 doi:10.20944/preprints201706.0086.v2

Article

Arsenate Phytoremediation-linked Genes in Egyptian Rice Cultivars as Soil Pollution DNA Geno-Sensor

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Abstract: Rice (Oryza sativa L.) is the most important crop all over the world. It is considered the main food of 50% of the world population especially in Egypt. However, rice not only accumulates some of heavy metals such as cadmium but also accumulate arsenate (As). Arsenic contamination in water and food resulted in many implications for millions of people leading cancer. For that reason, three local rice cultivars; Sakha 102, 103 and 104 were propagated on modified Murashige and Skoog Basal Medium (MS medium) containing elevated concentrations of arsenate (0.1, 1 and 10 mg/l). The three varieties showed different resistant attitudes against arsenate. Extracted messenger RNA (mRNA) from treated and untreated Sakha 104 plantlets was scanned using differential display to demonstrate the differentially expressed genes in response to arsenate contamination. About 100 different RNAs with molecular sizes ranged between 1500 bp - 50 bp were obtained. Seven up-regulated and several down regulated genes were observed. The seven up regulated genes were sequenced and the sequence analysis described four genes are; disease resistance protein RPM1, Epstein-Barr virus EBNA-1-like, CwfJ family protein and outer membrane lipoprotein OmlA while the other three genes were hypothetical proteins. The four up regulated genes showed different percentage of similarity with other well identified genes that play role in arsenate phytoremediation such as; arsenate reductase, oxidase and aminoacylase 1. These genes were specifically induced in respond to the presense of arsnate in rice soil and they share in pathway to phytoremidate arsenate by the rice. In future work these genes can be used as geno-sensors for rice grains and soil contamination with As (V).

Key words: Soil arsenic pollution, Differential Display, Genes resistance and Rice Crop.

1. Introduction

Rice (Oryza sativa L.) is among the most important cereals widely grown worldwide. Rice is by far the largest food dietary source of As beside drinking water with elevated As content [1]. It is estimated that 1–24% of the tolerable intake of inorganic As is solely due to the consumption of As-containing rice

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[2]. It reaches plants grains such as rice and wheat as well as some vegetables and fruits via irrigation with As-contaminated water [3]. The predominant species of As in rice was arsenite (As III) followed by arsenate (As V) with dimethylarsinic acid (DMA) [4].

Arsenic has been reported as one of the water and soil pollutants worldwide including Egypt where high arsenic concentrations were detected in the underground water at El-Sharqia governorate [5]. Shakoor et al. [6] recorded As concentrations ranged between 1.5–201 µg/l in the groundwater rural areas of Punjab in Pakistan [6] where 53% of groundwater samples (n=62) showed As values higher than WHO safe limit (10 µg/l), it was 50 µg/L in Vietnam [7, 8]. In USA (Hanford) arsenate was determined in the surface soils and ranged from 30 to 270 mg/kg dry wt in the collected samples [9]. Total arsenic concentrations in the soils ranged from 1.6 to 17.1 mg/ kg in chinease soils [10]. Arsenic availability generally increases under flooding conditions, whereas the increase in the redox potential of flooded soils generally reduces As availability to plants [11,12].

Arsenic (As) is a bio-available and highly abundant metalloid in the earth's crust (2 mg/kg) in soil [13]. Mining and smelting activities, application of As-based herbicides and insecticides, sheep dips, wood preservatives, dyestuffs and irrigation with As-contaminated ground water/wastewater are the main anthropogenic sources of As soil contamination [14-17]. Rinklebe et al. [19] reported that both of wetland and water-logged soils are a good image for specific ecosystem facilities including; plant habitat and biodiversity, storage of organic carbon and hydrological buffering. The same importance was demonstrated by sediments in aquatic environment at. Trace element such as arsenate (As), Boron, Zn, etc has anthropogenic origin and their distribution and concentration affected by the environmental conditions (18). There is little evidence to suggest that arsenic (As), cadmium (Cd), lead (Pb) and mercury (Hg) play a nutritive role in higher plants and animals (18). Moreover, Giller et al. [20] revealed that a presence of trace element in human or animal serum is not inductor on their uptake from soils or plants but they could be determined.

Some plant species exhibit phenotypic variation in response to arsenic species giving idea about arsenic toxicity and the way with which plants have evolved arsenic resistances [21]. Arsenate tolerance is achieved by the suppression of high affinity arsenate/phosphate co-transport. models of plant tolerance to As V have been recently found and reported in tomato and rice [22]. Several studies were performed to examine the arsnate tolerance genes in plants but these genes have not been mapped [23]. Dasgupta et al. [23] screened 20 diverse genotypes of rice against their resistance to arsenate contamination and they found that Dawn cultivar is highly susceptible to arsenic contamination of soil. However, studies reported that arsenate reductase gene (ACR2) may not control the arsenate reduction in the plants grown in arsenate contaminated solis [24,25]. Instead, two genes of arsnate reducatse; AsV reduction and AsV tolerance were identified in Arabidopsis [26]. Additionally, plants suddenly reduce AsV to AsIII in the roots followed by efflux out into external medium [27-30]. Consequently, Induction of AsIII efflux by plant roots considered as a good to decrease As accumulation in plants. Until now, the pathway control membrane transporters for AsIII efflux in plant roots have not been characterized. Thus, we aimed in this study, to examined the differentially expressed genes (up and/or down regulated) in the treated plant seedlings with three different concentration of arsenate. Among these genes we can identify one which could be selectable DNA marker for the arsenate susceptible cultivars instead of the resistance ones. By this way, we can avoid the rice grains contains high amount of arsenate and prevent its serious effects on human health.

2. Materials and Methods

Soil Sampling and Preparation

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Two representative soil samples were collected (0–20 cm) from an area prepared for rice cultivation at El-Sharkeya Governorate, Egypt. Soil samples were air dried and sieved to ≈ 2 mm. Soil pH was determined in a suspension of 1:2.5 (w:v) of soil: water using glass electrode. Soil was ground to≈ 0.45 mm, extracted with 0.01 mol/kg CaCl2 to determine total elemental As concentrations [31] using ICP-MS (Prodigy Spec., Leeman Labs, USA). The irrigation water was also analysed for the As concentration.

Determination of Soil Anions and Cations

The K+ and Na+ available in the soil samples were extracted with 1N ammonium acetate adjusted to pH 7.0 and determined using flame photometer model PFP.7 Jenway [32]. Calcium and magnesium were determined by titrating the saturated extract with EDTA (ethylenediamine tetra-acetate disodium salt) (versenate) by using buffers and EBT indicator (Eriochrome black-T) to end point when solution turned into bluish green or greenish blue. Chloride was estimated using 0.01N AgNO3 [33,34]. Nitrate, phosphate and sulphate were determined in the soil samples by colorimetric method described by Ben Mussa [35] using Spectronic 21D UV/VIS Spectrophotometer. Absorbance of the extracted nitrate was measured at 410 nm while 880 nm was used to measure phosphate after the addition of ascorbic acid (colour reagent). Sulfates were determined using BaCl2 crystals method and absorbance was measured at 420 nm. Standard calibration curves for NO3, PO4 and SO4 were prepared as recommended by Ben Mussa [35]. Soil total C and N were measured by a combustion method using carbon, nitrogen and sulphur LECO analyzer (LECO CNS 2000).

Plant Materials

Three local rice cultivars; Sakha 102-104 were used in the present study (they are widely cultivated in Delta region). They were kindly provided by Dr. Ameen El-Sayed, department of Agronomy, faculty of Agriculture, Alexandria University.

Rice Micropropagation and Treatments

Based on the data obtained from the soil profile, rice grains were dehusked; surface sterilized with ethanol 70% for 3 min, followed by immersion in NaClO 50% (v/v) for 45 min and then washed three times with sterile distilled water [36]. Sterilized grains were cultured on Murashige and Skoog (MS) germination medium [37]. After 7 days, the seedlings were sectioned below the first node and the upper part of the coleoptile, and an explant 2 cm long was obtained. Explants were cultured on MS medium [38] supplemented with either 5 mg/L 6-benzylamino purine (BAP) or 0.2 mg/L 2, 4-dichlorophenoxyacetic acid [1]. The pH of the medium was adjusted to 5.8 using either HCl or KOH prior to the addition of 0.75% Sigma agar (A-1296) and autoclaved at 1.46 kg/cm2 for 20 min [36]. Three treatments of sodium arsenate (0.1, 1, and 10 mg/L) added to the MS germination medium were investigated compared with control one (0.0 AS). Eight glass Jars, each containing 5 grains were used for each treatment. Cultures in the glass Jars were incubated in a growth room at 35°C under a 14 h photoperiod regime and provided by cool white fluorescent lamps. Data and samples were collected one week after culturing.

Differential Display-PCR

RNA extraction; Sakha 104 cultivar was selected based on its high As-resistance. Total RNAs of the three treatments (0.1, 1, and 10 mg/L) were extracted using RNA easy kit according to manufacturer's instructions (QIAGEN). The RNA was dissolved in DEPC-treated water, quantified spectrophotometrically and analyzed using 1.2% agarose gel. Genetic pools were performed from the extracted RNAs of all the survival plants from each treatment [39].

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cDNA synthes; Reverse transcription reactions were performed using oligo (dT) primer (5`TTTTTTTTTTTTTTT`3). Each 25 µl reaction mixture containing 2.5 µl of 5x buffer with 2 MgCl, 2.5 µl of 2.5 m mol/l dNTPs, 1 µl of 10 p mol/l primer, 2.5 µl RNA and 0.2 µl reverse transcriptase enzyme. PCR amplification was performed in a thermal cycler (Eppendorf) programmed at 95 °C for 5 min, 42 °C for 1 h, 72 °C for 10 min and a stored at 4°C [39].

Differential Display reaction

Differential reaction was performed using three different arbitrary primers (Table 1) according to [40]. Two µl of synthesized cDNA were added to 23 µl of Taq DNA polymerase reaction mixture containing 10 m mol/l Tris HCl (pH 8.3), 25 m mol/l KCI, 4 m mol/l MgCl, 2 µl from the primer and 1 unit of Taq polymerase (AmpliTaq, Perkin- Elmer) and cycled first in a 9700 thermal cycler (Perkin-Elmer) programmed at 94 °C for 5 min, 56 °C for 5 min, and 72 °C for 5 min, followed by 40 cycles at 94 °C for 1 min, 56 °C for 1 min, and 72 °C for 2 min. Reaction was then incubated at 72 °C for 10 min for final extension. Two µl of loading dye was added prior to loading of 10 µl per gel pocket. Electrophoresis was performed at 80 Volt with 0.5 x TBE buffer in 1.5% agarose gel. Gel was stained in 0.5 µg/ml (w/ v) Ethidium bromide solution and destained in deionized water. Finally, gel was visualized and photographed using gel documentation system.

Table 1: Oligonucleotide sequence of the primers used in this study.

Primer	Sequence
CH R	5-TGCCTTTGATTCAGTCATC-3
DFR f	5-CAAAAAGCCCGAATACGATG-3
F3H F	5-AGAGAGGGGAAATATGTAGG-3

Cloning, Sequencing and Sequence Analysis of Up-regulated DNA Genes

To sequencing a PCR product amplified by arbitrary primers (contains more than one gene in the same molecular weight), the selected PCR bands were excised from the gel and the DNA was purified using a QIA quick gel extraction kit (Qiagen Inc., Germany). Purified DNAs were ligated into the pGEM-T vector (Promega Co., USA) to sequence only one copy of the target DNA. The ligation reaction was transformed into E.coli competent cells and the recombinant plasmids were selected and the plasmid DNA was directly sequenced using automated sequencer (Macrogene Company, Korea), with vector universal primer (Sambrook et al. 1998). DNA homology searches were carried out with the NCB1 data bases, using the BLAST network service [40].

Sequence Accession Number and Phylogeny Construction

Blast search for the obtained sequence was performed with the published homologous genes on database of National Centre for Biotechnology Information (NCBI). The obtained DNA nucleotide sequences were submitted into EST gene Bank under the accession numbers HO054970, HO054971, HO054972 and HO054973. Phylogentic analysis was carried out using MEGA4 program (http://www.megasoftware.net) [40].

3. Results

Physical and Chemical Characterization of Soil

Some physicochemical properties of two representative soil samples collected from El-Sharkeya Governorate, Egypt are illustrated in Tables 2-3. In that area, agricultural drainage water is As-contaminated and reused for soil irrigation. The slightly alkaline soils (X= 7.95 pH) showed high salt concentrations (Mean CEC= 153 mmolc/kg) dominated by Na+, Cl-, Ca2+, Mg2+, SO42-, K+ and finally PO43- with means of 1.2, 0.56, 0.44, 0.24, 0.21, 0.12 and 0.001 molc/kg, respectively (Table 2). These high

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salts concentration have strong effects not only on the microbial biodiversity, but also on the sensitive and semi-sensitive plant crops. In addition, they also disturb the relations between plant roots and microbial communities. Results revealed low organic matter content (X=1.9 %) as well as total N (X= 0.2 %) indicating low fertility (Table 3). The average total As content in soil was recorded 0.27 mg/kg.

Table 2 Soluble Cations and Anions in the 1:10 Aqueous Extract of the Horizon (A) of the selected soils.

Soil No.	Na+	K+	Mg2+	Ca2+	Cl-	NO3-	PO43-	SO42-	Σ cations	Σ anions
	molc/kg									
1	1.10	0.11	0.25	0.42	0.60	0.02	0.001	0.23	1.88	0.85
2	1.30	0.13	0.24	0.47	0.53	0.02	0.001	0.19	2.21	0.74

Readings are averages of 3 replicates

Table 3 Soil Properties and Arsenic content of the two collected soil samples (Aggregate Fraction ≈ 1 mm).

Soil No.	pH	Organic C	Total N	CEC	Total As Content
		g/kg		mmolc/ kg	mg/kg
1	8.0	20	1.5	120	0.24
2	7.9	18	0.9	186	0.30

Readings are averages of 3 replicates

Survival and Resistance of Rice Cultivars

Based on the soil analysis of the two collected samples, the jars MS experiment was designed and the number of survived rice plants cultured on MS medium supplemented with different concentration of sodium arsenate showed that Sakha 104 was the highest resistant cultivar with the two high arsenate concentration (1 and 10 mg/L), followed by Sakha 103 and Sakha 102 respectively (Fig. 1). This may be attributed to the genome of Sakha 104 which is able to express arsenate resistant genes more efficiently than the other two examined cultivars especially with the high concentrations of arsenate. On the other

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hand, the three cultivars showed the same response toward the low arsenate concentration (0.1mg/L).

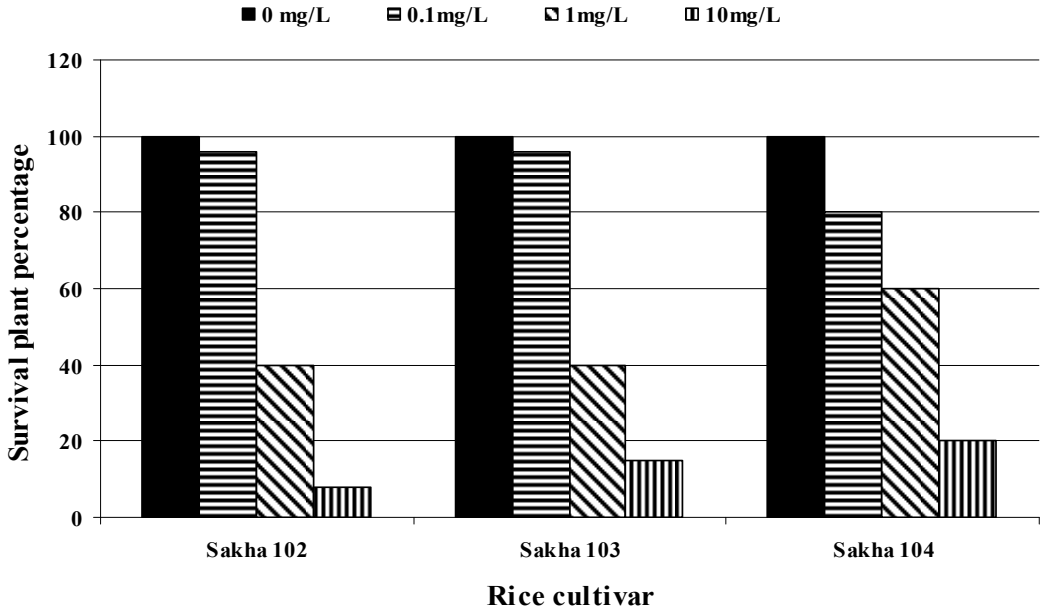

Figure.1 Number of survival Rice Plant Cultivars cultivated on MS medium and treated with different concentrations of Sodium Arsenate.

Molecular Characterization of Sakha 104 Rice Cultivar

The highest As resistance expressed by Sakha 102 (for the high concentration of As) rice cultivar was confirmed by the number of genes differentially displayed when treated with elevated concentrations of sodium arsenate (Fig. 2). Results revealed the presence of different band patterns with the three arbitrary primers used. About 100 bands were obtained with different molecular sizes ranged from 100 bp to 1.8 kbp. All the obtained bands were polymorphic except one monomorphic band that was shown with the F3H F primer at molecular weight ~800 bp. Seven up-regulated bands (indicated by the arrows) were cut, cloned and sequenced The sequence analysis revealed the isolated genes are; disease resistance protein RPM1, Epstein-Barr virus nuclear antigen 1 (EBNA1)-like, CwfJ family protein and outer membrane lipoprotein OmlA. The other three genes were hypothetical protein with more than 80% identity. The DNA band pattern explained how many genes were up-regulated (induced) when the plant was cultivated on medium contains arsenate. In addition, there are some down regulated genes (shut down) which suppressed in the treated plants but still active in the non-treated ones. Therefore it is concluded that, arsenate is able to induce specific genes and in the same time shut down others in the resistant cultivars.

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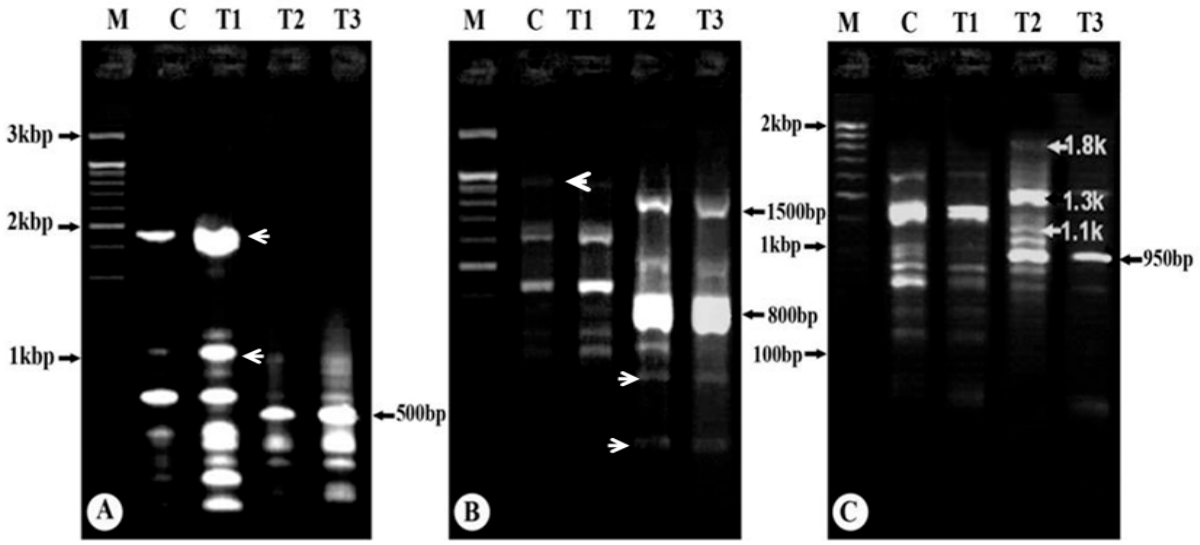

Figure 2 Differential display band patterns obtained by tested three arbiatary primers on RNS of Sakha 104 rice plants treated with elevated concentrations of sodium arsenate. The three arbitrary primers; CH R (A), DFR f (B) and F3H F (C). lanes; M: ladder DNA Marker 3kbp, C: control (0 mg/L) and T:Treated; T1: 0.1 mg/L, T2: 1 mg/L and T3: 10 mg/L of sodium arsenate. The arrows indicated to sample of the up-down regulated genes.

Phylogenetic analysis revealed that the is homology between the four induced genes in treated rice plant with arsenate. The four genes were divvied into two main groups; group one contains 3 PRM1, EBNA and CWFJ but the second group contains the fourth one (OmIA gene). The homology between PRM1 and EBNA is 100%, while the homology between both of PRM1& ENBA and CWFJ less than 80%.(Fig. 3A).

To prove the close relation between the isolated genes and the other published genes which playing an important role in arsenate reducation [23], arsnate oxidation [2] and accumulation using amynocylase-1 genes were compared with the four genes isolated in this study. Resulted presented in figure (3B) revealed that both the genes (PRM1, EBNA) showed similarity with the two genes (reductase ARC2 and aminoacylase 1) and all formed three clusters in one group. On the other hand, OmIA gene grouped with two amynoacylase 1 genes isolated from rice. Whereas, CWFJ gene grouped with the amine oxidase genes and similarity between them was 67%.The sequence alignments support the present results and prove that the isolated four genes from the treated rice playing an important role in arsenate; reduction, oxidation and/or accumulation in the plant tissues. Under in vitro (Murashige and Skoog (MS) agar medium) conditions supplemented with different concentrations of arsenic, the plant biomass was able to tolerate and accumulate 300 mg/l. They attributed such ability to arsenate reductase, oxidase and/or amynoacyase 1 genes that the plant might contains. These findings confirm the arsenate induced many specific genes that effect of rice tolerant to arsenate pollution and the potentiality of their use as arsenic pollution marker in rice grains as well as cultivated soil.

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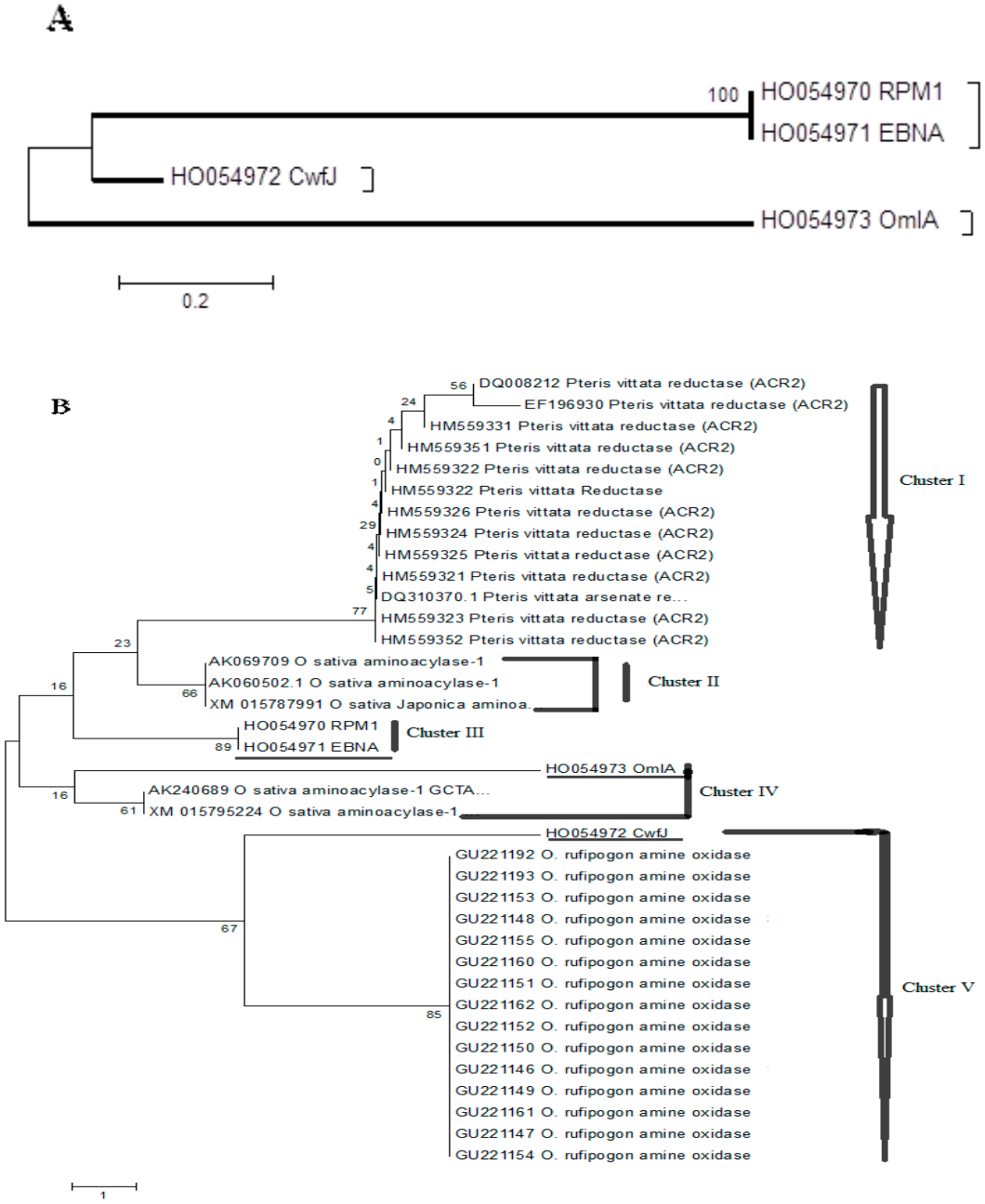

Fig. 3: Phylogentic trees constructed based on the obtained DNA neculotide sequence of the four induced genes in response to arsenat accumulation and the published genes which play roles in arsenate accumulation inside plants. A; phylogenetic between the four isolated genes with each other. B; phylogenetic tree of the four genes in compared with the arsnate reductase, oxidase and aminoacylase genes which published in Gene Bank and play roles in arsnate oxidation and reduction in plants such as; *Pteris vittata* and different cultivars of Rice. The phylogeny was constructed using MEGA 4 program (https://www.ncbi.nlm.nih.gov).

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4. Discussion

Physical and chemical analysis of soil in the study area revealed low fertility, high salt content and hazardous levels of As which are direct results of the severe nearby industrial pollution and the use of contaminated irrigation water. Arsenic contamination not only affecting soil biotic and abiotic elements, but also rendering the soil unfit for agricultural practices or of low quality for mass production. The expected results of As soil pollution become worse attributed to the fact that factors controlling distribution and transfer of heavy metals within the soil and vegetation systems are not always well defined [41]. Also because As speciation in the environment is complex, existing in both organic and inorganic forms, with inter-conversion between species regulated by biotic and abiotic processes [21].

Elevation of arsenic levels in soils causes considerable concern with respect to plant uptake and subsequent entry into wildlife and human food chains. Uptake of As by rice plants depends on the As species and soil conditions as reported by Abadan [42]. It was stated that arsenate became a dominant species in paddy soils under aerobic conditions, whereas under the submerged soil conditions the predominant species was arsenite. Mocrobial methylation of As is a well-known process in paddy soil systems, where inorganic species are converted into organic form [43]. Arsenate was found as the major component with lower levels of arsenite, monomethylarsenic acid (MMAA), and dimethylarsenic acid (DMAA). Although no experiment for arsenate uptake from polluted soils was carried out during the present study, similar results as those of Abadan are expected especially with the fact that arsenate is the dominant species in the tested Egyptian soil of El-Sharkeya Governorate [42]. It was shown that MMAA and DMAA can be taken up by rice roots at a slow rate, as a result and due to the restricted translocation of MMAA from roots to the areal parts of plant while DMAA could be a major component of total As in rice grains [44].

Rice cultivars tested in the present study showed variable resistance to As with the variant Sakha 104 being more efficient to express arsenate resistant genes than the other tested cultivars. Moreover, the defence system in the rice plant can be expanded to accumulate more concentration of arsenate if it is amended to the cultivation medium as well as that already present in the contaminated soils. This phenomenon in the arsenate- highly resistant rice cultivar represents a threat to human health, therefore it is recommended to avoid cultivation of rice in the contaminated areas. Also, it is well known that generally rice grains are much more efficient at accumulating As compared with other cereals [45] as a result of soil flooding during rice cultivation that leads to a rapid mobilization of As, mainly as arsenite. Xu reported that arsenic accumulation in rice grains was markedly increased (10-15- fold) under flooded conditions compared to aerobically grown rice [46]. Therefore, growing rice aerobically can dramatically decrease the As transfer from soil to grain due to the great reduction of As bioavailability.

The achieved results offer testable hypotheses for genes related o As tolerance (phytoremediation) that might offer strategy for mitigating As accumulation in consumed rice. Results indicated combination between the plant disease resistance genes with outer membrane lipoprotein, which affect the physiological parameters for the cell to diminish the effect of the (As). These results are in agreement with those obtained by Norton who demonstrated three-genes in rice contaminated with (As) and the involvement of epistemic interaction between these genes [47]. They proved also physiological evidence that genes related to phosphate transport are unlikely to be behind the genetic loci conferring tolerance which confirmed the induction of the Om1A among genes discovered in the present study.

It was reported that two genes (aminoacylase-1 and aquaporin NIP4) were induced when the rice plant was treated with (As) and both of them showed higher levels of expression in Bala cultivar [22, 45, 48]. The aquaporin gene is of interest as the class of this gene has been implicated in the transport of As (III) into roots [49]. Five genes have significant differences in gene expression under As (V) treatment in the candidate gene region on chromosome 10. Induction of three different hypothetical proteins in the

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treated rice cultivars especially those grown on medium contains high As concentrations (1 and 10 mg/l) is supported by results obtained by [50]. In that study two genes showed higher levels of gene expression in the rice cultivar (Bala) i.e. hypothetical protein and peptide transporter PTR2, and three have higher levels of expression in the rice cultivar (Azucena) i.e. glutathione S-transferase, cellulase-containing protein and protein CutA.

Boyes reported that RPM1 protein was localized using an epitope tag [50]. In contrast to previous suggestions, RPM1 is a peripheral membrane protein that likely resides on the cytoplasmic face of the plasma membrane. Furthermore, RPM1 is degraded coincident with the onset of the hypersensitive response, suggesting a negative feedback loop controlling the extent of cell death and overall resistance response at the site of infection. Moreover, Belkhadir [51] reported that most plant disease resistance proteins contain a series of leucine-rich repeats (LRRs), a nucleotide-binding site (NBS), and a putative amino-terminal signalling domain. They are termed NBS-LRR proteins and RPM1 is an NBS-LRR protein. The LRRs of a wide variety of proteins from many organisms serve as protein interaction platforms, and as regulatory modules of protein activation. Genetically, the LRRs of plant resistance proteins are determinants of response specificity, and their action can lead to plant cell death in the form of the familiar hypersensitive response. These genes may work in harmony with the outer membrane lipoprotein A (OmlA). It was documented [52] that in the citrus canker pathogen, Xanthomonas axonopodis pv. citri (X. citri), OmlA is co-regulated with the ferric uptake regulator and their expression is enhanced when X. citri is grown on citrus leaves, suggesting that these proteins are involved in plant-pathogen interaction. Therefore, the structure of OmlA does suggest that this protein may be implicated in protein–protein interactions required during X. citri infection. It can be concluded that Om1A protein may play an important role with membrane protein to resist the dangerous effect of (As) or at least prevent its entrance inside the cell. Moreover, CwfJ may be precursor for both Om1A and the outer membrane protein. But if the three genes failed to resist the (As) hassles, the LRRs then guide the cell into the death phase.

Epstein-Barr virus EBNA1 binds to the terminal repeat sequences of the viral genome and can activate DNA replication [53]. Till now there are no literature deals with the function of the CwfJ family protein except some reports which considered it as one of the defence proteins. It was suggested that these genes work in combination to resist the toxic effect of the arsenate and this agree with what postulated by Raab [54]. The mechanism of how they tolerate high (As) burdens in their tissues is not well understood, although tolerance is not due to enhanced phytochelatin production or metabolism of inorganic As to organic species. The role of methylation as a detoxification mechanism in plants has not been fully investigated, although it is clear that >10% of a plant's (As) burden can be dimethyl arsenate DMA(V) depending on As exposure concentrations and on nutrition [46, 54]. In the present study it is proved that at least three rice cultivars are able to accumulate high amounts of As. Results also confirmed that the genetic DNA marker obtained in this study can be efficiently used as diagnostic kit for arsenate contamination. The high percentage of homology of the four isolated genes from the treated rice cultivar (104) and the other published genes which share in arsenate reduction, oxidation and accumulation confirm they role in arsenate phytoremediation meanwhile the plant can survive and grow in contaminated soils with arsenate. These genes and their role in arsenate phytoremediation were not previously reported and this piece of work considered that is a new record in addition they can be used as new DNA markers for arsenate pollution discovery.

5. Conclusion

Among the three investigated local rice cultivars (Sakha 102-104); Sakha 104 showed the highest arsenate resistant over the other varieties (only with high conc. Of arsnate). Our finding is; the four genes (disease resistance protein RPM1, Epstein-Barr virus EBNA-1-like, CwfJ family protein and outer

membrane lipoprotein OmlA) are associated with the arsenate rsesistanceand/or accumulation in Rice. Such genes can be used as DNA markers for examining As (V)-contaminated soil and rice grains (Chao et al. 2014). Future work, new DNA markers for arsenate resistant genes should be arrayed, identified and used as a tool for characterizing sensitivity/susceptibility of rice genotypes.

Conflict of interest : The authors declare that they have no conflict of interest.

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