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Journal of Agroalimentary Processes and Technologies 2024, 30 (3), 274-281

Journal of Agroalimentary Processes and Technologies

Genetic DNA polymorphism induced by samarium (Sm) in the macrophyta *Ulva lactuca* alga as revealed by TU-DAMD marker

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Abstract

DNA change profile in the green *Ulva lactuca* alga after 2 days of exposure to different Samarium (Sm) (0.25, 0.5, 0.75 and 1 mg/L) concentrations, has been evaluated using touch-up directed amplification of minisatellite DNA (TU-DAMD) marker. Genomic template stability (GTS%) and band-sharing index (BSI) values as qualitative measure of DNA changes followed Sm treatment were calculated under the above applied Sm concentrations. Data showed that GTS% and BSI values followed similar tendency; in this regards, GTS% value increased from 37.891 to 50.865%, whereas, BSI value increased from 0.505 to 0.581 as Sm applied concentration increased from 0.25 to 1 mg/L Sm. The present study could be considered as the first report describes genomic instability induced by Sm in U. lactuca alga. Indeed, TU-DAMD marker could serve as a potent tool for monitoring genomic instability induced by Sm in *U. lactuca* alga in ecosystems. This data revealed new insight concerning genomic instability induced by Sm on marine algae.

Keywords: Genomic template stability (GTS%), samarium (Sm), touch-up directed amplification of minisatellite DNA (TU-DAMD), *Ulva lactuca*.

1. Introduction

The use of lanthanides in agriculture and in aquatic cultures is in gradual augmentation all over the world. Thereby, growing demand on their application in modern technology caused increased in their amount on the environment and consequently, arise as a serious environmental threat. Lanthanides as nonessential elements can induce either positive or/and negative physiological responses in the living organism [1].

According to the International Union of Pure and Applied Chemistry (IUPAC), rare earth elements (REEs) can be classified into two categories based either on their atomic weight or on their position on the Periodic Table of elements: Light rare earth elements (LREEs) including lanthanum, cerium, praseodymium, neodymium, promethium, and samarium with the atomic numbers from 57 to 62; and heavy rare earth elements (HREEs) including europium, gadolinium, terbium, dysprosium, holmium, erbium, thulium, ytterbium, and lutetium with the atomic numbers from 63 to 71 [2].

Samarium (Sm) among lanthanides**,** a rare earth element is the sixth element in the chemical periodic table and the 40th most abundant element in the Earth's crust and is more common than such metals as tin. It displayed different application forms *e.g.* in samarium–cobalt magnets, as catalyst and chemical reagent. World resources of samarium are estimated at two million tonnes; they are mostly located in China, US, Brazil, India, Sri Lanka and Australia, and the annual production is about 700 tonnes [3]. Samarium is the hardest and most brittle of the rare earth

10.59463/JAPT.2024.2.23

elements, with diverse applications. It serves as an absorber in nuclear reactors and as a catalyst in ethanol dehydrogenation and dehydration processes. The radioactive isotope ^{153}Sm is used in cancer treatment. Samarium is also widely used in the production of samarium-cobalt alloy magnets, commonly found in electric guitars, small motors, and headphones. Additionally, it aids in doping calcium fluoride crystals for optical lasers, and its oxide [4].

The impact of lanthanides depended on the combination of species, element, and light intensity. Bioaccumulation of lanthanides metals in algae has been reported at physiological level; *e.g.* biosorption of lanthanum (La), europium (Eu) and ytterbium (Yb) by *Sargassum polycystum* brown alga [5]; biosorption of lanthanum (La), cerium (Ce), europium (Eu) and ytterbium (Yb) REEs by *Turbinaria conoides* brown alga [6]; biosorption of europium (Eu), gadolinium (Gd), lanthanum (La), Neodymium (Nd), praseodymium (Pr) and Sm ERRs by *Sargassum* sp. brown macroalga [7]; biosorption of praseodymium (Pr) REE by *U. lactuca* green alga [8]; cerium (Ce), gadolinium (Gd), and lutetium (Lu) mixture effects on *Raphidocelis subcapitata* and *Chlorella vulgaris* microalgae [9]. Recently, Bergsten-Torralba et al. (2020) [10] reported La, Nd and Sm toxicity on algae, microcrustaceans, and fungi; the previous study revealed that Nd was the most toxicant element against all studied organisms.

It is been documented that metals stress caused DNA damage (single and double strands breaks, modified base pairs, point and deleted mutations) in plants, reflected in DNA changes pattern (appearance of new DNA bands or/and disappearance of normal DNA bands) [11]. These changes profile has been successfully investigated in living organisms including some plants (aquatic and terrestrial), lichens and algae, using different molecular markers systems; *e.g.* random amplified polymorphic DNA (RAPD) marker in *Hydrilla verticillata* aquatic plant exposed to cadmium (Cd) and mercury (Hg) heavy metals [12]; RAPD marker in *Pseudevernia furfuracea* lichen [13]; RAPD marker in maize (*Zea mays* L.) exposed to Chromium (Cr) [14]; RAPD merker in cumin (*Cuminum cyminum*) exposed to Cd metal [15]; RAPD marker in rye (*Secale cereale* L.) exposed to Pb metal [16]; random amplified microsatellite polymorphism (RAMP) marker in the green *U. lactuca* alga exposed to copper (Cu), lead (Pb), cadmium (Cd) and zinc (Zn) heavy metals [17]; RAPD marker in *U. lactuca* green alga exposed to Cd heavy metal [18] and *Padina pavonica* brown alga exposed to Cd heavy metal [19] and recently, RAPD marker in rice (*Oryza sativa* L.) cultivars exposed to Arsenic (As) heavy metal [20].

To our knowledge, the majority of experiments deal with lanthanides and algae was focused on physiological algal response (growth parameters) regardless understanding their beneficial effects mechanisms. Their specific effects in environmental ecosystems on plants particularly on algae, has poorly discussed. To date, little is known about Sm induced alteration in GTS% value on the green *U. lactuca* alga. Thereby, the current study deal with DNA genetic variation induced by Sm metal in *U. lactuca* macroalga using TU-DAMD marker for the first time.

2. Materials and method

2.1. Algal samples collection and chemicals treatment

U. lactuca alga samples were collected along the Syrian coast of the Mediterranean Sea. Algal sampling and cultivation were performed as reported by Saleh (2016a) [17]. Samples were treated with different Samarium (Sm) [1006 ppm of $Sm(NO_3)_3.6H_2O$ from AccuStandard-USA] (0.25, 0.5, 0.75 and 1 mg/L) concentrations with five replicates/treatment. Experimental assay has been carried out under laboratory conditions for 2 days as previously reported by Saleh (2016a) [17]. Then samples were collected for molecular study.

2.2. DNA Isolation

Algal genomic DNA was isolated from (bulk of 5 replicates/ treatment) tissues for both of the control and Sm-treated alga by a CTAB (cetyltrimethylammonium bromide) protocols as previously described by Doyle and Doyle (1987) [21]. DNA concentration was quantified by DNA Fluorimeter at 260/280 nm and adjusted to final concentration of 10 ng/µL. DNA was stored at -80 °C until needed.

2.3. TU-DAMD assay

To investigate DNA genetic variation induced by Sm metal in *U. lactuca*

macroalga, TU-DAMD test has been carried out as recently reported by Saleh (2021) [22] using 20 DAMD primers (Table 1). Final PCR products were separated by electrophoresis at 85 V for 2.5 h, and visualized with a UV transilluminator. PCR amplification products size was estimated with a VC 100 bp Plus DNA Ladder (Vivantis) ladder standard.

Table 1. Selected DMAD primers employed in the current study

Primer N°	Primer name	Primer sequence 5'-3'
1	URP1F	ATCCAAGGTCCGAGACAACC
2	URP4R	AGGACTCGATAACAGGCTCC
3	URP25F	GATGTGTTCTTGGAGCCTGT
4	URP38F	AAGAGGCATTCTACCACCAC
5	HVR(-)	CCTCCTCCCTCCT
6	FVIIex8C	CCTGTGTGTGTGCAT
7	FVIIex8	ATGCACACACACAGG
8	HBV5	GGTGTAGAGAGGGGT
9	YNZ22	CTCTGGGTGTGGTGC
10	14C2	GGCAGGATTGAAGC
11	33.6	GGAGGTGGGCA
12	6.2H(+)	AGGAGGAGGGGAAGG
13	PM13	GAGGGTGGCGGCTCT
14	HBVb	GGTGTAGAGAGAGGGGT
15	HVRc	CCTCCTCCCTCCT
16	HVR	GGAGGTTTTCA
17	URP6R	GGCAAGCTGGTGGGAGGTAC
18	URP17R	AATGTGGGCAAGCTGGTGGT
19	HVA	AGGATGGAAAGGAGGC
20	HVV	GGTGTAGAGAGGGGT

2.4.Genomic template stability (GTS%)

Genomic template stability (GTS%) value was calculated as previously described by Atienzar et al. (2002) [11] according to the following formulate:

GTS% = (1 – a/n) * 100

Where (a) was TU-DAMD polymorphic profiles detected in each samples treated and (n) the number of total bands in the control. Polymorphism observed in TU-DAMD profiles included disappearance of a normal band and induction of a new band in comparison to the control TU-DAMD profiles.

2.5. Band sharing index (BSI)

Band sharing index (BSI) value between Sm-treated alga and non-treated one; has been calculated as reported by Savva (2000) [23] as following:

$BSI = 2s / (a + b)$

Where *s* is the shared bands between two samples, *a* is the presented bands in the first sample and *b* is the presented bands in the second sample. Where, BSI value of 1 refers that two samples are identical, whereas, a BSI value of zero refers that two samples are totally different.

3. Results and discussion

DNA change profiles in *U. lactuca* alga induced by different Sm concentrations were detected through TU-DAMD marker. TU-DAMD pattern yielded by HBVb, URP17R, HVA and HVV DAMD primers into *U. lactuca* green alga exposed to 0.25, 0.5, 0.75 and 1 mg/L Sm has been presented in Figure 1

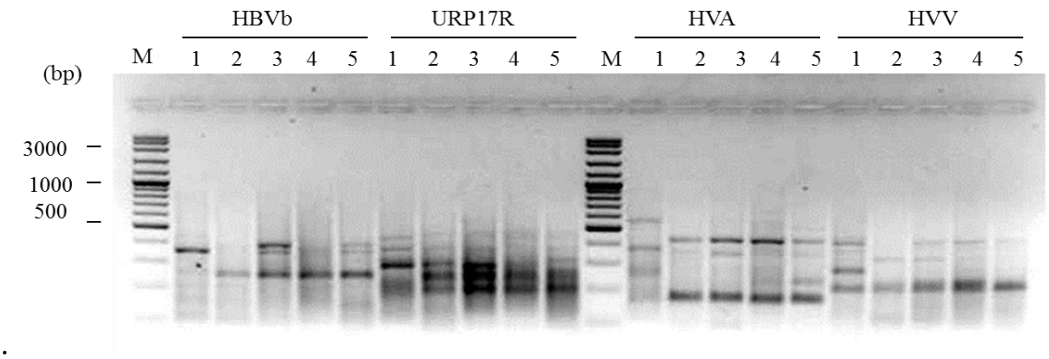

Figure 1. TU-DAMD pattern yielded by HBVb, URP17R, HVA and HVV primers into the *U. lactuca* green alga exposed to 0.25, 0.5, 0.75 and 1 mg/L Sm. Line 1: Control, 2: 0.25 mg/L Sm, 3: 0.5 mg/L Sm, 4: 0.75 mg/L Sm and 5: 1 mg/L Sm. M: A VC 100 bp Plus DNA Ladder (Vivantis) ladder standard

Polymorphic bands (PB) [(a: gain bands - appearance of new bands and b: loss bands-disappearance of normal control bands)] were recorded at each Sm applied concentration. Sm treatment induced changes in bands number in treated alga compared to non-treated one using
TU-DAMD marker (Table 2). In this regards, PB were recoded to be 71, 65, 60 and 56 bands under 0.25, 0.5, 0.75 and 1 mg/L Sm, respectively (Table 2). GTS% as a qualitative measurement of DNA changes induced by Sm treatment has been also evaluated. Calculated GTS% value was recorded to be 37.891, 43.377, 48.522 and 50.865% under 0.25, 0.5, 0.75 and 1 mg/L Sm, respectively (Table 3). As for BSI value, it was recorded to be 0.505, 0.555, 0.555 and 0.581 under 0.25, 0.5, 0.75 and 1 mg/L Sm, respectively (Table 4).

Table 2. Banding pattern of TU-DAMD amplified fragments scored

Primer	C	T1		T2		T3		T4	
		a	b	a	b	a	b	a	b
URP1F	3	0	1	0	0	0	0	0	0
URP4R	3	2	0	1	0	1	0	1	0
URP25F	6	0	4	0	4	0	3	1	2
URP38F	9	4	3	3	2	2	2	3	2
HVR(-)	7	0	3	2	2	2	2	2	1
FVIIex8C	6	2	2	2	2	2	2	2	2
FVIIex8	7	4	2	3	2	3	2	2	1
HBV5	3	0	1	0	1	0	1	0	1
YNZ22	8	3	4	3	3	3	2	2	2
14C2	4	0	0	1	0	0	0	0	0
33.6	7	3	4	3	4	3	3	3	2
6.2H(+)	2	1	0	1	0	0	0	0	0
PM13	4	2	1	0	1	0	1	2	1
HBVb	6	1	2	0	2	1	3	0	2
HVRc	4	1	1	1	1	2	2	2	1
HVR	6	2	2	3	2	2	2	2	2
URP6R	6	0	3	1	3	1	3	1	3
URP17R	5	2	3	2	3	2	2	2	2
HVA	5	2	3	3	2	2	2	2	2
HVV	5	0	2	0	2	0	2	0	3
Total bands	106	29	42	29	36	26	34	27	29
a+b			71		65		60		56

C: Control, T1: 0.25 mg/L Sm, T2: 0.5 mg/L Sm, T3: 0.75 mg/L Sm and T4: 1 mg/L Sm. (a): Appearance of new bands compared to their respective control and (b): Disappearance of normal control bands.

Table 3. Genomic template stability (GTS%) value yielded by Sm treatment as revealed by TU-DAMD marker in *U. lactuca* alga

Primer	C	T1	T2	T3	T4
URP1F	100.000	66.667	100.000	100.000	100.000
URP4R	100.000	33.333	66.667	66.667	66.667
URP25F	100.000	33.333	33.333	50.000	50.000
URP38F	100.000	22.222	44.444	55.556	44.444
HVR(-)	100.000	57.143	42.857	42.857	57.143
FVIIex8C	100.000	33.333	33.333	33.333	33.333
FVIIex8	100.000	14.286	28.571	28.571	57.143
HBV5	100.000	66.667	66.667	66.667	66.667
YNZ22	100.000	12.500	25.000	37.500	50.000
14C2	100.000	100.000	75.000	100.000	100.000
33.6	100.000	0.000	0.000	14.286	28.571
6.2H(+)	100.000	50.000	50.000	100.000	100.000
PM13	100.000	25.000	75.000	75.000	25.000
HBVb	100.000	50.000	66.667	33.333	66.667
HVRc	100.000	50.000	50.000	0.000	25.000
HVR	100.000	33.333	16.667	33.333	33.333
URP6R	100.000	50.000	33.333	33.333	33.333
URP17R	100.000	0.000	0.000	20.000	20.000
HVA	100.000	0.000	0.000	20.000	20.000
HVV	100.000	60.000	60.000	60.000	40.000
Average	100.000	37.891	43.377	48.522	50.865

C: Control, T1: 0.25 mg/L Sm, T2: 0.5 mg/L Sm, T3: 0.75 mg/L Sm and T4: 1 mg/L Sm.

Table 4. Band sharing index (BSI) value yielded by Sm treatment as revealed by TU-DAMD marker in *U. lactuca* alga.

Primer	T1	T2	T3	T4
URP1F	1.000	1.000	1.000	0.800
URP4R	0.571	0.571	0.857	0.857
URP25F	0.222	0.222	0.250	0.250
URP38F	0.500	0.625	0.667	0.714
HVR(-)	0.333	0.462	0.667	0.400
FVIIex8C	0.667	0.667	0.667	0.667
FVIIex8	0.714	0.857	0.857	1.077
HBV5	0.800	0.800	0.800	0.800
YNZ22	0.286	0.286	0.286	0.267
14C2	1.000	1.000	0.889	1.000
33.600	0.182	0.143	0.143	0.133
6.2H(+)	1.000	1.000	0.800	0.800
PM13	0.250	0.500	0.250	0.286
HBVb	0.800	1.000	1.000	1.111
HVRc	0.000	0.000	0.000	0.000
HVR	0.182	0.182	0.167	0.200
URP6R	0.667	0.444	0.444	0.667
URP17R	0.400	0.600	0.600	0.800
HVA	0.250	0.250	0.250	0.222
HVV	0.286	0.500	0.500	0.571
Average	0.505	0.555	0.555	0.581

T1: 0.25 mg/L Sm, T2: 0.5 mg/L Sm, T3: 0.75 mg/L Sm and T4: 1 mg/L Sm.

Different PCR based molecular markers techniques were extensively employed for detection DNA profile changes induced by heavy metals [12, 13, 17-19, 24, 25]. However, unlike to heavy metals, no reports up tell now deal with DNA changes induced by REEs metals exposure in algae.

In the current study, PB number was decreased from 71 to 56 bands, along with increased applied Sm concentration from 0.25 to 1 mg/L Sm. Whereas, GTS% and BSI values as a qualitative measurement of DNA changes induced by Sm treatment have been also evaluated. Calculated GTS% and BSI values were followed similar tendency inversely to PB pattern. In this regards, GTS% value was increased from 37.891 to 50.865%; similarly, BSI value was also increased from 0.505 to 0.581 along with increased applied Sm concentration from 0.25 to 1 mg/L Sm.

Regarding GTS% increase tendency along with increased applied Sm concentrations; our findings were coherent with other investigations; *e.g.* Aydın et al. (20212) [26] reported that GTS% increased from 15.98 to 38.69% when Cu+Zn concentrations increased from 40 to 640 ppm in cucumber (*Cucumis sativus* L.) using RAPD marker. Moreover, Aydin et al. (2015) [25] reported that GTS% value increased from 78.14 to 90.08% when Pb concentration increased from 40 to 240 mg/L after 21 days exposure in tomato (*Lycopersicum esculentum* L.) using RAPD marker. Similarly, Saleh (2016b) [24] reported that GTS% value increased from 45.4 to 72.8% in *U. lactuca* alga exposed to Cd treatment for 4 days, when Cd concentration increased from 2.5 to 10 mg/L, using RAPD marker. Furthermore, Saleh (2018b) [19] reported that GTS% value increased from 30.696 to 42.724% when applied Cd concentration increased from 2.5 to 10 mg/L Cd in *P. pavonica* brown marine alga after 4 days Cd exposure using RAPD marker. Indeed, Aydin et al. (2013) [27] reported that GTS% value increased from 59 to 72.5% when Cd applied concentration increased from 30 to 120 mg/L Cd in Okra (*A. esculantus* L.) after 21 days Cd treatment, using RAPD marker.

However, other reports showed inverse tendency regarding GTS% value followed heavy metals stress. In this regards, Erturk et al. (2013) [14] reported increase of PB from 33.58 to 45.25 and decrease GTS% from 66.42 to 54.75% when Chromium (Cr) applied concentrations increased from 5 to 40 mM Cr in maize (*Z. mays* L.) using RAPD marker. Similarly, Salarizadeh and Kavousi (2015) [15] reported decrease GTS% from ~80 to ~70% for the both cumin (*C. cyminum*) ecotypes with increased PB from 11 to 22 and from 10 to 12 polymorphic bands in Isfahan and Khorasan cumin (*C. cyminum*) ecotypes, respectively, when Cd applied concentration increased from 0.300 to 1.050 mg/L after 7 days exposure using RAPD marker. Whereas, Ozyigit et al. (2016) [16] reported decrease GTS% value from ~80 to ~45% when Pb applied concentration increased from 100 to 400 µmol/L Pb in rye (*S. cereale* L.) using RAPD marker. Moreover, Saleh (2018a) [18] reported decease in GTS% value from 87.274 to 70.667 % and that PB increased from 60 to 101 when Pb applied concentrations increased from 2 to 8 mg/L in *U. lactuca* alga after 2 days exposure using RAPD marker. Recently, Majumder et al. (2020) [20] reported decrease GTS% value from 91.11 to 82.22%, from 95.56 to 88.89%, from 70.73 to 60.97% and from 97.50% to 85.00% when Arsenic (As) applied concentrations increased from 50 to 75 µM in rice (*O. sativa* L.) cultivars of IR-64, TN-1, Tulaipanji and IR-20, respectively, after 18 days exposure to Arsenic (As) using RAPD marker. The previous study showed also increase in PB from 4 to 8, from 2 to 5, from 12 to 16 and from 1 to 6 when Arsenic (As) applied concentrations increased from 50 to 75 µM In IR-64, TN-1, Tulaipanji and IR-20 in rice cultivars, respectively, after 18 days exposure to Arsenic (As) using RAPD marker.

Our data were comparable with others those that previously reported, these differences could be related to many factors *e.g.* studied living organism (plant, alga…..), tested metal, metal concentration and exposure time as well as employed molecular marker.

It worth noting that, the progressive kinetic increase observed in GTS% and BSI values along with increased applied Sm concentrations could consider as a potent indicator for introduction of one or more an

effective repair system/s or any other approach developed by *U. lactuca* alga as a defense system or cellular mechanism adaptation against Sm metal effects.

In conclusion, overall, Sm treatment affected DNA pattern in *U. lactuca* alga reflecting in GTS% and BSI values, even at Sm applied concentrations of ≤ 1 mg/L. It worth noting that, when *U. lactuca* alga exposed to Sm, it tendency to progressive increase of GTS% and BSI values as protective mechanism against Sm metal. This report could consider as the first findings regarding genomic instability induced by Sm in marine algae, as a novel assay. Moreover, TU-DAMD marker application provides to be an effective tool for detection DNA polymorphism induced by Sm treatment in the studied alga. However, Sm concentrations up to 1 mg/L effect on DNA polymorphism in the studied alga, is requested.

Acknowledgement

I thank Dr. I. Othman (Director General of AECS) and Dr. A. Almariri (Head of Molecular Biology and Biotechnology Department in AECS) for their support, and also the Plant Biotechnology group for technical assistance.

Compliance with Ethics Requirements

Author declares that he respects the journal's ethics requirements. Author declares no conflict of interest.

Author Contributions Statement

Basel Saleh (B.S) declares his responsibility for the entire manuscript (*U. lactuca* alga sampling, all manipulation, data analysis, data interpretation, manuscript drafting and writing by Basel Saleh).

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