for variety rice of Yasamin three bands ranged between molecular weights (100-400 bp), the concentration has 200 $mL.L^{-1}$ gave four band molecular weight reached (100-500 bp). The concentration 300$mL.L^{-1}$ gave five bands molecular weight reached (100-700 bp). The given treatment comparison of three bands molecular weight reached (100- 400 bp), which did not differ from concentration 100 $mL.L^{-1}$ this is shown in fig. 3.

The lostbands of the beams or the difference in their location due to the change of the complementary sites to the DNA, the samples of the studied treatments indicate the effect of the fruit *Citrullus colocynthis* extract in the induction of mutations, this assured (Hanacek *et al*., 2002). Different DNA fragments may result from differences in the binding of the nuclei to the plant DNA, deletion or addition of a base or number of nitrogen bases forming a DNA bar. These processes result in changes in the number and molecular weight of the beams that occur after the separation of the bands depending on

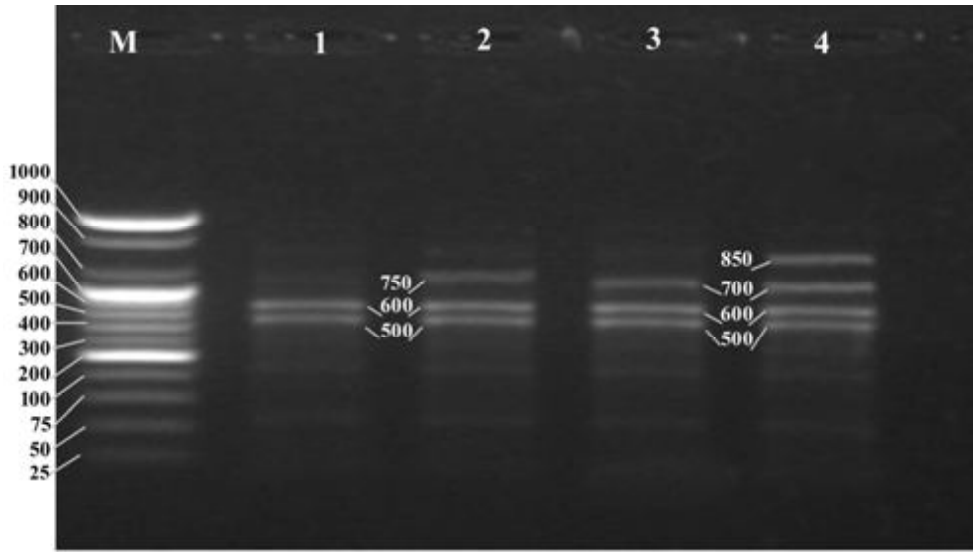

Fig. 1 : The results of the analysis RAPD- PCR using the primer OPA-12 in the variety Furata-1.
M: (DNA Ladder).
1. Treatment comparison(Distilled sterile water).
2. 100 mL.L^{-1} fruits of (*Citrullus colocynthis*) extract.
3. 200 mL.L^{-1} fruits of (*Citrullus colocynthis*) extract.
4. 300 mL.L^{-1} fruits of (*Citrullus colocynthis*) extract.

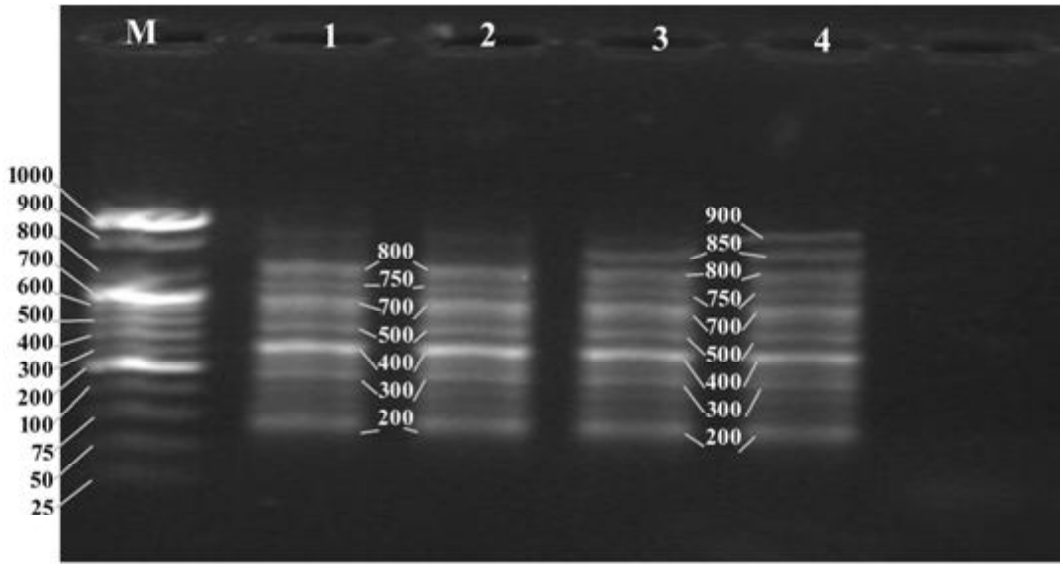

Fig. 2 : The results of the analysis RAPD- PCR using the primer OPB-11in the variety Furata-1.
M: (DNA Ladder).
1. Treatment comparison (Distilled sterile water).
2. 100 mL.L^{-1} fruits of (*Citrullus colocynthis*) extract.
3. 200 mL.L^{-1} fruits of (*Citrullus colocynthis*) extract.
4. 300 mL.L^{-1} fruits of (*Citrullus colocynthis*) extract.

their molecular weight in agarose gel using an electric relay, This would be able to activate specific genes or silence other genes and thus change in one or more of the traits present in the plant, thus identifying the genetic differences between the varieties and coefficients (Al-Arrqdi, 2013; Muler *et al*., 1990). The genetic differences that resulted of the use of fruit (*Citrullus colocynthis*) extract by using two rice varieties may be due to a change in the sequence of nucleotides as a result of the addition, deletion or rearrangement of nucleotides in the treated DNA of the used rice such as changes in the chromosomes of the body or point of link mutations (Brar and Jain, 1998). Figs. 1 to 3 showed that the RABD marker is an effective marker of genetic variation between species, plant varieties and (*Citrullus colocynthis*) concentration, which is consistent with Al. shemary and Baday, 2015). More than one primer can be used to know the sequence, differences and differences in DNA. Electrophoresis device can easily separate the DNA fragments depending on molecular weight this is in line with many researchers (Mehfuz and Raihan, 2014; Rajani *et al*., 2013; Malik, 2008 and Nantawan *et al*., 2011), who have referred to

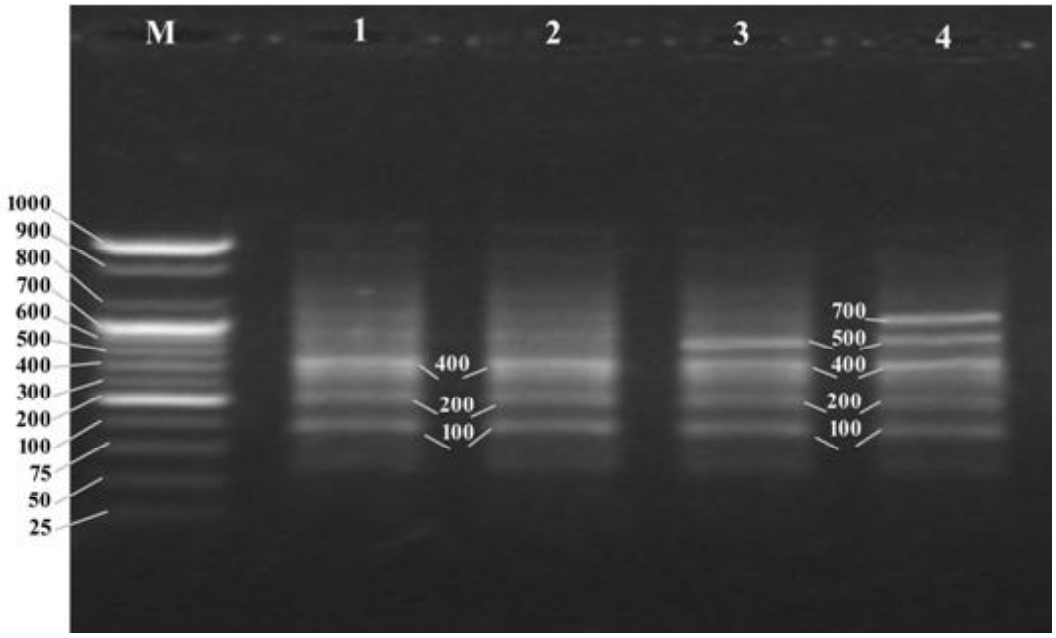

Fig. 3 : The results of the analysis RAPD- PCR using the initiator OPC-13in the variety Yasamin.
M: (DNA Ladder).
1. Treatment comparison (Distilled sterile water).
2. 100 mL.L^{-1} fruits of (*Citrullus colocynthis*) extract.
3. 200 mL.L^{-1} fruits of (*Citrullus colocynthis*) extract.
4. 300 mL.L^{-1} fruits of (*Citrullus colocynthis*) extract.

the efficacy of PCR-RADP technique to identify genetic differences.

Conclusion

We conclude from the research that the extract of *Citrullus colocynthis* fruits the ability to stimulate genetic variations in all concentrations under studyin both varieties of rice.

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