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Article

Arsenate-Resistant Genes in Egyptian Rice Cultivars as Soil Pollution Sensors

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Abstract: The main objective of the present study was to investigate arsenate [As (V)] resistance genes in rice cultivars grown in arsenic contaminated Egyptian soil in order to genetically induce resistance against arsenic in the local rice varieties as well as defining contaminated rice grains and/or soil. Three local rice cultivars; Sakha 102-104 were cultivated 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 with Sakha 104 being the most resistant. Extracted messenger RNA (mRNA) from treated and untreated Sakha 104 plantlets was scanned using differential display to demonstrate the arsenate resistant genes using three different arbitrary primers. About 100 different RNAs with (1500 bp - 50 bp) were obtained from which seven were up-regulated genes, subjected to DNA cloning using TOPO TA system and the selected clones were sequenced. The sequence analysis described four genes out of the seven namely 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. It is concluded the four induced genes in the resistant rice cultivar considered as a direct response to arsenic soil pollution. Genes detected in the present study can be used as geno-sensors for rice grains and soil contamination with As (V). Moreover, local rice cultivars may be genetically modified with such genes to induce high resistance and to overcome *arsenic* soil pollution.

Keywords: arsenic pollution; differential display; genes; resistance; rice crop; soil contamination

1. Introduction

Arsenic (As) is a bio-available and highly abundant metalloid in the earth's crust (2 mg/kg in soil). It is of great environmental and public concern since it is highly toxic and class 1 carcinogenic metalloid. It has a wide distribution in groundwater and all soils naturally contain As due to both anthropogenic activities and/or mineral dissolution as well as pedogenic content [51]. 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 [2, 3, 7, 16, 60]. Geochemical sources of As-contaminated soils include As-rich parent material where As easily substitutes for Si, Al or Fe in silicate minerals [11] or commonly associated with sulfides, e.g. in sulfidic ore deposits. Wind born soil particles, sea salt sprays and microbial volatilisation of As represent other natural sources of As [23, 42]. Arsenic has been reported as one of the water and soil pollutants worldwide including Egypt

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where high arsenic concentrations were detected in the underground water at El-Sharqia governorate [21]. Shakoor *et al.* (2015) recorded As concentrations ranged between 1.5–201 µg/l in the groundwater rural areas of Punjab in Pakistan [48] where 53% of groundwater samples (n=62) showed As values higher than WHO safe limit (10 µg/l). Arsenite {As(III)} constituted 13%–67% of total As in groundwater samples while arsenate {As(V)} ranged from 33% to 100%. Hazard quotient and cancer risk values of As were 11–18 and 46–600 times higher than the recommended values of US-EPA (i.e., 1.00 and 10−6, respectively). Consumption of As-contaminated groundwater induces a real health threat to the surrounded communities; therefore, remediation of the contaminated water resources is a must.

In a recent study by Azizur Rahman *et al.* (2015), chemical remediation of arsenic (As)-contaminated water using Fe-oxide-coated sand (IOCS) showed substantial decrease in As removal efficiency with time [6]. Moreover, residual As and other constituents (e.g. Fe, nitrate) in the remediated water had a significant effect on freshwater organisms such as *Lemna disperma*, *Chlorella* sp. CE-35, and *Ceriodaphnia cf. dubia*). This type of remediation was less efficient to suppress As(V) toxicity to *L. disperma* compared to As(III) while was highly efficient in reducing both As(III) and As(V) toxicity to *C. cf. dubia*. Results indicated that As(V) is more toxic microalga *Chlorella* sp. than As(III). Bioremediation using biological masses is however highly active technology for removal toxic metals including As. Teclua *et al.* (2008) reported that sulphate-reducing bacteria mixed culture (2 g/L) could efficiently remove about 77 and 55% of As(V) and As(III) respectively (1 mg/l of either species) within 24 h at pH 6.9 [55].

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 [56]. It is estimated that 1–24% of the tolerable intake of inorganic As is solely due to the consumption of As-containing rice [29]. It reaches plants grains such as rice and wheat as well as some vegetables and fruits via irrigation with As-contaminated water [10]. Compared with other cereals (wheat, barley and maize), rice is much more efficient at accumulating As into its grain [62]. The accumulation of As in rice was viewed as a recognized disaster for South-East Asia, where rice is a staple food. Meharg and Rahman reported accumulation of 1.7 mg As/kg in three rice samples from Bangladesh which is far exceeding the permissible limit in rice [39]. The predominant species of As in rice was arsenite (As III) followed by arsenate (As V) with dimethylarsinic acid (DMA) [61].

Arsenic levels in the edible parts of crops depend on As availability in soil and the uptake ability of a crop with subsequent translocation into the target organs. The availability of soil arsenic is determined by soil properties, mainly mineral composition, organic matter content, pH, redox potential and phosphate content [15, 17, 33, 57, 60]. Arsenic availability generally increases under flooding conditions, whereas the increase in the redox potential of flooded soils generally reduces As availability to plants [33, 34]. Because of the strong adsorption by Fe, Mn and Al oxides/hydroxides and clays [22, 25, 33, 34, 59], availability of arsenic in soils is generally low. The effects of chemical factors and the speciation of this metalloid on the bioavailability of As in soil have been the focus of many studies [24, 52]. However, few reports discussed the potential effect of soil physical conditions on the uptake of As by plants. Soil physical conditions, such as compaction, aeration and water availability, can have a significant influence on the growth and function of roots, transport of ions to the root surface, and leaching of solutes out of the rooting zone. Soil compaction and water availability have been shown to affect the uptake of major nutrients (N, P, K, Ca, Mg and S) and micronutrients (Mn, Fe, Zn and Cu) [5, 27, 31, 32, 43, 48, 67].

Toxicity of arsenate (the dominant form of arsenic phyto-available in aerobic soils) in un-resistant plant species ranges from inhibition of root growth to death. It is evident that exposure to inorganic arsenic species results in the generation of reactive oxygen species (ROS) through the conversion of arsenate to arsenite [37]. Arsenate tolerance is achieved by the suppression of high affinity arsenate/phosphate co-transport. It was shown that arsenate hyper-tolerance in *Cytisus striatus* and grasses are mainly due to reduced arsenate uptake through suppression of phosphate transporter activity [13]. A single allele detected in all populations of the native grass *Holcus lanatus* but at increased frequency in populations from As-contaminated soils, suppresses high affinity

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arsenate/phosphate co-transport. This adaptive tolerance is also reliant on constitutive detoxification of As within the plant [37]. However, other models of plant tolerance to As V have been recently found and reported in tomato and rice [64]. 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 [44]. 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. This could be used to produce plant cultivars that are more arsenic resistant or that have reduced arsenic uptake [37]. Recently, Casas-Flores *et al*. (2015) isolated arsenic tolerant bacterial community from two mine heap samples at up to 66 m depth and identified it using 16S rDNA gene sequence analysis. It was dominated by taxa of three main metabolic groups: chemolithotrophs (*Leptospirillum, Sulfobacillus*); chemolithoheterotrophs and organoheterotrophs (*Acidovorax temperans, Pseudomonas alcaligenes, P. mendocina, Sphingomonas* spp., *Leptospirillum* spp. and *S. thermosulfidooxidans* in the deepest sample of the oldest, highest-temperature heap. Results indicated arsenic resistance in the microbial community, therefore specific primers were used to amplify ars (arsenic resistance system), aio (arsenite oxidase), or arr (arsenate respiratory reduction) genes from total sample DNA. Presence of arsB genes in *S. thermosulfidooxidans* in the Q63–66 cultures permits H3AsO4-As(V) detoxification and strengthens the community's response up to 0.5 g/l As [18].

Since it is highly important to find a modern technology for detection of As contamination in rice grains, the present study was aimed to investigate arsenate (As) resistant genes in rice cultivars grown in As-contaminated Egyptian soil. Such genes can be used as DNA markers for examining As-contaminated soil and rice grains and further to induce resistance against As in the local rice varieties.

2. Materials and Methods

2.1. Soil Sampling and Preparation

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 M $CaCl_2$ to determine total elemental As concentrations [28] using ICP-MS (Prodigy Spec., Leeman Labs, USA). The irrigation water was also analyzed for the As concentration.

2.2. 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 [45]. 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 $AgNO_3$ [12, 45]. Nitrate, phosphate and sulphate were determined in the soil samples by colorimetric method described by Ben Mussa 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) [9]. Sulfates were determined using $BaCl_2$ crystals method and absorbance was measured at 420 nm. Standard calibration curves for NO_3, PO_4 and SO_4 were prepared as recommended by Ben Mussa [9]. Soil total C and N were measured by a combustion method using carbon, nitrogen and sulphur LECO analyzer (LECO CNS 2000).

2.3. 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

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2.4. Rice Micropropagation and Treatments

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 [40]. 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 [46] supplemented with either 5 mg/l 6-benzylamino purine (BAP) or 0.2 mg/l 2, 4-dichlorophenoxyacetic acid [65]. 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/cm^2 for 20 min [36]. Four treatments of sodium arsenate (0, 0.1, 1, and 10 mg/l) added to the MS germination medium were investigated. Eight glass flasks, each containing 5 grains were used for each treatment. Cultures in the glass flasks 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.

2.5. RNA Extraction and Preparation

Sakha 104 cultivar was selected based on its high As-resistance. Total RNAs of the four treatments (0, 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.

2.6. Reverse Transcription of RNA

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.

2.7. Differential Display

Differential display was performed using three different arbitrary primers [35] (Table 1). Two micro liter 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: Sequences of Arbitrary Primers Used in the Present 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

The resultant PCR product was excised from the gel and purified using a QIA quick gel extraction kit (Qiagen Inc., Germany). Purified DNAs were legated into the pGEM-T vector (Promega Co., USA). Recombinant plasmids were then directly sequenced using automated sequencer

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(Macrogene Company, Korea), with vector universal primer. DNA homology searches were carried out with the NCB1 data bases, using the BLAST network service [4].

2.8. 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) [54].

3. Results

3.1. 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 $mmol_c/kg$) dominated by Na^+, Cl^-, Ca^{2+}, Mg^{2+}, SO_4^{2-}, K^+ and finally PO_4^{3-} with means of 1.2, 0.56, 0.44, 0.24, 0.21, 0.12 and 0.001 mol_c/kg, respectively (Table 2). These high 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. Whether or not such levels correlate with the maximum permissible limits for good soil [21, 30, 49], the severe toxicity of As considers these figures as risky levels plus the potential of the soil to build it up to extremely toxic levels. This is true since soils with low total concentrations of trace metals showed higher relative mobility than soils with elevated concentrations [19].

Table 2: Soluble Cations and Anions in the 1:10 Aqueous Extract of the Horizon (A) of the Selected Soils

Soil	Na^+	K^+	Mg^{2+}	Ca^{2+}	Cl^-	NO_3^-	PO_4^{3-}	SO_4^{2-}	Σ cations	Σ anions
						mol_c/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: Selected Soil Properties and Arsenic content of the Two Soils (Aggregate Fraction ≈ 1 mm)

Soil	Soil Properties				
	pH	Organic C	Total N	CEC	Total As Content
		g/kg		$mmol_c/kg$	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

3.2. Survival and Resistance of Rice Cultivars

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 even at the highest investigated contamination level of arsenate (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.

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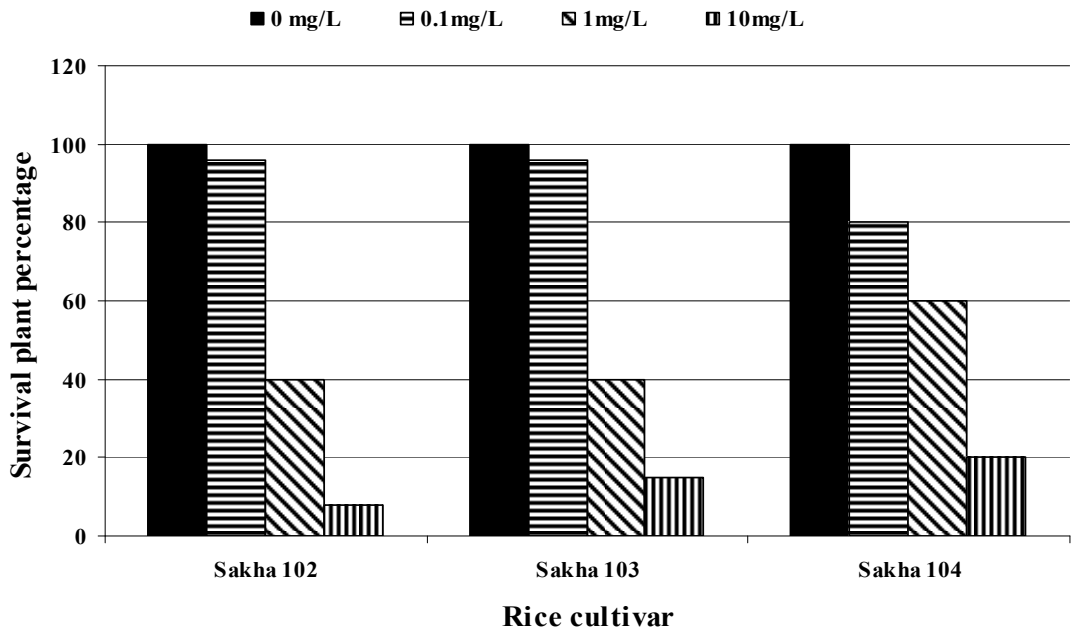

Fig. 1: Survival Percentage of the Rice Plant Cultivars at Different Arsenate Concentrations

3.3. Molecular Characterization of Sakha 104 Rice Cultivar

The highest As resistance expressed by Sakha 104 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 subjected to DNA sequencing. The DNA nucleotide sequence revealed the following genes; 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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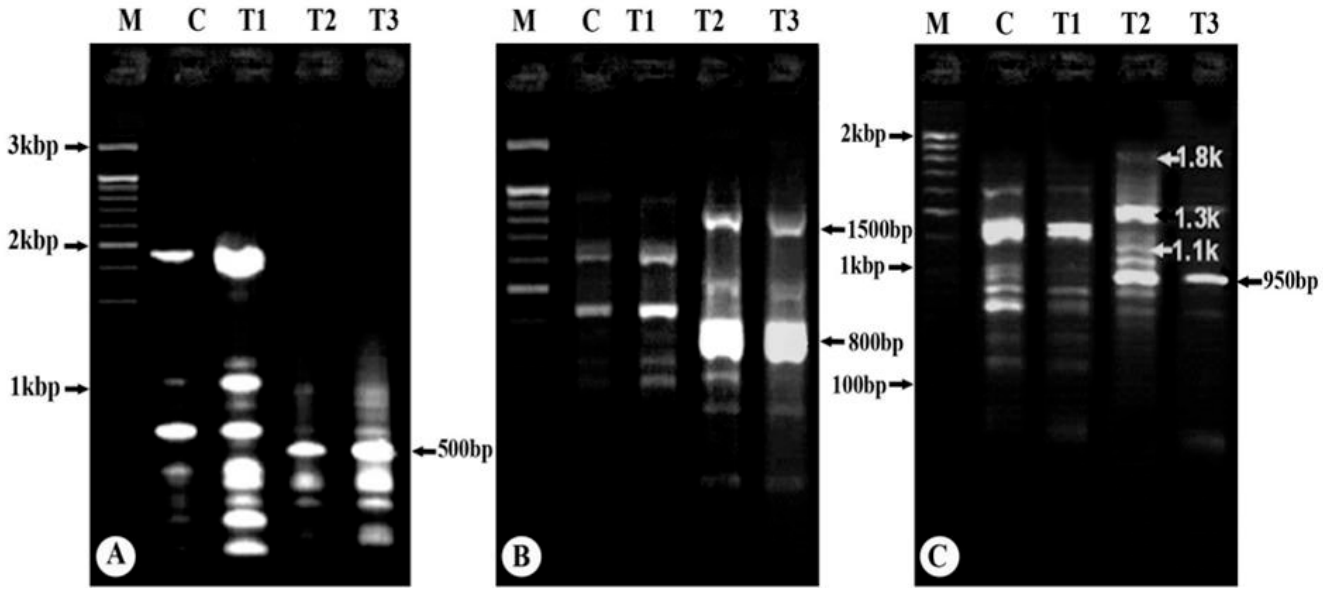

Fig. 2: Differential display for Sakha 104 rice cultivar samples treated with elevated concentrations of sodium arsenate using three arbitrary primer, CH R (A), DFR f (B) and F3H F (C). lanes; M: ladder DNA Marker, C: control (0 mg/l), T1: 0.1 mg/l, T2: 1 mg/l and T3: 10 mg/l of sodium arsenate

Phylogenetic analysis revealed that the disease resistance protein and the Epstein-Barr virus nuclear antigen 1 are closely related to each other with 100% identity while CwfJ is less similar to the outer membrane lipoprotein.

To prove the close relation between the isolated genes and the arsenate efflux in the rice plant only two arsenate resistant genes isolated from rice were aligned with arsenate resistant genes in brake ferns (*Pteris vittata*) and bacteria (Fig. 3 A & B). Data presented in the Figure 3A showed the high similarity between the isolated gene and the reducatse gene (gi|310768411) isolated from *Pteris vittata* ecotype. The sequence alignments support the present results and prove that the isolated rice gene play an important role in arsenate reduction and it might help the plant to resist the accumulated arsenic in the soil. Sarangi and Chakrabarti reported that brake fern ecotype characterized by remarkable arsenic tolerance and accumulation ability [47]. Under *in vivo* (soil) and *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 gene that the plant might contain. Moreover, Figure 3B revealed that similarity between the cfwj and the arsenate efflux gene is so high. These findings confirm the specific effect of rice resistant isolated genes and the potentiality of their use as arsenic pollution marker in rice grains as well as cultivated soil.

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Fig. 3A

Fig. 3B

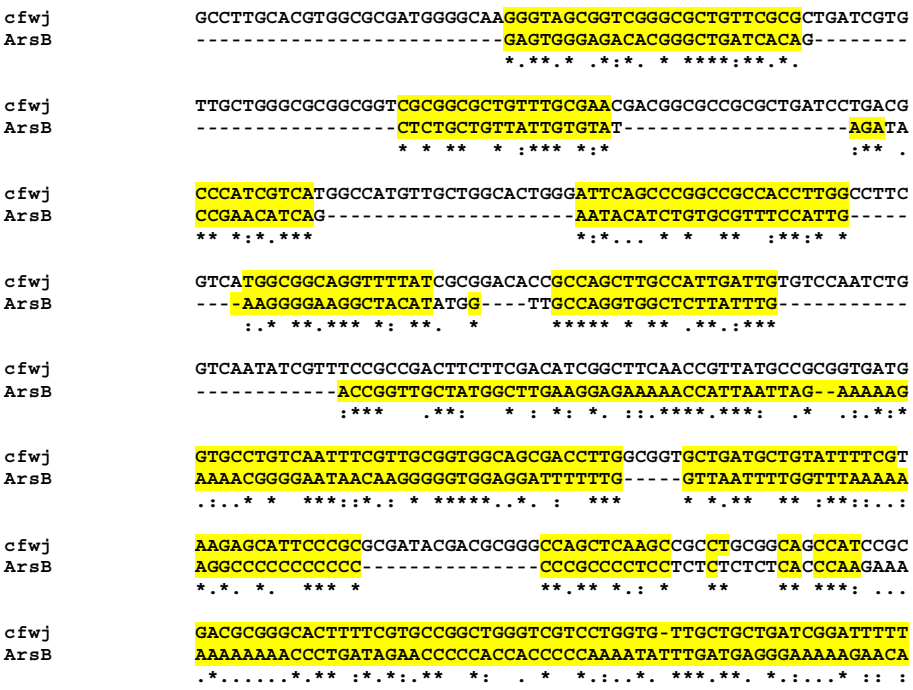

Fig. 3: Sequence Alignment between A) Arsenate Resistant Rice Genes and Arsenate Reductase Gene Isolated from *Pteris vittata* Ecotype, and B) ArsB gene for Putative Arsenate Efflux Pump Isolated from *Achromobacter* sp and the cfwj Isolated from the Rice Plant Treated with Arsenate. Highlighted Blocks Indicate the Similarity between Genes

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 [19]. 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 [37].

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 [1]. 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

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process in paddy soil systems, where inorganic species are converted into organic form [53]. 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 [1]. 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 [26].

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 [62] 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 [63]. 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 to As tolerance 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 [41]. 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.

Xu and Zhang discovered that two genes (aminoacylase-1 and aquaporin NIP4) were induced when the rice plant was treated with (As) and both of them have higher levels of expression in Bala cultivar [63, 64, 66] . The aquaporin gene is of interest as the class of this gene has been implicated in the transport of As (III) into roots [38]. 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 treated rice cultivars especially those grown on medium contains high As concentrations (1 and 10 mg/l) is supported by results obtained by Norton [41]. 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 [14]. 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 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 [8]. 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 [58] that in the citrus canker pathogen, *Xanthomonas axonopodis* pv. *citri* (*X. citri*), OmlA is co-regulated with the ferric uptake

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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 [20]. 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 [44]. 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 [41, 44]. 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.

5. Conclusion

Among the three investigated local rice cultivars (Sakha 102-104); Sakha 104 showed the highest arsenate resistant over the other varieties. DNA sequence analysis of the resistant rice cultivars described four Arsenate-resistant genes (disease resistance protein RPM1, Epstein-Barr virus EBNA-1-like, CwfJ family protein and outer membrane lipoprotein OmlA) in addition to three genes for hypothetical proteins. Induction of the four identified genes in the resistant rice cultivar considered a direct response to arsenic soil pollution. Such genes can be used as DNA markers for examining As(V)-contaminated soil and rice grains. Moreover, local rice cultivars may be genetically modified with such genes to induce high resistant cultivars and overcome *arsenic* soil pollution problem

Future Expectations of the Achieved Results

Results of the present study would help in the future research to screen all the available rice genotypes and can be used to identify the ability of the examined genotypes to resist and accumulate arsenate. Consequently, the farmers will be aware about selecting the suitable genotypes (sensitive genotypes) for cultivation in the contaminated sites. Moreover, the accumulated arsenate will never transfer to the human through rice. On the other hand, new DNA markers for arsenate resistant genes will be tested and used as a tool for identifying sensitive rice genotypes.

Conflict of interest :

The authors declare that they have no conflict of interest.

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