ISSN: 2230-9926

Available online at http://www.journalijdr.com

IJDR

International Journal of Development Research
Vol. 15, Issue, 02, pp. 67705-67726, February, 2025
https://doi.org/10.37118/ijdr.29214.02.2025

RESEARCH ARTICLE OPEN ACCESS

MOLECULAR CHARACTERIZATION OF GENETIC DIVERSITY INDUCED IN CERATOPHYLLUM DEMERSUM L. GROWN IN WASTEWATER

***Mervat I. Kamal, K. A. Zaied, A. S. Abd El-Mohsen and A. H. Abd El–Hady**

Department of Genetics, Faculty of Agriculture, Mansoura University, Egypt

ARTICLE INFO

Article History:
Received 05th December, 2024
Received in revised form
29th December, 2024
Accepted 20th January, 2025
Published online 27th February, 2025

Key Words:
Genotoxicity, Ceratophyllum demersum L., genetic divergence, heavy metals removal, SCoT primers, wastewater, UV-B, polymorphism, amplified fragments.

****Corresponding author: Mervat I. Kamal***

ABSTRACT

The adaptability of aquatic plants to changing environments depends on their genetic diversity. These were influenced by the rate of sexual reproduction, mutation, as well as, gene flow from distant areas. So, the aim of this study was to investigate the genotoxicity of wastewater on the molecular level of *Ceratophyllum demersum* L., in addition to improving the quality of these effluents resulting from the chemical fertilizer industry. Six doses of ultraviolet (UV-B) irradiation were used to induce genetic diversity in *C. demersum* L, in addition to unirradiated plants served as control. For this purpose, ten SCoT primers were used against 14 samples of DNA isolated from irradiated *C. demersum* L. grown in Nile water and wastewater. Irradiated plants from the same dose of UV irradiation consumed higher quantities of wastewater than those from Nile water. The greatest value of wastewater consumed was achieved by the plants irradiated for 40 minutes. This allows irradiated plants to greatly bioaccumulate heavy metals. A high level of genetic diversity was explored by SCoT-6 and SCoT-2 primer sets. The SCoT-2 primer appeared 85% of the loci were found to be polymorphic. This primer appeared the highest number of amplified fragments reached 20 and unique bands reached five. The greatest genetic variability was detected by SCoT-6, which found that 85.71% of the loci were polymorphic without unique bands. Two main clusters were obtained one for each kind of water. The results reflected that the greatest genetic variability was revealed in the populations treated with wastewater due to their genotoxicity.

Citation*: *Mervat I. Kamal, K. A. Zaied, A. S. Abd El-Mohsen and A. H. Abd El–Hady. 2025. *"*Molecular Characterization of genetic diversity induced in Ceratophyllum Demersum l. Grown in Wastewater*". International Journal of Development Research*, 15, (02), 67705-67726

INTRODUCTION

Ceratophyllum demersum L. is a macrophyte with a worldwide distribution, also known as coontail or hornwort. It is a completely submerged aquatic plant, commonly seen in quiet waters of lakes, ponds, marshes and streams, located just under the surface of water and grows quickly. *C. demersum* L. is one of many aquatic plants used for bioremediate wastewater. This plant belongs to the family Ceratophyllaceae, rootless, dicotyledon's seed grows well in tropical and subtropical areas (Di Tomaso *et al*. 2013). It can cause blockages at hydroelectric power stations, as well as, cause problems in waterways for recreational activities. In Egypt, *C. demersum* L. is commonly available and grows in the water of the Nile River and lakes. It is flowering at the beginning of spring (Afaj *et al*. 2016). Aquatic plants such as *Ceratophyllum demersum* L. are dispersed by water in the rivers. The water is the main dispersal factor producing the dispersal of this plant in the downstream direction (Barrat-Segretain 1996). Animals, fishes and water birds in particular play an important role in the dispersal of aquatic plants (Green *et al*. 2002). Both sexual and clonal reproduction revealed significant dispersal ability within water lakes. The asexual propagules are best suited for dispersal (Duarte *et al*. 1994).

Clonal reproduction probably contributed to low levels of genetic differentiation observed in aquatic species of macrophyte (Santamaría 2002). The low viability observed within the *Ceratophyllum* population was due to the predominance of clonal growth. Most macrophyte species were dispersed by water flow with either seed or vegetative propagules that can spread long distances by wind and water bodies in rivers. These transporting processes resulted in a low level of genetic diversity in aquatic macrophyte clones and their populations (Santamaría 2002). *Ceratophyllum* lacks roots and seldom produces seeds. Most of the populations originated from shoot fragments (Les 1988). King *et al*. (2002) applied RAPD and ISSR molecular techniques on *Ceratophyllum* and found that genetic distance between populations increased with geographic distance increased. The levels of differentiation within the population could be correlated with waterfowl migrations. *Ceratophyllum demersum* L. has stems that reach lengths of 1-3 m. The leaves are composed of six to twelve whorls. It is a monoecious plant, producing male and female flowers separated on the same plant. The flowers are tiny reach up to 2 mm long containing 8 or more greenish-brown petals. The flowers are formed in the axils of the vine. The plants form turions and buds that sink in the water bottom to stay during the winter and form new plants in spring. Aquatic plants have great importance in freshwater and marine ecosystems. Climate change threatens aquatic plants in

several ways as CO2, dissolved organic carbon, rising temperature, nutrient availability, light conditions and salinity. Climate change greatly affected physiology, species composition and ecosystem functioning (Reitsema *et al*. 2020). Sexual reproduction, as well as, gene inflow from distant areas can significantly increase the genetic variations in *Ceratophyllum demersum* L. populations. This can improve their ability to adapt to changing environments (Cao *et al*. 2021). Alteration in the genetic makeup of *Ceratophyllum demersum* L. populations can be induced by various environmental vectors among which dispersal can play a decisive role (Davis *et al*. 2018). Environmental factors are important drivers of genetic variation in aquatic plants (Li *et al*. 2022). The main reproductive mechanism in *Ceratophyllum demersum* L. is shoot fragmentation rather than pollen or seeds (Capers 2003). Most of the molecular studies on *C. demersum* L. are phylogenetic (Yang *et al*. 2020). Some studies concern the genetic alteration in response to heavy metal tolerance (Khaleel *et al*. 2022) and isolation of enzymes encoding genes potentially required for heavy metal accumulation (Shukla *et al*. 2012).

Recently, geneticists have used a molecular marker technique termed start codon targeted (SCoT) polymorphism as a recent tool of DNA marker methodology. This technique was developed by Collard and Mackill (2009), and based on the short conserved region flanking the start codon (ATG) in plant genomes. This technique can produce more information about biological traits than random DNA molecular markers (Collard and Mackill 2009). SCoT markers generate high polymorphism through longer primers which is reproducible (Mulpuri *et al*. 2013). The annealing temperature and primer length are not the sole vectors that determine the reproducibility of the SCoT tool (Collard and Mackill 2009). SCoT makers have been used to evaluate genetic diversity and fingerprinting variation since 2009 (Mulpuri *et al*. 2013). SCoT techniques use single 18-mer primers in a polymerase chain reaction (PCR) with an annealing temperature of 50 ^{0}C. SCoT markers do not consume time, are technically simple, require no prior sequence information and target functional regions (Xiong *et al*. 2011). This technique was used successfully in a wide range of plants. It is a beneficial tool for assessing DNA fingerprinting and genetic diversity in plant populations (Vivodík *et al*. 2016). Different molecular markers studied were used for the genetic differentiation of *Ceratophyllum demersum* L., ranging from karyology to AFLP and ISSR molecular markers (Gargiulo *et al*. 2022). Molecular studies based on microsatellite primers have not yet been published for this species. Microsatellites as short tandem repeats have been the most widely used genetic markers for genotyping plants over the past 20 years (Vieira *et al*. 2016). The elevated levels of polymorphism have made them powerful for investigating genetic diversity and structure (Kim and Sappington 2013). The benefits of microsatellite studies in population genetics are investigated in macrophyte species (Kong *et al*. 2019). DNA-based molecular marker techniques are the most powerful diagnostic tools used to determine genetic polymorphisms at the molecular level of DNA. Different molecular markers have been employed to estimate genetic diversity for phylogenetic analysis to identify the germplasm (Hu *et al*. 2017). Molecular markers were used to reveal individual or group differences among aquatic plants as SSR (simple sequence repeat), RFLP (restriction fragment length polymorphism), RAPD (random amplified polymorphic DNA), AFLP (amplified fragment length polymorphism), as well as, ISSR (intersimple sequence repeat). SSR technique known as microsatellite was widely used to study the genetic diversity in aquatic plants due to its high variability, high abundance and codominance (Pollux *et al*. 2007). If two molecular markers were used in combination they enhanced the effectiveness and achieved good results. For example, AFLP markers combine the advantages of RFLP and RAPD. This is another effective technique to investigate genetic diversity. The difficulties of phylogenetic studies in aquatic plants are due to the high variability and complexity of their traits and morphology. Aquatic plants were used in phytoremediation which is an inexpensive, effective and ecofriendly technology leading to environmental cleanup by plants (Salt *et al*. 1998). There are three genomes were exist in plant cells including, mitochondrial, chloroplast and nuclear. Their evolutionary rate is different because of differences in their structure and function, providing alternative traits for phylogenetic studies. DNA-based molecular marker technique has obvious advantages in overcoming morphological limitations and detecting the evolutionary relationship among different species (Kumar *et al*. 2016). During the last three decades, solar ultraviolet (UV-B) radiation has increased on the Earth's surface as a degradation of the stratospheric ozone layer (Häder *et al*. 1998). Organisms including aquatic plants exposed to enhanced UV-B radiation could cause damage to DNA, photosynthetic apparatus, membranes and proteins. Plants protect themselves from UV-B radiation through the repair of any damage by different mechanisms (Gaberščik *et al*. 2002). Testing water quality is an important tool in agriculture water management. Insufficient quality of water irrigation could retard plant growth and contaminated soil making it less suitable in the agricultural sector. Therefore, phytoremediation is mainly applied on contaminated soils and waters if the material to be treated is low or medium in depth and the area is large (Berti and Cunningham 2000). Thus, aquatic plans were important keys in the bioremediation treatment of wastewater before use in gardening purposes such as irrigation of plants. Phytoremediation is an efficient tool for wastewater treatment in terms of pollutant removal. It has been described the utilization of plants for water contaminated cleaning up from heavy metals or organic contaminants. This technology is considered eco-friendly, efficient, effective, pollution and low-cost technology. Wastewater treatment and its proper disposal are the main problems all over the world (Patel and Kanungo 2010). *Ceratophyllum demersum* L. removing excess heavy metals from wastewater. The biological effects are high in the higher water contaminant and prolonged exposure time (Jawad *et al*. 2018). RAPD methodology was mostly used to detect genetic relationships. Banding DNA patterns can be used to detect DNA damage and mutations (Atienzar *et al*. 1999). The RAPD method was used to assess the effects of toxicants on organisms to diagnose genotoxicity. The presence of bands, intensity and absence are linked to DNA damage. Cadmium is a genotoxic heavy metal that directly induces alteration in DNA function and structure (Cambier *et al*. 2010). Molecular marker techniques require DNA isolation with a suitable purity as a prerequisite for molecular research. Successful polymerase chain reaction (PCR) is based on the efficient recovery of good quality and quantity of DNA (Abbasi and Afsharzadeh 2016). Aquatic plants have great economic value with health benefits. They have lower quantities of DNA(Abbasi and Afsharzadeh 2016). The traces of heavy metals in aquatic environments produced DNA alteration in the plants. These include inhibition of chlorophyll biosynthesis, visible morphological traits, inhibition of enzyme activity and growth rate (Vaillant *et al*. 2005). Aquatic plants accumulate heavy metals (Rai *et al*. 1995). Heavy metal pollution is of major ecological concern due to its impact on human health through the food chain and its high persistence in the environment. The present study was carried out to investigate the genotoxicity of wastewater in UV-B irradiated *Ceratophyllum demersum* L. using SCoT molecular markers.

MATERIALS AND METHODS

Genetic materials: *Ceratophyllum demersum* L. was used in this study as an aquatic plant found in the rivers. It was collected from the Nile River in the winter season of 2023/2024 behind the campus of Mansoura University. Plants were thoroughly washed with tap water before being used to remove any sediment particles attached to their surfaces. The plants were grown in the Nile water with three replications for one week of acclimatization in the Experimental Farm of the Plant Pathology Department, Faculty of Agriculture, Mansoura University. After acclimatization, healthy plants of the same weight (20 g) were selected to be irradiated with UV-B and then regrown in Nile water for eight days followed by wastewater as an environmental stress to test their genotoxicity induced in the *Ceratophyllum* population on the molecular level.

Wastewater collection : Wastewater samples were collected from the main pipe of the chemical fertilizer factory before being mixed with the surface water during the summer season of 2023. This sewage of wastewater contains all industrial contaminants.

Ultraviolet irradiation: The artificial source of ultraviolet rays was the UV lamp in the laminar air cabinet located in the Microbial Genetics Laboratory, Faculty of Agricultural, Mansoura University. The spectrum of this UV lamp belongs to a high-energy source named UV-B (280-320 nm) (Barta *et al.* 2004). The spectrum of the UV lamp used in this investigation was 300 nm, leading to be classified as UV-B. Each minute of exposure time to a UV lamp was equal to 188.2 joules/m2 according to Kondrateva *et al.* (2021). The joules were defined as one watt of irradiated power for one second. Twenty grams of *Ceratophyllum demersum* were grown in a 500 ml beaker containing 400 ml Nile water for eight days to be acclimatization on Experimental Farm conditions. After this time the plants were exposed to ultraviolet irradiation for 0, 10, 20, 30, 40, 50 and 60 minutes of exposure time.

Experimental setup: *Ceratophyllum demersum* L. plants were placed in 500 ml beakers filled with 400 ml Nile water or wastewater. Each replicate provided 20 grams of the wild-type *Ceratophyllum demersum* L. After acclimatization in Nile water for eight days, the plants were ultraviolet irradiated. The irradiated plants were also re-acclimatization in Nile water for another eight days before being transferred to wastewater. Then the plants were transferred into wastewater for eight days to test their genotoxicity induced as shown in Table 1. Specific plant parameters were measured before and after the experiments to investigate the biochemical changes that occurred according to Al-Nabhan and Al-Abbawy (2021). This experiment was conducted in the Experimental Farm of Plant Pathology Department during the winter season of 2023/2024 based on kindly acceptance from Prof. Dr. Mohamed El-wakil, Professor of Plant Pathology, Faculty of Agriculture, Mansoura University.

Table 1. Designation of treatments with *Ceratophyllum demersum* L.

Treatments	Treatment code
Original Nile water	T_1
Nile water treated with non-irradiated plants	T_2
Nile water treated with UV-irradiated plants for 10 minutes	T_3
Nile water treated with UV-irradiated plants for 20 minutes	T_4
Nile water treated with UV-irradiated plants for 30 minutes	T_5
Nile water treated with UV-irradiated plants for 40 minutes	T_6
Nile water treated with UV-irradiated plants for 50 minutes	T_7
Nile water treated with UV-irradiated plants for 60 minutes	T_8
Original wastewater	T_9
Wastewater treated with non-irradiated plants	T_{10}
Wastewater treated with UV-irradiated plants for 10 minutes	T_{11}
Wastewater treated with UV-irradiated plants for 20 minutes	T_{12}
Wastewater treated with UV-irradiated plants for 30 minutes	T_{13}
Wastewater treated with UV-irradiated plants for 40 minutes	T_{14}
Wastewater treated with UV-irradiated plants for 50 minutes	T_{15}
Wastewater treated with UV-irradiated plants for 60 minutes	T_{16}

Fresh weight: After eight days of each experiment, the plants were taken to measure the changes in fresh weight after washed with tap water to remove any debris and then air dried before weighting. They were oven-dried at 70 0C until reached constant weight in grams. Each of the three experiments done in this study was taken eight days in non-aeration conditions. In the second experiment, the plants were irradiated with different exposure times of ultraviolet irradiation and then regrown in Nile water for eight days. In the third experiment, the irradiated plants were transferred from Nile water to wastewater and then grown in wastewater for another eight days. After eight days of each experiment, the elements were measured in the Department of Chemistry, Genetic Engineering Institute, Menofya University according to the Standard Methods of Examination of Water and Wastewater (APHA, 19th edition 1995).

Chlorophyll content: hlorophyll and carotenoid pigment concentrations were measured according to Oron *et al*. (1988). The absorption spectrum of different pigments of chlorophyll a, b and carotenoids was determined according to Lichtenthaler and Wellburn (1983). In addition, total chlorophyll was determined according to Ahmed *et al*. (2020).

Removal efficiency of pollutants: The removal efficiency percent of pollutants uptake by *Ceratophyllum demersum* L. was measured according to Kumar and Deswal (2020).

Relative growth rate: The relative growth rate of the plants was determined at the end of each experiment to be compared with the initial plant weight according to Lu *et al*. (2004).

Tolerance index rate: The tolerance index ratio was assessed according to Wilkins (1978) using the following equation below.

$$\text{Tolerance index} = \frac{\text{Treated plant dry weight (g)}}{\text{Control dry weight (g)}}$$

Net primary productivity (NPP): The net primary productivity of cultured aquatic plant before and after phytoremediation of nutrients, for eight days interval was determined as air dried fresh weight by Harvest method according to Patel and Kanungo (2010) using the following formula:

$$\text{NPP} = \frac{\text{Final biomass} - \text{Initial biomass}}{\text{Number of culture days}}$$

SCoT "Start Codon Target"- PCR Reactions: Ten SCoT primers were used in the characterization of polymorphism according to Ibrahim *et al*. (2019) as listed in Table 2. PCR amplification reaction was performed in 20 µl reaction volume containing 10 µl Master Mix (sigma), 2µl primer (10 pcmol), 2 µl template DNA (10 ng) and 6µl d H_2O.

Table 2. Base sequences of ten SCoT primers used in this study.

Primer code	Primer sequence (5'-3')
SCoT-1	ACGACATGGCGACCACGC
SCoT-2	ACCATGGCTACCACCGGC
SCoT-3	ACGACATGGCGACCCACA
SCoT-4	ACCATGGCTACCACCGCA
SCoT-6	CAATGGCTACCACTACAG
SCoT-10	ACAATGGCTACCACTACC
SCoT-11	ACAATGGCTACCACCAGC
SCoT-12	AAGCAATGGCTACCACCA
SCoT-13	ACGACATGGCGACCAACG
SCoT-14	AACCATGGCTACCACCAC

Thermocyling Profile PCR: PCR amplification was conducted in a Perkin-Elmer/Gene Amp® PCR System 9700 (PE Applied Biosystems) programmed to fulfill 40 cycles after an initial denaturation cycle for five min at 94ºC. Each cycle consisted of a denaturation step at 94 ºC for 45 s, an annealing step at 50ºC for 50 s and an elongation step at 72 ºC for one minute. The primer was extended to seven min at 72 ºC in the final cycle.

Plant material and DNA extraction: Genomic DNA was extracted from 14 samples, seven of them numbered 1, 2, 3, 4, 5, 6 and 7 were grown in Nile water after irradiation. Meanwhile, the others numbered 14, 15, 16, 17, 18, 19 and 20 were grown after irradiation in Nile water for eight days to be acclimatization followed by wastewater for another eight days. DNA was extracted by DNeasy Plant Mini Kit (Qiagen Santa Clarita CA) according to Sathapondecha *et al*. (2021).

Detection of PCR products: The amplified products were run using 1.5% agarose gel containing ethidium bromide (0.5 mg/ml). 1X TBE buffer was taken using the run at 95% volts. PCR-amplified products are visualized using UV light. A gel documentation system (BIO-RAD 2000) was used for photographing the results.

Data analysis: The banding profiles generated from SCoT molecular markers were compared to assess the genetic relatedness of 14 samples used in this study. Amplified products were termed as '1' for the presence and '0' for the absence of bands. Through the similarity matrix cluster analysis was performed. It was used for organizing the obtained data into meaningful structures to develop the taxonomies according to Sneath and Sokal (1973). The binary statistic matrix was constructed. Dice's similarity matrix coefficients were assessed between genotypes using the unweighted pair group method with arithmetic averages (UPGMA). The matrix was used to construct a phylogenetic tree (dendrogram) according to the Euclidean similarity index using PAST software version 1.91 after each accession its own cluster was represented according to Hammer *et al*. (2001).

Statistical analysis: Analysis of variance (ANOVA) was employed to detect significant differences between or among sample means according to Steel and Torrie (1960).

RESULTS AND DISCUSSION

Water consumption: *Ceratophyllum demersum* L. consumed high amounts of Nile water by non-irradiated plants if compared with irradiated plants over eight days of acclimatization (Table 3). This indicated that irradiated plants consumed lower values of Nile water than non-irradiated plants. This may be due to genetic alteration induced by UV-B irradiation which leads to reduced water uptake from aquatic bodies. This plant is rootless having forked leaves and thin cuticles on the plant surface. These features facilitate the uptake of water from aquatic bodies through the large surface area of the plant with no complication of root-shoot water partitioning. These features contributed non-irradiated plants to adequate water accumulation observed than irradiated plants. Therefore, there were significant differences between the doses of UV-irradiated plants for water absorbed. This may be due to genetic diversity induced by UV-irradiation which may impact the large surface area. The general mean appeared insignificant increase in water consumption by irradiated plants if compared with that in non-irradiated plants.

Water consumption was increased gradually over the experimental time among irradiated and non-irradiated plants. The water consumption by irradiated plants ranged between 3.33 ml after two days to 35 ml after eight days. Meanwhile, the water consumption by non-irradiated plants ranged between 6.67 ml after two days to 70 ml after eight days. The general mean of water consumption by irradiated plants ranged between 6.67 ml after two days to 27.78 ml after eight days. Furthermore, the general mean of water consumption by non-irradiated plants ranged between 16.11 ml after two days to 56.11 ml after eight days. This indicated that non-irradiated plants grown in Nile water responded positively to absorb more water quantities upon irradiation grown in the same water. Longer duration time increased water consumption among non-irradiated and irradiated plants but with a higher rate in non-irradiated plants. These results are in line with Sharma and Dubey (2005), who reported that contamination results in adverse effects on the morphology of aquatic plants, growth and photosynthesis process and causes alterations in membrane permeability, inhibition in enzyme activity, disturbs water, water imbalance, and mineral nutrition. In this study irradiated plants were subjected to Nile water for eight days to be adaptive population divergence after irradiation. The irradiated plants may have diverse physiological and genetic differences. The plants were grown in Nile water after irradiation before transferring to wastewater for acclimatization. These plants provide good and effective filters for removing many pollutants as heavy metals from wastewater. According to Gondek *et al*. (2020), aquatic plants decrease the concentration of total dissolved solids as basic elements needed for building their tissues as calcium, magnesium, sodium, potassium, chlorides, sulfates, carbonates and bicarbonates. The results tabularized in Table 4 showed significant differences between irradiated plants that consumed Nile water and wastewater after eight days. This indicated that the plants may genetically diverge after UV irradiation. Consumed Nile water by irradiated plants ranged between 3.33 ml after two days to 35 ml after eight days. The general mean of consumed Nile water ranged between 6.67 after two days to 27.78 ml after eight days. The wastewater consumed by irradiated plants ranged between 36.67 ml after two days to 78.33 ml after eight days. The general mean of wastewater consumed ranged between 30.0 ml

Table 3. Nile water consumption by non-irradiated and irradiated *Ceratophyllum demersum* L. over eight days of acclimatization

UV-B irradiation time (minutes)		Water consumption (ml/days)							
		2		4		6		8	
		NIR	IR	NIR	IR	NIR	IR	NIR	IR
0		6.67	5.00	11.67	13.33	18.33	21.67	26.67	28.33
10		13.33	6.67	20.00	13.33	26.67	20.00	48.33	25.00
20		10.00	3.33	15.00	8.33	20.00	13.33	41.67	18.33
30		10.00	3.33	20.00	10.00	25.00	16.67	46.67	23.33
40		18.33	6.67	23.33	13.33	30.00	20.00	61.67	30.00
50		20.00	8.33	26.67	15.00	33.33	23.33	68.33	35.00
60		25.00	11.67	31.67	18.33	38.33	23.33	70.00	35.00
Mean		16.11	6.67	22.78	13.05	28.89	19.44	56.11	27.78
F-test		**	*	*	*	*	NS	**	**
LSD	0.05	7.85	4.34	9.61	5.71	10.81	6.91	7.48	8.12
	0.01	11.00	6.09	13.47	8.01	15.15	9.69	10.48	11.39

NIR: Non-irradiated.
IR: Irradiated.

Table 4. Waste water consumption by irradiated *Ceratophyllum demersum*L. over eight days

UV-B irradiation time (minutes)		Water consumption (ml/days)							
		2		4		6		8	
		Nile water	Wastewater	Nile water	Wastewater	Nile water	Wastewater	Nile water	Wastewater
0		5.00	31.67	13.33	41.67	21.67	46.67	28.33	58.33
10		6.67	21.67	13.33	33.33	20.00	38.33	25.00	46.67
20		3.33	25.00	8.33	35.00	13.33	41.67	18.33	51.67
30		3.33	33.33	10.00	43.33	16.67	50.00	23.33	60.00
40		6.67	36.67	13.33	50.00	20.00	60.00	30.00	78.33
50		8.33	28.33	15.00	40.00	23.33	45.00	35.00	53.33
60		11.67	35.00	18.33	45.00	23.33	53.33	35.00	65.00
Mean		6.67	30.00	13.05	41.11	19.44	48.06	27.78	59.17
F-test		*	NS	*	NS	NS	NS	**	**
LSD	0.05	4.34	10.48	5.71	13.50	6.91	13.33	8.12	13.40
	0.01	6.09	14.70	8.01	18.92	9.69	18.69	11.39	18.79

NS: Not significant.
*,**: significance at 0.05 and at 0.01 probability levels, respectively.

after two days to 59.17 ml after eight days. This indicated that the absorption of water was gradually increased over time among Nile water and wastewater. A significant increase in wastewater consumption after eight days was achieved by the plants irradiated for 40 minutes. This reflected the importance of genetic diversity induced in C. *demersum* L. by UV-B irradiation. The results indicated that irradiated plants consumed higher quantities of wastewater than that consumed from Nile water at the same corresponding doses of UV irradiation. The current shortage of wastewater by irradiating plants is one of the most important issues due to genetic diversity induced in the growing population of aquatic plants which is the basic necessities of life. Given that over 80% of the weight of living was made of water. Therefore, aquatic plants such as *C. demersum* L. were used to remediate contaminated water. This plant was successful to be used in decreasing turbidity levels, ammonia and nitrates in addition to other pollutants, as well as boosting dissolved oxygen in the water (Beheary *et al*. 2019). The agricultural and industrial wastewater reuse strategy is the ideal solution in the biotechnological process to overcome irrigated water shortages. It is considered an as economical tool, the fastest, as well as, most effective to provide acceptable water traits to be re-used for irrigation purposes (Elshemy 2017). Thus, aquatic plants play a major role in the filtration of polluted environments and increased dissolved oxygen in the water.

Table 5. Relative growth rate of irradiated *Ceratophyllum demersum*L. grown for eight days in Nile and wastewater

UV-B exposure time (minutes)		Fresh weight of non-irradiated plants grown in Nile water (g)	Fresh weight of irradiated plants (g)		Relative growth rate of irradiated plants (g)		Decrease of relative growth rate in wastewater (g)
			Nile water	Wastewater	Nile water	Wastewater	
0		20.69	21.86	14.56	1.06	0.70	0.66
10		20.80	21.54	15.41	1.04	0.74	0.71
20		20.76	21.60	15.90	1.04	0.77	0.74
30		20.21	20.68	14.31	1.02	0.71	0.70
40		20.67	21.30	17.46	1.03	0.84	0.82
50		20.56	20.77	14.22	1.01	0.69	0.68
60		20.20	21.05	13.61	1.04	0.67	0.65
Mean		20.53	21.16	15.15	1.03	0.74	0.72
F-test		NS	NS	NS	NS	NS	NS
LSD	0.05	0.99	1.32	2.61	0.06	0.13	0.13
	0.01	1.38	1.85	3.67	0.09	0.18	0.18

NS: Not significant.

Table 6. Net primary productivity (g/day) of irradiated *Ceratophyllum demersum* L.grown in Nile water and wastewater for eight days

UV-B exposure time (munites)		Nile water	Wastewater	Decline ratio	
				Nile water	Wastewater
0		0.163	- 0.913	1.00	1.00
10		0.123	- 0.767	0.76	0.84
20		0.131	- 0.713	0.80	0.78
30		0.028	- 0.796	0.17	0.87
40		0.113	- 0.480	0.70	0.53
50		0.056	- 0.819	0.35	0.90
60		0.095	- 0.930	0.58	1.02
Mean		0.091	- 0.751	0.56	0.82
F-test		NS	NS	NS	NS
LSD	0.05	0.14	0.33	0.86	0.35
	0.01	0.20	0.46	1.20	0.49

NS: Not significant.

The greatest value of wastewater consumed after eight days was observed by the plants irradiated for 40 minutes. Meanwhile, the greatest value of Nile water consumed after eight days was observed by the plants irradiated at 50 and 60 minutes. Thus, bioremediation of wastewater by *C. demersum* L. Is considered a relatively less expensive biotechnology and eco-friendly tool for cleaning up pollutant environments from toxic heavy metals (Singh *et al*. 2010). The importance of this aquatic plant was due to the phytochelatin synthase (PCS) gene that encodes the key enzyme for heavy metal detoxification and accumulation which is used as an important task to develop the technology of bioremediation. This gene (PCS) was earlier isolated from *Ceratophyllum demersum* L., as a metal bioaccumulator by Shukla *et al*. (2013). Expression of this gene in transgenic tobacco-induced PCS synthesis and metal accumulation. Many efforts were made before to increase the metal accumulation in bacterial cells via genes expressing PCS and metallothioneins (Say *et al*. 2003). The expression of metallothioneins in bacteria faced difficulties because of the instability of cysteine-rich proteins (Bae *et al*. 2001). Generally, *C. demersum* L. can bioaccumulate, and remove trace metal contaminants unselectively by acting as filters (Osmolovskaya and Kurilenko 2005). The plants have very thin cuticles and take up metals readily through their entire surface. Certain essential trace metals were accumulated in aquatic plants with very small values for healthy growth and development. The same ability allows the plants to bioaccumulate other nonessential trace metals such as Pb, Cr and Cd (Djingova and Kuleff 2000). Any marked increase in heavy metals may cause alterations and physiological disturbances with different levels (Vaillant *et al*. 2005). The gradual increases in wastewater consumption by irradiated plants over the phytoremediation time allow aquatic plants to bioaccumulate a marked increase of certain trace metals may lead to physiological disturbances (Vaillant *et al.* 2005). The results obtained herein are in line with the findings of Al-Nabhan and Al-Abbawy (2021), who found that C. *demersum* L. has improving wastewater quality and it can be considered as an effective key in phytoremediation biotechnology. This technology uses sunlight as a source of energy without generating secondary waste. Phytoremediation is a safe alternative to conventional methods of wastewater treatment (Al-Nabhan and Al-Abbawy 2021).

The growth rate in wastewater: Relative growth rate did not show any significant differences between irradiation doses among the plants grown in Nile water or wastewater (Table 5). The fresh weight of unirradiated plants grown in Nile water ranged between 20.20 to 20.76 g. The fresh weight of irradiated plants grown in Nile water was ranged between 21.05 to 21.86 g. The fresh weight of irradiated plants grown in wastewater ranged between 13.61 to 17.46 g. Meanwhile, the relative growth rate of irradiated plants grown in Nile water and wastewater ranged between 1.01 to 1.04 and 0.67 to 0.84 g, respectively. The decrease in the relative growth rate of irradiated plants grown in wastewater ranged between 0.65 to 0.82 g. The results indicated a reduction in the fresh weight of irradiated plants grown in wastewater than that in Nile water at the corresponding doses. The results indicated that the growth of C. *demersum* L. was better in Nile water than in wastewater at the same doses of UV irradiation. This is an indicator of water quality. The toxic effects of

wastewater were a decline in plant growth rate depending on the plant's susceptibility to water pollutants. Reduction in relative growth rate in wastewater may be due to high pollutants of heavy metals in wastewater, which harms the cell by affecting amino acid content, and nitrogen, as well as, decreased DNA and RNA in plant tissues. The reduction in plant growth rate grown in wastewater may be due to the decline in protein content which may also increase the activity of protease responsible for protein degradation (Al-Nabhan and Al-Abbawy 2021). The results obtained in this study agrees with the work of Pandey and Verma (2020), who found that increase in wastewater level up to 40% decreases dry matter yield of *C. demersum*L.This wastewater level was toxic effect for dry matter yield.The experimental time used in this study was not enough to appear significant differences between treatments concerning the growth and relative growth rate. This agrees with Pandy and Verma (2020), who found highly significant decline in dry matter yield of *C. demersum*L. grown in 80% wastewater level for 15, 30 and 60 days. As shown from the results tabularizedin Table 6, net primary productivity was decreasedin irradiated plants grownin Nile water. It was ranged between 0.056 to 0.131g/day in irradiated plants if compared with the control (0.163).

The wastewater used in this study was discharged from the chemical fertilizer industry. This kind of wastewater contains high values of ammonia. Total ammonia in this kind of wastewater consists of two principal forms, the ammonium ion (NH_4^+), as well as, unionized ammonia (NH_3). Ammonia is an important vector present in freshwater ecosystems. Ammonia exhibits toxicity to aquatic plants. It causes internal carbon-nitrogen imbalance in *Ceratophyllum demersum* L. (Ge *et al*. 2012). If the concentration of free ammonia is higher than 0.41 mg L-1, then the growth of *C. demersum* L. is decreased (Fan *et al*. 2009). Therefore, the relative growth rate and net primary productivity of irradiated *C. demersum* L. declined in wastewater. This may be due to the high concentration of ammonia which caused internal carbon-nitrogen imbalance. This is in line with the finding of Gao *et al*. (2012), who reported that *C. demersum* L. has a certain stress resistance and adaptive capacity within a limited range of ammonium concentrations. The same authors also reported that higher concentrations of ammonium and pH levels of nine or above can lead to growth inhibition of *C. demersum* L. and stress resistance. Monselise and Kost (1993) decided that both forms of ammonia are toxic at high concentrations. Wang *et al*. (2013) decided that pH-dependent on ammonia relative concentrations was often not

Table 7. Chlorophyll and carotenoid pigments in non-irradiated and irradiated *Ceratophyllum demersum* L. grown in Nile water at zero time

UV-exposure time (minutes)		Pigments (mg/g FW) in unirradiated plants				Pigments (mg/g FW) in irradiated plants			
		Chl a	Chl b	Total	Carotenoids	Chl a	Chl b	Total	Carotenoids
0		0.31	0.13	0.44	0.18	0.31	0.13	0.44	0.18
10		0.25	0.08	0.32	0.12	0.22	0.11	0.34	0.15
20		0.20	0.13	0.33	0.13	0.22	0.09	0.31	0.14
30		0.20	0.11	0.31	0.13	0.18	0.08	0.26	0.12
40		0.29	0.12	0.41	0.16	0.23	0.10	0.33	0.15
50		0.33	0.14	0.46	0.20	0.28	0.11	0.39	0.19
60		0.27	0.12	0.39	0.14	0.20	0.09	0.30	0.13
Mean		0.26	0.12	0.37	0.15	0.22	0.10	0.32	0.15
F-test		**	**	**	**	**	**	**	**
LSD	0.05	0.01	0.01	0.015	0.003	0.01	0.02	0.019	0.006
	0.01	0.02	0.02	0.022	0.004	0.02	0.03	0.027	0.008

** : Significance at 0.01 probability level.

Table 8. Chlorophyll and carotenoid pigments in irradiated *Ceratophyllum demersum* L. grown in Nile water and wastewater for eight days

UV-exposure time (minutes)		Pigments (mg/g FW) in irradiated plants grown in Nile water for eight days				Pigments (mg/g FW) in irradiated plants grown in wastewater for eight days			
		Chl a	Chl b	Total	Carotenoids	Chl a	Chl b	Total	Carotenoids
0		0.29	0.12	0.42	0.18	0.029	0.040	0.068	0.024
10		0.31	0.13	0.44	0.19	0.046	0.043	0.090	0.023
20		0.36	0.11	0.47	0.20	0.027	0.059	0.086	0.026
30		0.36	0.15	0.51	0.22	0.034	0.037	0.071	0.031
40		0.30	0.14	0.44	0.18	0.105	0.088	0.193	0.063
50		0.25	0.12	0.36	0.16	0.012	0.026	0.038	0.012
60		0.29	0.14	0.42	0.17	0.040	0.045	0.085	0.028
Mean		0.31	0.13	0.44	0.19	0.044	0.050	0.094	0.031
F-test		**	*	**	**	**	**	**	**
LSD	0.05	0.016	0.022	0.021	0.004	0.010	0.023	0.019	0.005
	0.01	0.023	0.030	0.029	0.005	0.014	0.032	0.026	0.007

*, **: Significance at 0.05 and 0.01 probability levels, respectively.

Meanwhile, net primary productivity declined in plants grown in wastewater if compared with that grown in Nile water. It ranged between - 0.480 in plants irradiated with 40 minutes to - 0.930 in plants irradiated with 60 minutes if compared with unirradiated plants (- 0.913). The decline ratio in irradiated plants grown in Nile water ranged between 0.17 to 0.80. Meanwhile, this decline in irradiated plants grown in wastewater ranged between 0.53 to 1.02 g/day. Therefore, wastewater discharge into the environment and water bodies as a result of developing agriculture, global population expansion and industrial activity, has a significant adverse effect on the growth of aquatic plants leading to reduce net primary productivity. This also influences water quality and creates substantial health problems (Barceló *et al*. 2011). So, many aquatic plants as *C. demersum* L. are used to remediate wastewater. This leads to minimizing wastewater toxicity via phytoremediation technology.

controlled, which precludes any distinction between the effects of NH_3 and NH_4^+. Therefore, the higher concentration of ammonia in wastewater has a marked influence on *C. demersum* L. biomass. The metabolism of carbon (C) may be negatively influenced by ammonia stress leading to reduced biomass. So, nutrient uptake and storage by aquatic plants is the main component of the biogeochemical cycle of natural ecosystems (Mitsch and Gosselink 2000). The rapid industrialization in developing countries resulted in heavy metal losses to economic welfare which impacted human health, agriculture activities and the ecosystem through water and air pollution. Water pollution influenced a large number of economic activities. This has a direct impact on human health and livelihoods (Reddy and Behera 2006). Therefore, it is necessary to protect public health through phytoremediation of wastewater as seen in this study. Thus, this study focuses on particular attention of metals removal from wastewater by

C. demersum L. (Miretzky *et al*. 2006). Nitrate is not stable in wastewater and it is absorbed by aquatic plants to be immobilized as part of their protein (Patterson 2003). *C. demersum* L. needs nutrient uptake from the water bodies (Mjelde and Faafeng 1997). The plants were initially acclimatization for eight days in Nile water after UV-B irradiation before transferring into wastewater. The quantitative reduction of nutrients in wastewater will lead to *C. demersum* L. as an efficient bioreactor for the phytoremediation of organics and nutrients from industrial wastewater.

Chlorophyll content: According to the results tabularized in Table 7, there were significant differences among irradiated plants grown in Nile water at zero time for pigment concentrations. This indicated that the plants used in this study after UV-irradiation were genetically diverse in chlorophyll and carotenoid pigments. The general mean of chlorophyll and carotenoid pigments of irradiated plants was significantly decreased than the control. This reflected that UV-irradiation have a toxic effect on chlorophyll formation in *C. demersum* L. in relation to the control. UV irradiation-induced H_2O_2 which accumulates in plant cells. This accumulation is prevented by catalase and peroxidases. Hydrogen peroxide (H_2O_2) is a very reactive oxygen-toxic species (Gill and Tuteja 2010). The reason chlorophyll and carotenoid decreased may be due to increased activities of peroxidases that are detoxifiers of H_2O_2. This are in line with the findings of Pandey and Verma (2020), who found significant decreases in chlorophyll content in *Ceratophyllum demersum* L. grown in over 60% of wastewater due to the toxic effects of wastewater. Regarding Table 8, there were significant differences in chlorophyll and carotenoid pigments between irradiation doses among the plants grown in Nile water and wastewaterfor eight days.

The effects of UV-irradiation on *C. demersum* L. may include marked increase or decrease in chlorophyll and carotenoid pigments.Generally, the results appeared that the aquatic plant*Ceratophyllum demersum* L. is extremely sensitive to some doses of UV irradiation.If chlorophyll and carotenoids were decreased, this owing to the phytotoxicity of UV-irradiation.Increased or decreased in chlorophyll and carotenoid contents due to irradiation or wastewater resulted in altered plant morphology and growth changes. The effects are dependent on the dose of UV irradiation.This indicated that chlorophyll and carotenoid contents in *C. demersum* L. are sensitive to UV-B irradiation, as well as, to the chemical composition of water, that affecting on the inhibition of chlorophyll formation.The reduction and inhibition of chlorophyll and carotenoid contents prevents photosynthetic activity, resulting in the breakdown of photosynthesis, as well as, in different morphological and growth alteration depending on the duration of exposure (Afaj *et al*. 2016). The results reflected that the dose of 50 minutes of UV duration time showed significant decline in photosynthetic pigments among Nile water and wastewater.Some doses of UV-irradiation as the dose of 50 minutes may adversely affects on photosynthesis through causing distortion of chloroplast ultrastructure leading to inhibiting the synthesis of photosynthetic pigments,as well as, the enzymes involved in calvin cycle.The reduction in chlorophyll concentrations may be attributed to the inhibition in α-aminolevulinic acid dehydrates (ALAD) affected by UV irradiation or wastewater (Prasad and Prasad 1987). Rebechini and Hanzely (1974) found that the damage of chloroplast in *C. demersum* L. involving grana and stroma. Meanwhile, Sharma and Dubey (2005) reported that damage in chloroplast may resulted from the damage in photosyntheticapparatas or chlorophyll degradation by increased chlorophyllase

Table 9. Pigments yield (mg/g FW) in irradiated plants grown in Nile water and wastewater for eight days in relation to zero time

UV- exposure time (minutes)		Nile water				Wastewater			
		Chl a	Chl b	Total	Carotenoids	Chl a	Chl b	Total	Carotenoids
0		0.95	0.96	0.96	1.02	0.10	0.33	0.16	0.13
10		1.40	1.21	1.31	1.29	0.15	0.33	0.20	0.12
20		1.63	1.19	1.49	1.44	0.08	0.54	0.18	0.13
30		1.99	1.88	1.99	1.84	0.09	0.25	0.14	0.14
40		1.30	1.39	1.32	1.23	0.35	0.63	0.44	0.35
50		0.88	1.07	0.93	0.85	0.05	0.22	0.10	0.08
60		1.43	1.54	1.43	1.27	0.14	0.32	0.20	0.16
Mean		1.44	1.38	1.41	1.32	0.14	0.38	0.21	0.16
F-test		**	**	**	**	**	**	**	**
LSD	0.05	0.03	0.23	0.06	0.02	0.03	0.17	0.04	0.03
	0.01	0.05	0.32	0.09	0.03	0.04	0.24	0.06	0.04

** : Significance at 0.01 probability level.

The general mean of chlorophyll a, total chlorophyll and carotenoid pigments was significantly increased over the control in irradiated plants grown in Nile water and wastewater. This means that irradiated plants were more genetically diverse than unirradiated control. These results agree with Pandey and Verma (2020), who investigated that *Ceratophyllum demersum* L. grown in a 20% level of wastewater showed maximum chlorophyll content at 30 and 60 days of growth. The reduction in total chlorophyll and carotenoids in 50 minutes irradiated plants grown in Nile water may be related to the toxic effect of 50 minutes exposure time irradiation that inhibit chlorophyll formation. The same dose of 50 minutes achieved a significant decrease in chlorophyll a, total chlorophyll and carotenoids in plants grown in wastewater. This indicated that this decrease in chlorophyll and carotenoid pigments in irradiated plants grown in Nile water and wastewater was mainly due to the effect of UV irradiation. Along with the reduction in chlorophyll content at the dose of 50 minutes, the irradiated plants exhibited symptoms of growth changes as the result of UV phytotoxicity (Kaur *et al*. 2010). The most visible morphological changes, growth changes and other alterations were observed before in this study in relative growth rate and net primary productivity. The inhibition in growth may be due to decreases in average branch length, chlorosis, decreases in the number of whorled leaves, leaf disconnection, as well as, finally the death of plants (Jassim 2009).

activity. Therefore,chlorophyll and carotenoid contents can be used as an indicators of genetic diversity in the population of *C. demersum* L. The results tabularized in Table 9 achieved that the yield of chlorophylla and carotenoid pigments in irradiated plants grown in Nile water and wastewater in relation to the crossepoding values at zero time were significantly decline at the dose of 50 minutes UV irradiation. In contrast, the doses of 10, 20, 30, 40, and 60 minutes duration time to UV-irradiation achieved significant increase in the yield of chlorophyll a, b, total and carotenoid contents in irradiated plants grown in Nile water over the control at zero time. Meanwhile, the doses of 10, 40 and 60 minutes appeared significant increase in the yield of chlorophyll a and total chlorophyll in irradiated plants grown in wastewater. Besides, the doses of 40 and 60 minutes appeared significant increase in carotenoid contents of irradiated plants grown in wastewater. The increase in photosynthetic pigments by irradiated plants as shownherein may leading to increase the aerobic conditions for submerged aquatic plants as *C. demersum*L. These aerobic conditions are due to increasing the rate of photosynthetic activity through the oxygen released in Nile water and wastewater and depletion of carbon dioxide (Al-Nabhan and Al-Abbawy 2021).

Removal of heavy metals from natural water: Regarding to the results tabularized in Table 10, the removal efficiency of selenium

was obtained only by irradiated plants. This removal was ranged between 10-20%. The highest removal efficiency of selenium was obtained by the plants irradiated with 50 and 60 minutes UV. This means that irradiated *C. demersum*L. has contributed in the phytoremoval rate of selenium from Nile water. Both irradiated and non-irradiated plants do not achieve any removal of mercury and cobalt probably to their lower concentrations in the natural water.The lowest removal efficiency of lead was achieved by unirradiated plants. The removal efficiency of lead was ranged between 38.46-76.92%.The highest removal efficiency of lead was obtained by the plants irradiated with 20 minutes (76.92%) followed by that irradiated with 40 minutes (61.54%), 30, 50 and 60 minutes (69.23%). The lowest removal efficiency of chromium was obtained by the plants irradiated with 10 and 20 minutes.Meanwhile,the greatest removal of chromium (31.58%) was obtained by unirradiated plants and that irradiated with 30, 40, 50, and 60 minutes UV. These results agreed with Kadhim and Kareem (2023), who found that *C. demersum* L. recorded the highest removal of organic nitrogen reached its concentration to 26.33% in relation to the control of about 36.99%. It is observed that *C. demersum* L. helps to reduce the inorganic load in wastewater to a lower significant level as indicated by the removal efficiency.

As shown in this study the decline of inorganic pollutants by irradiated plants was more efficient than the reduction in the control treatments.This reduction can be due to higher uptake by irradiated plants. The results are in line with the finding of Germ *et al*. (2002), who reported that *C. demersum*L. produced significant values of UV-B absorbing compounds providing a protective filtering against harmful UV-B radiation.The complexity of these compounds has been increasing during evaluation. Lead toxicity inhibit plant growth, causing distortion in chloroplast ultrastructure, inhibiting the formation of photosynthetic pigments, as well as, enzymes involved in calvin cycle (Prasad and Prasad 1987). The results agreed with Abdulwahid (2023), who reported that aquatic plants as *C. demersum* L. remove heavy metals through absorption or surface absorption and integrated its into their system to be accumulate in certain bounded forms. On the basis of the present findings, it can be suggested that *C. demersum* L. is an efficient key agent for phytoremediation of wastewater via removal nutrients.

Heavy metals removal from wastewater

Ceratophyllum demersum L. is a potentially useful aquatic plant used in this study for bioremediation of heavy metals in wastewater (Table 11).

Tabel 10. Removal efficiency percent of heavy metals from Nile water by irradiated *Ceratophyllum demersum* L.

Sample code	Selenium (Se)		Indium (In)		Mercury (Hg)		Lead (Pb)		Chromium (Cr)		Cobalt (Co)	
	Con.	RE	Con.	RE	Con.	RE	Con.	RE	Con.	RE	Con.	RE
T_1	0.010	0.0	0.045	0.0	0.001	0.0	0.013	0.0	0.019	0.0	0.001	0.0
T_2	0.010	0.0	0.012	73.33	0.001	0.0	0.008	38.46	0.013	31.58	0.001	0.0
T_3	0.009	10	0.011	75.56	0.001	0.0	0.005	61.54	0.015	26.67	0.001	0.0
T_4	0.009	10	0.020	55.56	0.001	0.0	0.003	76.92	0.015	26.67	0.001	0.0
T_5	0.009	10	0.026	42.22	0.001	0.0	0.004	69.23	0.013	31.58	0.001	0.0
T_6	0.009	10	0.019	57.78	0.001	0.0	0.005	61.54	0.013	31.58	0.001	0.0
T_7	0.008	20	0.010	77.78	0.001	0.0	0.004	69.23	0.013	31.58	0.001	0.0
T_8	0.008	20	0.023	48.89	0.001	0.0	0.004	69.23	0.013	31.58	0.001	0.0

Con: Concentration by ppm. RE: Removal efficiency percentage.

Tabel 11. Removal efficiency percent of heavy metals from wastewater by irradiated *Ceratophyllum demersum* L.

Sample code	Strontium (Sr)		Indium (In)		Barium (Ba)		Mercury (Hg)		Lead (Pb)	
	Con.	RE	Con.	RE	Con.	RE	Con.	RE	Con.	RE
T_9	5.361	0.0	0.984	0.0	8.767	0.0	0.008	0.0	0.067	0.0
T_{10}	1.858	65.34	0.170	82.72	2.339	73.32	0.008	0.0	0.021	68.66
T_{11}	1.173	78.12	0.064	93.50	2.133	75.67	0.007	12.5	0.024	64.18
T_{12}	1.533	71.40	0.032	96.75	2.356	73.13	0.007	12.5	0.043	35.82
T_{13}	1.975	63.16	0.022	97.76	2.352	73.17	0.006	25.0	0.049	26.86
T_{14}	1.949	63.64	0.017	98.27	2.143	75.56	0.006	25.0	0.044	34.33
T_{15}	1.752	67.32	0.013	98.68	2.269	74.12	0.006	25.0	0.025	62.69
T_{16}	1.416	73.59	0.011	98.89	2.172	75.22	0.006	25.0	0.053	20.89

Sample code	Titanium (Ti)		Manganese (Mn)		Iron (Fe)		Cobalt (Co)		Nickel (Ni)	
	Con.	RE	Con.	RE	Con.	RE	Con.	RE	Con.	RE
T_9	31.612	0.0	1.365	0.0	2.688	0.0	0.014	0.0	0.423	0.0
T_{10}	14.842	53.05	0.683	49.96	2.247	16.41	0.011	21.43	0.170	59.81
T_{11}	12.280	61.15	0.355	73.99	2.205	17.97	0.009	35.71	0.184	56.50
T_{12}	12.913	59.15	0.481	64.76	2.390	11.09	0.011	21.43	0.252	40.42
T_{13}	14.258	54.90	1.356	0.66	2.422	9.90	0.010	28.57	0.151	64.30
T_{14}	13.073	58.64	0.521	61.83	2.311	14.03	0.012	14.28	0.398	5.91
T_{15}	12.181	61.47	1.062	22.20	2.555	4.95	0.011	21.43	0.332	21.51
T_{16}	12.146	61.58	0.316	76.85	2.505	6.81	0.010	28.57	0.267	36.88

Con: Concentration by ppm.
RE: Removal efficiency percentage.

The advantage of treating wastewater by *C. demersum* L. is the simultaneous removal of inorganicvectors containing water.Further there were no requirements for any pretreatment needed in this methodology. When the wastewater discharged in water bodies and then treated with *C. demersum* L. the dissolved oxygen level will not be depleted significantly to affected the aquatic life.It could be concluded that *C. demersum* L. can be used successfully as a phytoremediation key forremoving inorganic pollutants from wastewater.

Removal efficiency of strontium by *C. demersum* L. was ranged between 63.16 to 78.12 by plants irradiated with 30 and 10 minutes UV, respectively.The highest removal efficiency percent of strontium was obtained by the plants irradiated with 10 min (78.12%) followed by 60 min (73.59%) and 20 min (71.40%).This indicated that *C. demersum* L. can bioaccumulate,bioremove and biostabilize trace metal contaminants, as well as, other pollutants unselectively by acting as filters or traps (Osmolovskaya and Kurilenko 2005). These results indicated that plants irradiated with 10 minutes UV showed

the greatest removal efficiencies (78.12%). The removal efficiency present of barium was ranged between 73.13 to 75.67% by the plants irradiated with 20 and 10 minutes UV, respectively.The greatest removal efficiency of barium was obtained by plants irradiated with 10 minutes (75.67%) followed by that irradiated with 40 minutes (75.56%) and 60 minutes (75.22%), where non-irradiated plants achieved removal efficiency reached to 73.32%.Unirradiated plants achieved zero removal efficiency of mercury. Meanwhile, plants irradiated with 10 and 20 minutes UV appeared removal efficiency of mercury reached to 12.5%.Moreover, the plants irradiated with 30, 40, 50and 60 minutes showed removal efficiency of mercury reached to 25%. Furthermore, the greatest removal efficiency (68.66%) of lead was appeared by non-irradiated plants.So, the removal efficiency of lead was ranged between 20.89% to 68.66% by the plants irradiated with 60 minutes and non-irradiated plants, respectively.

The removal efficiency of titanium was ranged between 53.05to 61.58% by non-irradiated and irradiated plants with 60 minutes UV, respectively.Highest removal efficiency of titanium was obtained by the plants irradiated with 60 minutes (61.58%) followed by that irradiated with 50 minutes (61.47%) and 10 minutes (61.15%). Thus, any marked increase in heavy metals bioaccumulate may cause alterations and physiological disturbances with various levels. It is well known that lead (Pb) is very toxic to the plants. Thus, chlorophyll concentration in plant tissues is very sensitive to such metal toxicity (Gupta and Chandra and 1996). The highest biosorption of lead was shown by unirradiated *Ceratophyllum* plants. There were a gradually decrease in lead biosorption with increasing the doses of UV-irradiation which reached to 20.89% at 60 minutes UV. It is noteworthy that irradiated *C. demersum* L. can not tolerate Pb concentration in wastewater. This indicated that irradiated plants are extremely sensitive to Pb in solution. The results obtained herein agreed with Shukla *et al*. (2012), who found that *Ceratophyllum demersum* L. harboring phytochelatin synthase (PCS) genes encoding the key enzyme in heavy metal detoxification and accumulation, which used to develop transgenic plants for the purpose of phytoremediation. Earlier studies by Brunetti *et al*. (2011) suggested that overexpressing of PCS in transgenic plants leading to metal sensitive phenotypes. The removal efficiency of manganese from wastewater was ranged between 0.66 to 76.85%. The greatest removal efficiency (76.85%) was obtained by the plants irradiated with 60 minutes followed by that irradiated with 10 minutes (73.99%) and 20 minutes (64.76%). Meanwhile, iron removal efficiency was ranged between 4.95-17.97%. The highest removal efficiency of iron (17.97%) was obtained by the plants irradiated with 10 minutes (17.97%) followed by unirradiated plants (16.41%) and that irradiated with 40 minutes (14.03%). Furthermore, the removal efficiency of cobalt was ranged between 14.28 - 35.71%. The highest removal efficiency of cobalt (35.71%) was achieved by the plants irradiated with 10 minutes followed by that irradiated with 30 and 60 minutes (28.57%). Besides, the removal efficiency of nickel was ranged between 5.91 - 59.81%. The greatest removal efficiency of nickel (64.30%) was obtained by the plants irradiated with 30 minutes followed by non-irradiated plants (59.81%) and that irradiated with 10 minutes (56.50%) and 20 minutes (40.42%). Overall, the plants irradiated with 10 minutes revealed the greatest removal efficiency of strontium (78.12%), barium (75.97%), iron (17.97%), and cobalt (35.71%). Moreover, the plants irradiated with 60 minutes showed the highest removal efficiency of mercury (25.0%), titanium (61.58%) and manganese (76.85%). In addition, the greatest removal efficiency of lead (68.66%) was obtained by unirradiated plants. Besides, the highest removal efficiency of nickel (64.30%) was achieved by the plants irradiated with 30 minutes UV. Removal efficiency of heavy metals from industrial effluents is beneficial because it serves to decrease toxicity and readiness of heavy metals in water (Shelef and Rachmilevitch 2013). In most removal efficiency irradiated plants gave higher efficiency in removing heavy metals from wastewater. The decline in the concentration of total dissolved soilds as manganese and iron is a result of plants need to basic elements for building their tissues (Gondek *et al*. 2020). The presence of these aquatic plants in wastewater increases the value of dissolved oxygen as a by-product of photosynthesis which consumes carbon dioxide gas during this process. This leads to availability of aerobic conditions in wastewater that increases the efficiency of aerobic bacteria in decomposing organic material and decreasing water content from vital oxygen requirement (Mohan *et al*. 2023). No removal rate of 100% was obtained in this study for any element. The greatest removal rate reached to 78.12% for strontium by the plants irradiated with 10 minutes. The results obtained herein indicated that *C. demersum* L. can tolerate heavy metal concentrations in wastewater as a result of their possession of mechanisms to be accumulate these elements within its tissues. In the same time these plants also need minerals as micronutrients to be used in physiological processes. Therefore, the aquatic plants have a high ability to withdraw these elements from the wastewater, but not over than the acceptable values because their concentrations increased leads to toxicity and plants death (Mustafa and Hayder 2021). The results agreed with Abdulwahid (2023), who found less significant removal rate by*C. demersum*L. to decline Cd levels which reached to 6.9% in aquaculture and 10.8% in wastewater treated. These results reflected that this aquatic plant may require an appropriate adaptation time to bear the toxicity of high concentrations of heavy metals especially in wastewater (Nagajyoti *et al*. 2010). The results indicated that aquatic plants remove heavy metals through absorption or surface adsorption to be integrate them into their system and then bioaccumulate in certain bounded forms (Sas-Nowosielska *et al*. 2008). Thus, *C. demersum*L. is biological unchangeable filter used for purification of water bodies via bioaccumulating of toxins and dissolved metals in their tissues. So, this plant is relatively ecofriendliness and inexpensive material (Singh *et al*. 2012).Rai (2009) appeared the important role of leaves or leaves like branches to enhance the surface area of adsorption heavy metals to be bioaccumulate and store in intracellular spaces, cell walls and vacuoles. This was mentioned in a structural study by Sharma and Dubey (2005), who reported that the increase in the size of cortical tissues of internodes stems-like may important in the resistance of aquatic plants to heavy metals.

Molecular differentiation of genetic diversity induce: Some techniques of molecular markers application have been demonstrated by several investigators for extracting their patterns through using special reproducible primers for each technique. In the present study SCoT marker technique was employed for the molecular genetic analysis of fourteen samples. Seven samples numbered 1, 2, 3, 4, 5, 6 and 7 were treated with Nile water and the other seven samples numbered 14, 15, 16, 17, 18, 19 and 20 were grown first in Nile water followed by wastewater.

Start Codon Target (SCoT)-PCR technique: Start codon targeted (SCoT) polymorphism marker, is a molecular marker techniquegained a considerable significance in plant genetics due to its many desirable features. It was targets the region flanking the start codon ATG as a highly conserved region in plant genes. SCoT is targets functional genes. It was developed based on the shortconserved region flanking the initiation codon ATG named start codon (Collard and Mackill, 2009). In this study ten SCoT primers were used to analyze the DNAs isolated from fourteen samples using SCoT genetic analysis.

Analysis with SCoT- 01

As shown in Figure 1 and Table 12 which displayed the total number of amplified fragments by this primer that reached to four bands. Out of the total four bands two polymorphic were amplified. Then, the percentage of polymorphism was 50%. This primer showed the lowest number of the total amplified bands which reached to four bands if compared with the other nine SCoT primers. On the other hand, two monomorphic bands with a molecular size of 185 and 190 bp are presented in all the fourteen samples investigated. Regarding the two polymorphic bands amplified by the SCoT-01 Primer, one band of them was non-unique polymorphic, while the other band was positive unique with a molecular size at 330 bp. The results obtained herein agreed with Huang *et al*. (2014), who determined the level of polymorphism in six Hemarthria cultivars using seven SCoT primers, which produced amplified fragments reached to 105 bands with an

average 15 bands per sample. SCoT markers were used by Gajera *et al*. (2014) on 20 mango cultivars using 19 primer markers for genetic comparison among these cultivars. These SCoT markers were produced the total of 117 loci among 20 mango cultivars, out of them 96 (82.05%) were polymorphic. Therefore, primers of SCoT marker analysis were designed from the conserved region around the translation initiation codon ATG (Sawant *et al*. 1999). This molecular marker technique have been used to evaluate genetic diversity in the populations, quantitative trait locus analysis, fingerprinting and polygenetic studies (Vivodík *et al*. 2017).

Analysis with SCoT- 02: As shown in Figure 2 and Table 13 concerning the results of amplified bands obtained by SCoT-02, the total number of amplified bands were reached to 20. From which 12 bands were polymorphic with polymorphism 60 %. This primer appeared the highest number of total amplified bands which reached twenty bands if compared with the others. Three monomorphic bands were amplified at the molecular size of 335, 230 and 170 bp which are presented in all fourteen samples investigated. However, five bands were positive unique at molecular size of 1250, 840, 750, 640 and 210 bp.

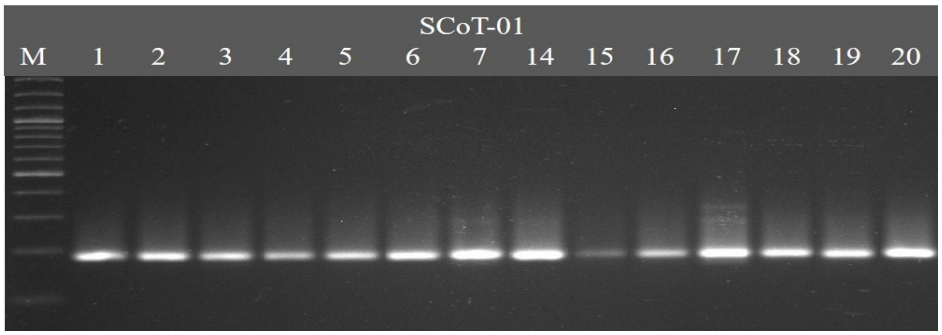

Figure 1. SCoT-01-PCR profile of fourteen samples, seven of them numbered 1, 2, 3, 4, 5, 6 and 7 were grown in Nile water, the others numbered 14, 15, 16, 17, 18, 19 and 20 were grown first in Nile water followed by wastewater

Table 12. Number and size of amplified DNA fragments generated from oligonucleotide SCoT- 01 used to establish SCoT-PCR fingerprints in fourteen samples

MW (bp)	1	2	3	4	5	6	7	14	15	16	17	18	19	20	Polymorphism
330	0	0	0	0	0	0	0	0	0	0	1	0	0	0	Unique
275	0	0	0	0	0	0	1	0	0	0	1	0	0	1	Polymorphic
190	1	1	1	1	1	1	1	1	1	1	1	1	1	1	Monomorphic
185	1	1	1	1	1	1	1	1	1	1	1	1	1	1	Monomorphic

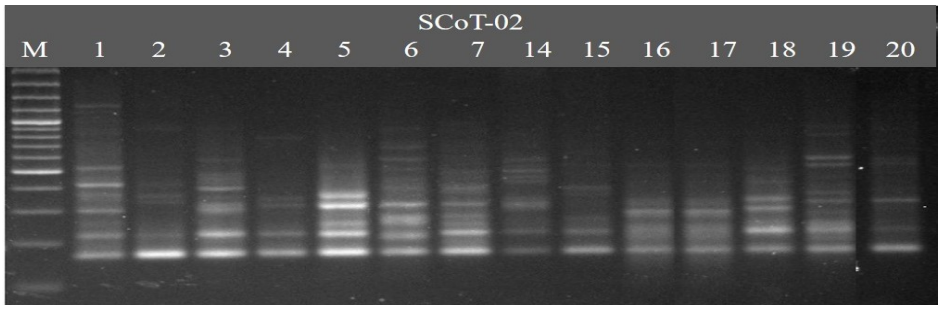

Figure 2. SCoT- 02 - PCR profile of fourteen samples, seven of them numbered 1, 2, 3, 4, 5, 6 and 7 were grown in Nile water, the others numbered 14, 15, 16, 17, 18, 19 and 20 were grown first in Nile water followed by wastewater

Table 13. Number and size of amplified DNA fragments generated from oligonucleotide SCoT-02 used to establish SCoT-PCR fingerprints in fourteen samples.

MW (bp)	1	2	3	4	5	6	7	14	15	16	17	18	19	20	Polymorphism
1250	1	0	0	0	0	0	0	0	0	0	0	0	0	0	Unique
890	1	1	0	0	0	0	0	0	0	0	0	0	0	0	Polymorphic
840	0	0	0	0	0	0	0	0	0	0	0	0	1	0	Unique
810	0	0	0	1	0	1	0	0	0	0	0	0	0	0	Polymorphic
750	0	0	0	0	0	1	0	0	0	0	0	0	0	0	Unique
640	0	0	0	0	0	0	0	0	0	0	0	0	1	0	Unique
590	0	0	1	0	0	1	0	1	0	1	1	0	1	1	Polymorphic
520	1	0	0	0	1	1	1	1	0	0	0	0	0	0	Polymorphic
480	0	0	1	0	1	0	0	1	0	0	0	0	0	0	Polymorphic
440	0	0	0	0	0	0	0	0	1	0	0	1	1	0	Polymorphic
430	1	1	1	0	1	1	1	0	0	0	0	0	0	0	Polymorphic
370	1	1	0	0	1	0	0	0	0	0	0	0	1	0	Polymorphic
335	1	1	1	1	1	1	1	1	1	1	1	1	1	1	Monomorphic
310	1	0	1	0	0	0	0	0	0	0	0	1	0	0	Polymorphic
300	0	0	0	0	0	0	1	0	0	1	1	0	1	0	Polymorphic
260	0	0	0	0	1	1	1	0	0	0	0	1	1	0	Polymorphic
230	1	1	1	1	1	1	1	1	1	1	1	1	1	1	Monomorphic
220	0	0	0	0	0	0	0	0	0	1	1	0	0	0	Polymorphic
210	0	0	0	0	0	0	0	0	0	0	0	0	1	0	Unique
170	1	1	1	1	1	1	1	1	1	1	1	1	1	1	Monomorphic

The twelve amplified polymorphic bands were at the molecular size of 890, 810,590, 520, 480, 440, 430, 370, 310, 300, 260 and 220 bp. So, there were a total of 17 bands were polymorphic. These including 5 unique and 12 polymorphic bands. Therefore, the percentage of total polymorphism was reached to 85 %. These results are in line with the findings of Que *et al*. (2014), who assessment the genetic diversity among 107 sugarcane accessions within the germplasm collection of a local sugarcane using 20 SCoT primers. These marker primers amplified 176 DNA fragments, out of them 163 (92.61%) were polymorphic. In addition, Gao *et al*. (2014) assessment the genetic diversity among 43 lycoris varieties. The authors found 23 SCoT primers out of 57 were detected to be high polymorphism. Furthermore, Jiang *et al*. (2014) analyzed genetic variations and genetic associations among 95 orchardgrass accessions using SCoT markers. The authors found total of 273 polymorphic bands were obtained with an average of 11.4 bands assessed per primer. In the same criteria, Zhang *et al*. (2015) studied the genetic diversity and genetic relationships among 53 *Elymus sibiricus* accessions using SCoT primer markers.

Analysis with SCoT- 03: As shown in Figure 3 and Table 14 concerning the results of amplified bands obtained by SCoT-03, the total number of amplified bands were reached to 16, from which 10 bands were polymorphic and one band was unique with polymorphism 68.75 %.

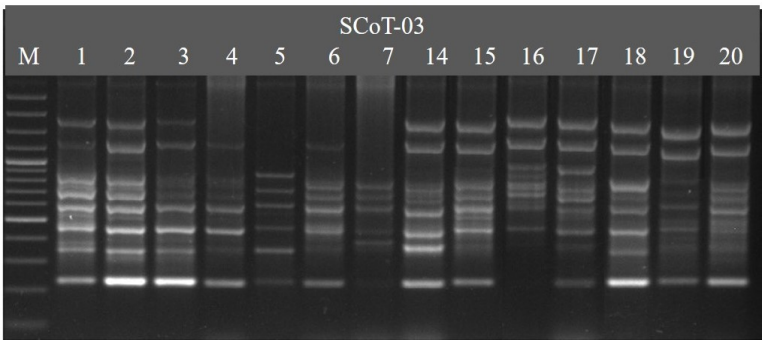

Figure 3. SCoT- 03 - PCR profile of fourteen samples, seven of them numbered 1, 2, 3, 4, 5, 6 and 7 were grown in Nile water, the others numbered 14, 15, 16, 17, 18, 19 and 20 were grown first in Nile water followed by wastewater

Table 14. Number and size of amplified DNA fragments generated from oligonucleotide SCoT- 03 used to establish SCoT-PCR fingerprints in fourteen samples

MW (bp)	1	2	3	4	5	6	7	14	15	16	17	18	19	20	Polymorphism
1600	0	0	0	0	0	0	0	1	1	1	1	1	1	1	Polymorphic
1500	1	1	1	0	0	0	0	0	0	0	0	0	0	0	Polymorphic
1150	1	1	1	1	0	1	0	1	1	1	1	1	1	1	Polymorphic
890	0	0	0	0	1	0	0	0	0	1	0	0	0	0	Polymorphic
720	1	1	1	1	1	1	1	1	1	1	1	1	1	1	Monomorphic
690	0	0	0	0	0	0	0	0	0	1	1	0	0	1	Polymorphic
630	1	1	1	1	1	1	1	0	0	0	0	0	0	0	Polymorphic
620	0	0	0	0	0	0	0	1	1	1	1	1	1	1	Polymorphic
540	1	1	1	1	1	1	1	1	1	1	1	1	1	1	Monomorphic
510	1	1	1	1	1	1	1	1	1	1	1	1	1	1	Monomorphic
460	0	0	0	0	0	0	0	1	1	0	0	1	1	1	Polymorphic
430	1	1	1	1	1	1	1	1	1	1	1	1	1	1	Monomorphic
370	0	0	0	0	0	0	1	0	0	0	0	0	0	0	Unique
340	1	1	1	1	1	1	1	1	1	1	1	1	1	1	Monomorphic
310	0	1	1	0	0	0	0	0	0	0	0	0	0	0	Polymorphic
210	1	1	1	1	1	1	0	1	1	0	1	1	1	1	Polymorphic

The five monomorphic bands were amplified at molecular size of 720, 540, 510, 430 and 340 bp which are presented in all fourteen samples investigated. The positive unique band was amplified at the molecular size of 370 bp. The ten polymorphic bands were amplified at the molecular size of 1600, 1500, 1150, 890, 690, 630, 620, 460, 310 and 210 bp. Genetic variations among plant individuals were studied using different PCR-based DNA marker techniques, one of them is SCoT marker technique. This is technically simple, cheap and realized a relatively large number of markers per sample (Molin *et al*. 2013). The higher reproducibility of SCoT markers if compared with RAPD markers ensure the higher primer lengths with subsequently higher temperatures annealing (Rajesh *et al*. 2015). Moreover, Gorji *et al*. (2011) stated that SCoT molecular markers are more effective and informative tool followed by ISSRs and AFLP techniques in the fingerprinting of potato varieties.

Analysis with SCoT- 04: As shown in Figure 4 and Table 15 concerning the results of amplified bands obtained by SCoT-04 in fourteen samples investigated, the total number of amplified bands were reached to 14. These included five bands were polymorphic one of them was unique with a polymorphism reached to 35.71 %. SCoT markers have been widely used to study genetic variations in different plant populations. Where, they are amplified multiple loci in a time as multiplex PCR, *Taq* polymerase system which resulting in accurate genotyping. SCoT primer markers were amplify more than one target genetic sequence in a single reaction. Therefore, PCR mix optimized for high yield performance is required (Sathapondecha *et al*. 2021). Therefore, the importance of genetic data increases because it can help for identification of species and populations which have a reduced genetic diversity (Allendorf and Luikart 2007). So, studying population structure of *C. demersum* L. is done using SCoT molecular technique based on nuclear DNA. Any changes in the niche environment may leading to mutation, genetic drift and polymorphism at the molecular level (Sbordoni 2010). Nine monomorphic bands were amplified at the molecular size of 970, 840, 610, 540, 520, 430, 400, 370 and 270 bp. These are presented in all fourteen samples investigated. The positive unique band was amplified at the molecular size of 290 bp. The polymorphic amplified bands were amplified at the molecular size of 1350, 1200, 470 and 330 bp. Furthermore, Gorji *et al*. (2011) used SCoT methodology for fingerprinting of 24 varieties and segregating population of tetraploid potato. The authors found that the number of scoreable and polymorphic bands produced for varieties were more than that of genotypes. In addition, some primers showed more than one allele at a given locus.

Analysis with SCoT- 06: As shown in Figure 5 and Table 16 concerning the results of amplified bands obtained by SCoT- 06 in

fourteen samples, the total number of amplified bands were reached to 14. Out of them 12 bands were polymorphic with a polymorphism reached to 85.71%. This primer achieved the high number of polymorphic bands. The other two monomorphic bands were amplified at the molecular size of 210 and 150 bp. The monomorphic bands are presented in all the samples investigated.

The polymorphic bands were amplified at the molecular size of 1300, 870, 720, 610, 380, 340, 310, 270, 190, 180 and 100 bp. The results obtained herein agreed with Abu Almaaty *et al*. (2020), who studied genetic similarity among four fishes species using ISSR molecular marker and SDS-PAGE. In addition, Abu Almaaty *et al*. (2020) used SCoT technique as a molecular tool for characterizing genetic

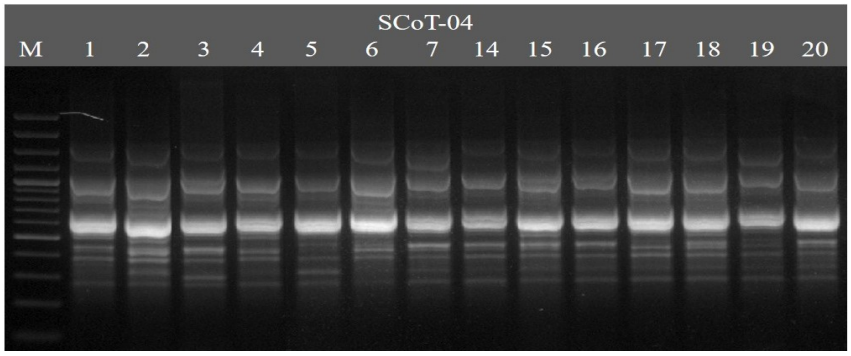

Figure 4. SCoT- 04 - PCR profile of fourteen samples, seven of them numbered 1, 2, 3, 4, 5, 6 and 7 were grown in Nile water, the others numbered 14, 15, 16, 17, 18, 19 and 20 were grown first in Nile water followed by wastewater

Table 15. Number and size of amplified DNA fragments generated from oligonucleotide SCoT- 04 used to establish SCoT-PCR fingerprints in fourteen samples

MW (bp)	1	2	3	4	5	6	7	14	15	16	17	18	19	20	Polymorphism
1350	0	0	0	0	0	0	0	1	1	1	1	1	1	1	Polymorphic
1200	1	1	1	1	1	1	1	0	0	0	0	0	0	0	Polymorphic
970	1	1	1	1	1	1	1	1	1	1	1	1	1	1	Monomorphic
840	1	1	1	1	1	1	1	1	1	1	1	1	1	1	Monomorphic
610	1	1	1	1	1	1	1	1	1	1	1	1	1	1	Monomorphic
540	1	1	1	1	1	1	1	1	1	1	1	1	1	1	Monomorphic
520	1	1	1	1	1	1	1	1	1	1	1	1	1	1	Monomorphic
470	0	0	0	0	0	0	0	1	1	1	1	1	1	1	Polymorphic
430	1	1	1	1	1	1	1	1	1	1	1	1	1	1	Monomorphic
400	1	1	1	1	1	1	1	1	1	1	1	1	1	1	Monomorphic
370	1	1	1	1	1	1	1	1	1	1	1	1	1	1	Monomorphic
330	0	1	0	0	1	1	1	1	1	1	1	1	1	1	Polymorphic
270	1	1	1	1	1	1	1	1	1	1	1	1	1	1	Monomorphic
290	0	0	1	0	0	0	0	0	0	0	0	0	0	0	Unique

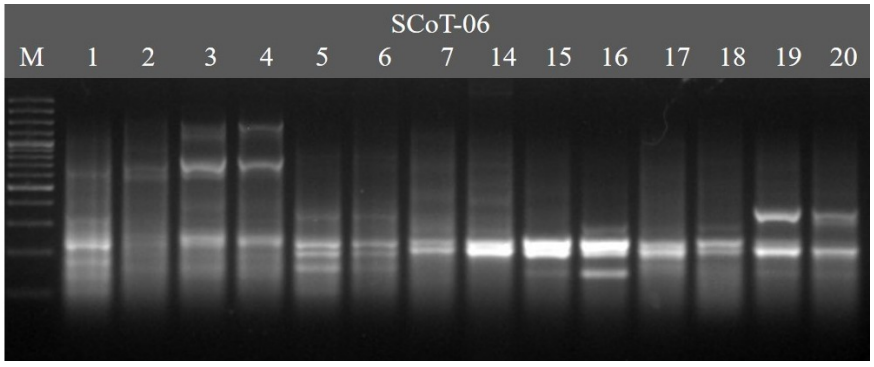

Figure 5. SCoT- 06 - PCR profile of fourteen samples, seven of them numbered 1, 2, 3, 4, 5, 6 and 7 were grown in Nile water, the others numbered 14, 15, 16, 17, 18, 19 and 20 were grown first in Nile water followed by wastewater

Table 16. Number and size of amplified DNA fragments generated from oligonucleotide SCoT- 06 used to establish SCoT-PCR fingerprints in fourteen samples

MW (bp)	1	2	3	4	5	6	7	14	15	16	17	18	19	20	Polymorphism
1300	0	0	1	1	0	0	0	0	0	0	0	0	0	0	Polymorphic
870	1	1	0	0	0	0	0	0	0	0	0	0	0	0	Polymorphic
720	1	1	1	1	0	0	0	0	0	0	0	0	0	0	Polymorphic
610	1	1	1	1	0	0	0	0	0	0	0	0	0	0	Polymorphic
380	0	0	1	1	0	0	0	0	0	0	0	0	0	0	Polymorphic
340	0	0	0	0	0	0	0	0	0	0	0	0	1	1	Polymorphic
310	1	0	0	0	1	1	1	0	0	0	0	0	0	0	Polymorphic
270	0	0	0	0	0	0	0	1	1	1	1	1	0	0	Polymorphic
210	1	1	1	1	1	1	1	1	1	1	1	1	1	1	Monomorphic
190	0	0	0	0	0	0	0	1	1	1	1	1	1	1	Polymorphic
180	1	1	1	1	1	1	1	0	0	0	0	0	0	0	Polymorphic
160	1	1	1	1	1	1	1	1	1	1	1	1	1	1	Monomorphic
140	1	0	0	0	0	0	1	0	0	0	0	0	0	0	Polymorphic
100	1	0	0	0	1	0	0	0	0	0	0	0	0	0	Polymorphic

variability between two fish species of family Moronidae. By using 11 SCoT primers, the authors found genetic similarity between the two fish species was reached to 75.5%. However, SCoT markers are PCR-based single primers formed through the short conserved sequence of plant genes flanking the start codon ATG (Collard and Mackill 2009). This codon regions are conserved among all the genes. Despite being similar to other single primers (used both as forward and reverse primer), SCoT markers are more reliable because annealing temperature is 50 ^{0}C if compared with 37 - 42 ^{0}C, reproducible and easy to design. Since 2009, SCoT is a novel method for generating plant DNA markers. SCoT primers generated promising polymorphic bands and rare bands as shown in this study.

Analysis with SCoT-10: As shown in Figure 6 and Table 17 concerning the results of amplified bands obtained by SCoT-10, the total number of amplified bands were reached to 15. Out of these, three bands were polymorphic and three are unique bands. The percentage of polymorphism reached to 40 %. Nine monomorphic bands were obtained and amplified at the molecular size of 960, 870, 830, 550, 380, 370, 290, 270 and 240 bp.

Analysis with SCoT-11: According to the results presented in Figure 7 and Table 18 concerning the amplified bands obtained by SCoT-11, the total number of amplified bands were reached to 14. Out of these nine bands were polymorphic without unique bands with a polymorphism percentage reached to 64 %. Five monomorphic bands were obtained and amplified at the molecular size of 1050, 680, 570, 350 and 260 bp. These are presented in all samples investigated. The polymorphic bands were amplified at the molecular size of 920, 870, 760, 750, 540, 530, 470, 380 and 330 bp.

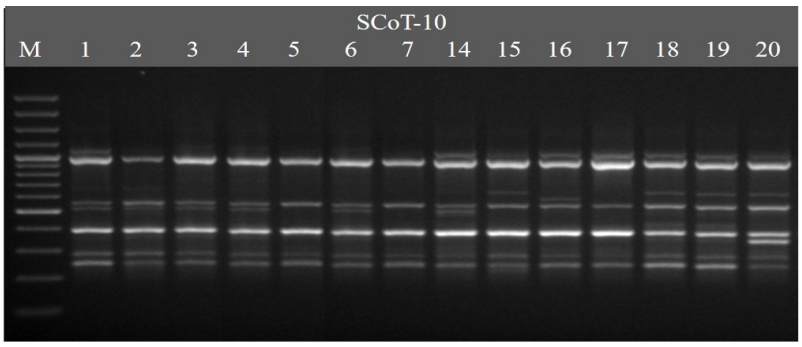

Figure 6. SCoT-10 - PCR profile of fourteen samples, seven of them numbered 1, 2, 3, 4, 5, 6 and 7 were grown in Nile water, the others numbered 14, 15, 16, 17, 18, 19 and 20 were grown first in Nile water followed by wastewater

Table 17. Number and size of amplified DNA fragments generated from oligonucleotide SCoT-10 used to establish SCoT-PCR fingerprints in fourteen samples

MW (bp)	1	2	3	4	5	6	7	14	15	16	17	18	19	20	Polymorphism
1100	1	0	0	0	0	0	0	0	0	0	0	0	0	0	Unique
1000	0	0	0	0	0	0	0	1	1	1	1	1	1	1	Polymorphic
960	1	1	1	1	1	1	1	1	1	1	1	1	1	1	Monomorphic
870	1	1	1	1	1	1	1	1	1	1	1	1	1	1	Monomorphic
830	1	1	1	1	1	1	1	1	1	1	1	1	1	1	Monomorphic
620	0	0	0	0	0	0	0	0	1	0	0	1	1	1	Polymorphic
590	0	0	0	0	0	0	0	0	0	1	0	0	0	0	Unique
550	1	1	1	1	1	1	1	1	1	1	1	1	1	1	Monomorphic
490	1	0	1	1	0	1	0	1	0	0	0	0	0	0	Polymorphic
380	1	1	1	1	1	1	1	1	1	1	1	1	1	1	Monomorphic
370	1	1	1	1	1	1	1	1	1	1	1	1	1	1	Monomorphic
320	0	0	0	0	0	0	0	0	0	0	0	0	0	1	Unique
290	1	1	1	1	1	1	1	1	1	1	1	1	1	1	Monomorphic
270	1	1	1	1	1	1	1	1	1	1	1	1	1	1	Monomorphic
240	1	1	1	1	1	1	1	1	1	1	1	1	1	1	Monomorphic

These are presented in all samples investigated. The positive unique bands were amplified at the molecular size of 1100, 590 and 320 bp. Whereas, the amplified polymorphic bands were amplified at molecular size of 1000, 620 and 490 bp. The results obtained herein agreed with Devi *et al*. (2019), who studied the polymorphism in the insect from *Chironomids* species by SCoT primer using standard agarose gel electrophoresis. The authors found 91.67% bands were polymorphic and 8.3% bands were monomorphic. Thus, Start Codon Targeted (SCoT) is a new tool for generating insect and plant DNA molecular markers. This technique was developed based on the conserved location flanking ATG start codon in the genes of plant species (Collard and Mackill 2009). This start codon is similarly applicable in animals. This technique used single 18-mer a forward and reverse primers in PCR and annealing temperature of 50 ^{0}C. Standard agarose gel electrophoresis was used to resolved PCR amplicons. SCoT methodology was used using genetically diverse genotypes in rice, as well as, backcross population.

The results obtained herein are in line with the findings of Vivodík *et al*. (2017), who genetically analysed 20 genotypes of maize using five SCoT markers. These primers generated 29 fragments, of which 22 (75.86%) were polymorphic with the average about 4.40 polymorphic fragments for each primer. Besides, general taxonomic classification was based on morphological parameters, but the availability of powerful and novel molecular genetic methods is helping taxonomicists in modifying the existing classification systems to establishing phylogenetic relationships depending on DNA fingerprinting and protein sequences specially in morphologically related species. PCR-based molecular markers are importants to be contributed in investigating phylogenetic relationships.

Analysis with SCoT-12: Regarding to the results obtained in Figure 8 and tabularized in Table 19 concerning the amplified bands obtained by SCoT-12, the total number of amplified bands were reached to 15. Out of these seven were polymorphic without unique bands with a

polymorphism percentage reached to 46.67%. Eight monomorphic bands were obtained and amplified at the molecular size of 600, 510, 490, 420, 410, 330, 210 and 200 bp. They are presented in all samples investigated. The polymorphic bands were amplified at the molecular size of 710, 460, 370, 250, 290, 260 and 170 bp.

The obtained results are in agreement with Devi *et al*. (2019), who used SCoT technique to evaluate genetic diversity of five *Chironomus* species using DNA isolated from either larvae or adults. The authors found that SCoT1 primer generated promising polymorphic bands, in addition to non-polymorphic bands.

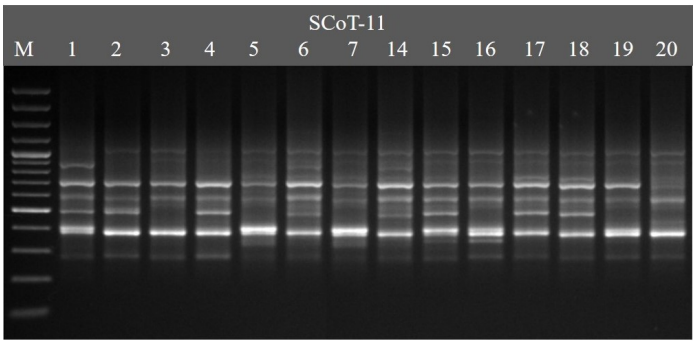

Figure 7. SCoT-11 - PCR profile of fourteen samples, seven of them numbered 1, 2, 3, 4, 5, 6 and 7 were grown in Nile water, the others numbered 14, 15, 16, 17, 18, 19 and 20 were grown first in Nile water followed by wastewater

Table 18. Number and size of amplified DNA fragments generated from oligonucleotide SCoT-11 used to establish SCoT-PCR fingerprints in fourteen samples

MW (bp)	1	2	3	4	5	6	7	14	15	16	17	18	19	20	Polymorphism
1050	1	1	1	1	1	1	1	1	1	1	1	1	1	1	Monomorphic
920	0	0	0	0	0	0	0	1	1	1	1	1	1	1	Polymorphic
870	1	0	0	0	0	1	0	1	0	0	0	0	0	0	Polymorphic
760	0	0	1	1	1	1	1	0	0	0	0	0	0	0	Polymorphic
750	0	0	0	0	0	0	0	1	1	1	1	1	1	1	Polymorphic
680	1	1	1	1	1	1	1	1	1	1	1	1	1	1	Monomorphic
570	1	1	1	1	1	1	1	1	1	1	1	1	1	1	Monomorphic
540	1	1	1	1	1	1	1	0	0	0	0	0	0	0	Polymorphic
530	0	0	0	0	0	0	0	1	1	1	1	1	1	1	Polymorphic
470	1	1	0	1	0	1	0	1	1	0	1	1	0	0	Polymorphic
380	0	0	0	0	1	0	1	0	1	1	0	0	1	0	Polymorphic
350	1	1	1	1	1	1	1	1	1	1	1	1	1	1	Monomorphic
330	0	0	0	0	1	0	1	1	1	1	0	0	0	0	Polymorphic
260	1	1	1	1	1	1	1	1	1	1	1	1	1	1	Monomorphic

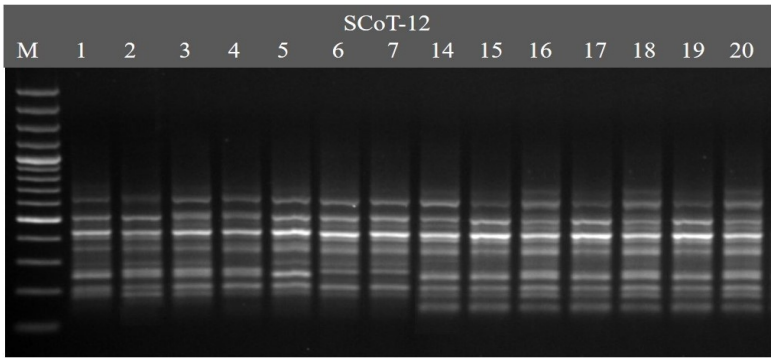

Figure 8. SCoT-12 - PCR profile of fourteen samples, seven of them numbered 1, 2, 3, 4, 5, 6 and 7 were grown in Nile water, the others numbered 14, 15, 16, 17, 18, 19 and 20 were grown first in Nile water followed by wastewater

Table 19. Number and size of amplified DNA fragments generated from oligonucleotide SCoT-12 used to establish SCoT-PCR fingerprints in fourteen samples

MW (bp)	1	2	3	4	5	6	7	14	15	16	17	18	19	20	Polymorphism
710	0	0	0	0	0	0	0	1	1	1	1	1	1	1	Polymorphic
600	1	1	1	1	1	1	1	1	1	1	1	1	1	1	Monomorphic
510	1	1	1	1	1	1	1	1	1	1	1	1	1	1	Monomorphic
490	1	1	1	1	1	1	1	1	1	1	1	1	1	1	Monomorphic
460	0	0	0	0	1	1	1	1	0	1	0	1	0	1	Polymorphic
420	1	1	1	1	1	1	1	1	1	1	1	1	1	1	Monomorphic
410	1	1	1	1	1	1	1	1	1	1	1	1	1	1	Monomorphic
370	0	0	0	0	0	0	0	1	1	1	1	1	1	1	Polymorphic
330	1	1	1	1	1	1	1	1	1	1	1	1	1	1	Monomorphic
250	1	1	1	1	1	0	0	1	1	1	1	1	1	1	Polymorphic
290	1	1	1	1	1	1	1	0	0	0	0	0	0	0	Polymorphic
260	1	1	1	1	1	1	1	0	0	1	0	1	0	1	Polymorphic
210	1	1	1	1	1	1	1	1	1	1	1	1	1	1	Monomorphic
200	1	1	1	1	1	1	1	1	1	1	1	1	1	1	Monomorphic
170	0	0	0	0	0	0	0	1	1	1	1	1	1	1	Polymorphic

On the basis of the present results, it can be noticed that SCoT technique could be used for evaluating genetic diversity in aquatic plants as *C. demersum* L. grown in polluted environments.

The future studies should focused on the increasing number of primers used to detect the genetic polymorphism or to delineate plant species to produce a promising results (Devi *et al*. 2019).

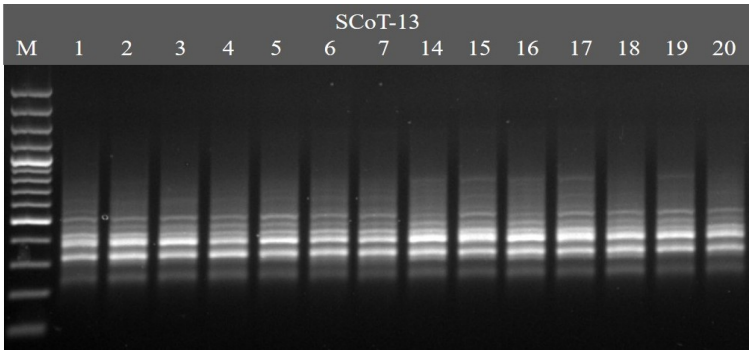

Figure 9. SCoT-13 - PCR profile of fourteen samples, seven of them numbered 1, 2, 3, 4, 5, 6 and 7 were grown in Nile water, the others numbered 14, 15, 16, 17, 18, 19 and 20 were grown first in Nile water followed by wastewater

Table 20. Number and size of amplified DNA fragments generated from oligonucleotide SCoT-13 used to establish SCoT-PCR fingerprints in fourteen samples

MW (bp)	1	2	3	4	5	6	7	14	15	16	17	18	19	20	Polymorphism
830	0	0	0	0	0	0	0	1	1	1	1	0	1	0	Polymorphic
690	0	0	0	0	0	0	0	1	1	1	1	1	1	1	Polymorphic
670	1	1	1	1	1	1	1	0	0	0	0	0	0	0	Polymorphic
560	1	1	1	1	1	1	1	1	1	1	1	1	1	1	Monomorphic
510	1	1	1	1	1	1	1	1	1	1	1	1	1	1	Monomorphic
490	0	0	0	0	0	0	0	1	1	1	1	1	1	1	Polymorphic
480	1	1	1	1	1	1	1	0	0	0	0	0	0	0	Polymorphic
460	1	1	1	1	1	1	1	1	1	1	1	1	1	1	Monomorphic
420	1	1	1	1	1	1	1	1	1	1	1	1	1	1	Monomorphic
380	1	1	1	1	1	1	1	1	1	1	1	1	1	1	Monomorphic
350	1	1	1	1	1	1	1	1	1	1	1	1	1	1	Monomorphic
330	1	1	1	1	1	1	1	1	1	1	1	1	1	1	Monomorphic
318	0	0	0	0	0	0	0	1	1	1	1	1	1	1	Polymorphic
270	1	1	1	1	1	1	1	1	1	1	1	1	1	1	Monomorphic
240	1	1	1	1	1	1	1	1	1	1	1	1	1	1	Monomorphic

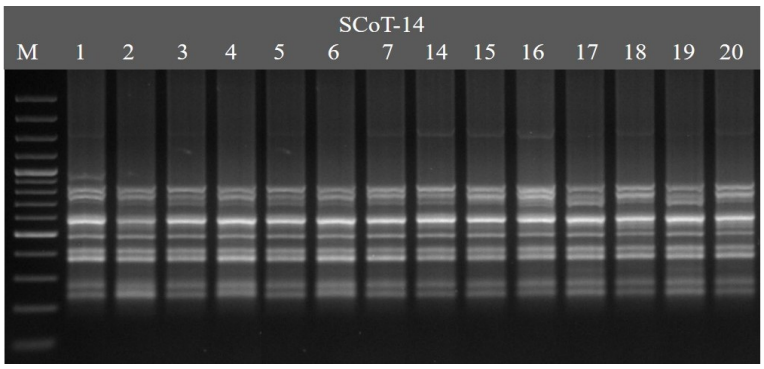

Figure 10. SCoT-13 - PCR profile of fourteen samples, seven of them numbered 1, 2, 3, 4, 5, 6 and 7 were grown in Nile water, the others numbered 14, 15, 16, 17, 18, 19 and 20 were grown first in Nile water followed by wastewater

Table 21. Number and size of amplified DNA fragments generated from oligonucleotide SCoT-13 used to establish SCoT-PCR fingerprints in fourteen samples

MW (bp)	1	2	3	4	5	6	7	14	15	16	17	18	19	20	Polymorphism
1600	1	1	1	1	1	1	1	1	1	1	1	1	1	1	Monomorphic
1500	0	0	0	0	0	0	0	1	1	1	1	1	1	1	Polymorphic
950	1	0	0	0	0	0	0	0	0	0	0	0	0	0	Unique
860	1	1	1	1	1	1	1	1	1	1	1	1	1	1	Monomorphic
780	1	1	1	1	1	1	1	1	1	1	1	1	1	1	Monomorphic
730	1	1	1	1	1	1	1	1	1	1	1	1	1	1	Monomorphic
710	0	0	1	0	1	1	1	1	1	1	1	1	1	1	Polymorphic
600	1	1	1	1	1	1	1	1	1	1	1	1	1	1	Monomorphic
580	1	1	1	1	1	1	1	1	1	1	1	1	1	1	Monomorphic
530	0	0	0	0	0	0	0	1	1	1	1	1	1	1	Polymorphic
480	1	1	1	1	1	1	1	1	1	1	1	1	1	1	Monomorphic
390	1	1	1	1	1	1	1	1	1	1	1	1	1	1	Monomorphic
350	1	1	1	1	1	1	1	1	1	1	1	1	1	1	Monomorphic
300	0	0	0	0	0	0	0	0	0	0	1	1	1	0	Polymorphic
260	1	1	1	1	1	1	1	1	1	1	1	1	1	1	Monomorphic
230	1	1	1	1	1	1	1	1	1	1	1	1	1	1	Monomorphic

Analysis with SCoT-13: According to the results obtained in Figure 9 and tabularized in Table 20 concerning the amplified bands obtained by SCoT-13, the total number of amplified bands were reached to 15. Out of them six were polymorphic without unique bands with a polymorphism percentage reached to 40%. Nine monomorphic bands were obtained and amplified at the molecular size of 560, 510, 460, 420, 380, 350, 330, 270 and 240 bp. They are presented in all samples investigated. The polymorphic bands were amplified at the molecular size of 830, 690, 670, 490, 480 and 318 bp. The results agreed with Rosengren *et al*. (2013), who reported that the levels of genetic variations are influenced gene flow, natural selection and adaptation. Meanwhile, Terracciano *et al*. (2012) decided that molecular markers was possible to estimate genetic diversity levels in various populations of aquatic plants and evaluate the viability potential of species grown in threatened environments. Furthermore, Amirmoradi *et al*. (2012) reported that SCoT technique was used based on the short conserved region flanking the sequence ATG start codon in plant genes. SCoT polymorphic markers as effective tool produce high reproducible and reliable bands to appear differences within populations exceptionally well. The results are also harmony with Baraki and Siahkolaee (2018), who used three SCoT primers to fingerprint nine samples of mosses and found a total of 80 amplified fragments, out of the 78 (97.5%) were polymorphic.

Genetic relationships among fourteen samples: Scored data obtained from the SCoT analysis were used to assessment the similarity matrices Table 22. Genetic similarity achieved were ranged between 0.70 to 0.96 with an average about 0.83. The highest genetic similarity was obtained between the samples numbered 15 and 18. While, the lowest genetic similarity was obtained between the samples numbered 1 and 16, as well as, between 1 and 19. Regarding SCoT markers, the results obtained herein agreed with Al-Yasi an Al-Qthanin (2024), who found that the genetic similarity values among 23 distinct species of *Juniperus procera* show significant genetic relatedness between the genotypes around from 0.76 - 0.95. The same authors reported that geological and climatic changes impact significantly on the genetic structure of *Juniperus* species genome's. On the other hand, Alotaibi and Abd-Elgawad (2022) stated that dominant markers only have two alleles signified as absent or present bands were detected by the variations in bands on electrophoretic gels. Thus, these findings appeared the utility of SCoT molecular markers in demonstrating the genetic relationships between wastewater treatments with *C. demersum* L. This will provide valuable for sustainable wastewater treatment in the future.

Cluster analysis as revealed by SCoT markers: The genetic relationships among 14 treatments of irradiated and nonirradiated *C. demersum* L. was studied using unweighted pair group method of arithmetic means (UPGMA) and Nei and Li's coefficients using free Tree/ Tree View Software **(Pavlicek *et al.* 1999)**. Model-based clustering has been performed to infer population structure of irradiated plants grown in Nile water and wastewater based on SCoT molecular markers. The dendrogram based on SCoT markers and a similarity matrix was presented in **Figure 11**. The dendrogram comprised two main clusters. The first cluster contains seven samples numbred 14, 15, 18, 20, 16, 17 and 19 pre-treated with UV-B irradiation followed by the plants were grown in wastewater. In addition, the second cluster contains the other seven samples numbered 5, 7, 6, 3, 4, 2 and 1 pre-treatment with UV-B irradiation followed by the plants were grown in Nile water.

Table 22. Similarity matrix among fourteen samples of C. *demersum*L. based on Dice coefficient as revealed by SCoT markers

	1	2	3	4	5	6	7	14	15	16	17	18	19	20
1	1.00													
2	0.93	1.00												
3	0.89	0.92	1.00											
4	0.90	0.93	0.94	1.00										
5	0.87	0.89	0.88	0.88	1.00									
6	0.88	0.89	0.89	0.91	0.92	1.00								
7	0.85	0.86	0.86	0.86	0.94	0.92	1.00							
14	0.74	0.76	0.76	0.76	0.78	0.80	0.75	1.00						
15	0.72	0.77	0.74	0.77	0.77	0.76	0.75	0.95	1.00					
16	0.70	0.74	0.74	0.74	0.78	0.75	0.77	0.93	0.93	1.00				
17	0.72	0.76	0.74	0.76	0.74	0.76	0.74	0.93	0.94	0.94	1.00			
18	0.74	0.77	0.75	0.77	0.77	0.78	0.76	0.93	0.96	0.91	0.93	1.00		
19	0.70	0.74	0.73	0.73	0.75	0.74	0.74	0.90	0.94	0.90	0.92	0.93	1.00	
20	0.72	0.76	0.76	0.76	0.76	0.77	0.76	0.93	0.93	0.93	0.93	0.94	0.92	1.00

Analysis with SCoT-14: As shown in Figure 10 and tabularized in Table 21 concerning the amplified bands obtained by SCoT-10, the total number of amplified bands we reached to 16. Out of them four were polymorphic and one band was unique with a polymorphism percentage reached to 31.25%. Eleven monomorphic bands were obtained and amplified at the molecular size of 1600, 860, 780, 730, 600, 580, 480, 390, 350, 260 and 230 bp. They are presented in all samples investigated. The positive unique band was amplified at the molecular size of 950 bp. Meanwhile, the other polymorphic bands were amplified at the molecular size of 1500, 710, 530 and 300 bp. The application of genomic diversity information has played an essential role in population genetic studies (Crespo *et al*. 2014). Banding-based molecular approaches were considered as useful tools in these studies. Sivaprakash *et al*. (2004) decided that the efficiency of molecular markers in genetic diversity studies was closely related with the level of its polymorphism. Baraki and Siahkolaee (2018) stated that SCoT primers that produced with an average about 97.5% and 91.66% polymorphism are more efficient as a molecular markers for genetic variations studies than ISSR (about 65%) polymorphism. Spagnuolo *et al*. (2002) found 20-68% polymorphism through population genetic studies of *Pleurochaete squarrosa* with ISSR marker. The results obtained herein exhibited that the genetic assessment using gene-targeted markers would be more applicable in population genetic structure between related species (Heidari *et al*. 2017). Ghorbanzadeh *et al*. (2021) noted the high degree of polymorphism in assessing the genetic diversity of *Juniper* populations. Sarmast *et al*. (2018) found 285 polymorphic DNA fragments were produced using four primers among eight populations of *Junipers* ssp. Parthiban *et al*. (2018) demonstrated that the values of genetic diversity studies reflected that genetic variations depending on the marker system applied.

The results are in line with Al-Yasiand Al-Qthanin (2024) who found two core clusters from the dendrogram, the first one has grouped nine genotypes and the second one has 14 genotypes. Shaban *et al*. (2022) stated that dendrograms generated by various molecular markers leading to produce inconsistent results as shown in bamboo, snake melons and sponge gourds. The results obtained herein are in line with the findings of Vivodík *et al*. (2017), who divided 20 genotypes of maize through cluster analysis into two main clusters, the first one included two maize genotypes and the second cluster containing 18 genotypes divided into two main subclusters. The same authors stated that SCoT markers shows effectiveness in maize genotypes analysis. It would be useful for further investigations in genotypes improvement and population genetics. Moreover, Cao *et al*. (2017) found that samples of aquatic plants analyzed by ISSR molecular markers originating from each lake tended to cluster together. In addition, the genetic relationships between distant aquatic plant populations reflected the effects of biotic, abiotic connectivity and

environmental selection pressure. The results are also agreed with Parkpoom *et al*. (2023), who found from the dendrogram that the two edible aquatic plants, *Ottelia alismoides* and *Ipomoea aquatic* collected near an electronic waste were separated into two main groups. This was correlated with the heavy metal quantity in the two plant samples. The same authors analyzed molecular changes in the two plant samples using ISSR patterns which achieved changes in the appearance of new DNA bands and the absence of DNA bands. The results of Parkpoom *et al*. (2023) indicated variations in the similarity index between each aquatic plant from the reference area and the electronic waste dumpsite.

Level of polymorphism: All indicesof polymorphism indicated a high level of variation in irradiated C. *demersum* grown in wastewater (Table23). Overall, more than 80% of the loci were found to be polymorphic as achieved by SCoT-2 and SCoT-6. The average number of rare bands were ranged between 1-5.The greatest number of rare bands were appeared by SCoT-2 followed by SCoT-10 primers sets. One rare band was appeared by SCoT-1, SCoT-3, SCoT-4 and SCoT-14 primer sets.The lower number of polymorphic bands was achieved by SCoT-14 primerwhich appeared the greatest number of monomorphic bands and produced polymorphism reached to 31.25%.

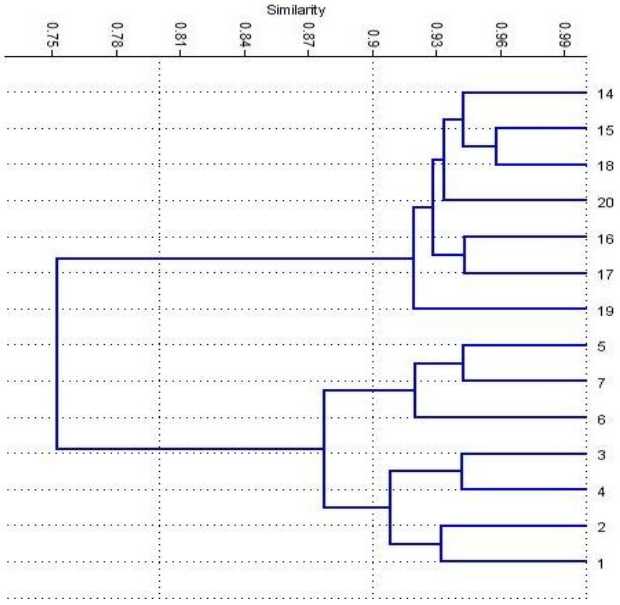

Figure 11. Dendrogram generated from SCoT analysis using UPGMA and similarity matrix estimated according to Dice coefficient over the fourteen samples tasted

Tabel 23. Total number of amplified fragments and polymorphic bands

Primer code	Total amplified fragments	Monomorphic bands	Polymorphic bands			Polymorphism %	Primer efficiency	
			Non-unique	Unique	Total		Non-unique	Unique
SCoT-1	4	2	1	1	2	50.00	0.25	0.25
SCoT-2	20	3	12	5	17	85.00	0.60	0.25
SCoT-3	16	5	10	1	11	68.75	0.63	0.06
SCoT-4	14	9	4	1	5	35.71	0.29	0.07
SCoT-6	14	2	12		12	85.71	0.86	0.00
SCoT-10	15	9	3	3	6	40.00	0.20	0.20
SCoT-11	14	5	9	0.0	9	64.28	0.64	0.00
SCoT-12	15	8	7	0.0	7	46.67	0.47	0.00
SCoT-13	15	9	6	0.0	6	40.00	0.40	0.00
SCoT-14	16	11	4	1	5	31.25	0.25	0.06

Therefore, the accumulation of heavy metals uptake in each group of two plants was related to genetic variations. Boonmee *et al*. (2015) reported that high heavy metals concentrations in plant tissues increased genetic variations or decreased genetic similarity. Thus, Neeratanaphan *et al*. (2016) stated that similarity index within species should be around 0.85 - 1.00. Parkpoom *et al*. (2023) found that similarity index in *O. alismoides* and *I. aquatic* achieved high genetic differentiation. Additionally, the difference in similarity index between the reference area and electronic waste dumpsite was influenced by heavy metals uptake from the wastewater. Moreover, heavy metals concentrations in aquatic plants from the electronic waste dumpsite exceeded those of the reference area. Heavy metals accumulated in plant tissues can induce genotoxicity via different mechanisms of action (Parkpoom *et al*. 2023). Meanwhile, Wahyudi *et al*. (2020) found that heavy metals induced DNA damage in *Phaseolus vulgaris* as appeared by RAPD profiles through the absence or presence of new bands in the developed profiles. In addition, Olafisoye *et al*. (2013) investigated that heavy metals absorbed from the electronic waste by aquatic plants exceed those of the reference area, which is the main factor associated with genetic diversity induced.

The data clearly show greater genetic variability in irradiated *C. demersum* L. grownin wastewater. The results demonstrated that genetic variability of *C. demersum* L. grown in wastewater was higher than that grown in Nile water. By comparing the samples, the plants grown in wastewater showed the highest variability due to the genotoxicity of wastewater. Although, SCoTprimers have become the most widely used markers for genotyping plants, the benefits of their use were demonstrated even in population genetic studies of *C. demersum* L. Based on the polymorphic bands, this study revealed differences in SCoT polymorphismof *C. demersum*L. population related to induced mutations by UV-B and wastewater. The weight of phytoremediation samples obtained from the population of *C. demersum* L. was exactly the same 20 g. Therefore, no differences is material size used for phytoremediation. The number of polymorphic bands make it possible to compare the genetic variability in the population of *C. demersum*L. with different SCoT primers sets. Calculated variability parameters showed greater genetic diversity in wastewater treatment than in Nile water. The observed genotypes were genetically closer to each other in the same kind of water treatments. The results obtained herein agreed with Eckert *et al*. (2016), who reported that genetic variation is highly dependent on the

ratio between sexual and asexual reproduction, in addition to the short and long distance dispersal of sexual and vegetative propagules. The low sexual reproduction in *C. demersum*L. and the predominance of clonal growth suggested that the revealed genetic diversity is the result of differences in vegetative propagation (Capers 2003). Furthermore, the clear relationship observed between the genetic diversity in each irradiated population treated with the same kind of water indicates the mode of action of each water treatment as Nile water or wastewater. Where,continuously following Nile water or wastewater provides a constant longitudinal connection with distance habitats.The irradiated population of *C. demersum*L. stands in Nile water showed the lowest genetic diversity than that grown in wastewater. These results reflected the genotoxicity of wastewater on aquatic plants. This are in line with the findings of Kong *et al*. (2019), who established that topography and geographic distance affected on the genetic structure of plant populations, especially in aquatic plants.The spread of aquatic plants between water bodies providing their populations high genetic diversity.Though, genetic diversity detected at marker loci in clonally *C. demersum* L. reproduction. maybe attributed to somatic mutations induced by UV-B irradiation, as well as,wastewater if irradiated plants were grown in wastewater (Kong *et al*. 2019).

So, there are no reports on somatic mutations related to *Ceratophyllum demersum* L. The harmful effects of wastewater on *C. demersum* L. may appear as a result of genotoxicity, which further increase the genetic diversity in the population.These altering reproductive system via reducing flowers and thus less sexual reproduction (Li *et al*. 2017).The results obtained in this study are in harmony with Engloner *et al*. (2023), who found that more than 80% of the loci in *C. demersum* L. were found to be polymorphic using 10 developed primer pairs. In addition, Khaleel *et al*. (2022) found genotoxicity of Cd,Ni and Pb in *C. demersum* L. as the descending order of Cd>Pb≥ Ni. The same authors observed three out of five primers of the RAPD markers revealed replication with the polymorphism percentage of 46.36%. Furthermore, Parkpoom *et al*. (2023) found that heavy metals from electronic waste accumulated intwo edible aquatic plants might be a factor in genetic differentiation. Genetic differentiation achieved that the plant can endure different environments may be including heavy metal contamination. In this respect,Boonmee *et al*. (2015) found that high heavy metal concentrations leading to increased genetic diversity as a consequence decreased genetic similarity.The contamination of heavy metals in aquatic plants leading to induce genotoxicity via several mechanisms of action as binding of sulfhydryl groups within proteins and enzymes. This agrees with Dutta *et al*. (2018), who found that heavy metals stimulate DNA repair inhibition leading to generate DNA damage and oxidative stress-related reactive oxygen species (ROS) that damaged DNA.

Furthermore, Moore *etal*.(2013) revealed that oxidative DNA damage induced slowly changes in DNA methylation schemes, DNA repair inhibition, gene amplification, as well as, affected on chromatin formation.Wahyudi *et al*. (2020) stated that heavy metals (Pb, Cu, Cd and Mn) induced damage in DNA of *Phaseolus vulgaris* that were observed in different RAPD profiles via the absence or presence of new bands in the detected profiles are due to genotoxicity.The wastewater under investigation containing values of heavy metals that exceed those of the reference limets which is the main factor associated with genetic variations obtained in this study (Olafisoye*et al*. 2013). Similarly, Correia *et al*. (2014) found that DNA polymorphisms checked by ISSR molecular markers are more unstable in *Plantago almogravensis* than in *Plantago lagopus*. So, *P. lagopus* were exposed longer to Althan *Plantago almogravensis.* This indicated that the DNA damage in *P. lagopus* maybe repaired by the mechanisms itself. The high level of genomic stability in *P. lagopus* reflected that DNA replication was not completely inhibited. Therefore, the communities near the chemical fertilizer industry dumpsite should increase awareness toward improvements the system of wastewater disposal.Thus, aquatic plants consumed polluted water should be avoided.

Concluding remarks: This study was performed to evaluate genotoxicity induced in irradiated *Ceratophyllum demersum* L. by wastewater, in addition the suitability and efficacy of this aquatic plant in treating wastewater.The results of testing water quality revealed that *C. demersum* L. was quite effective in treating wastewater.So, it is advisable to use in treating wastewater as a good candidate for heavy metal bioremediation or phytoremediation.*C. demersum* L. can also increased oxygen dissolved in water and decreased CO_2.This aquatic plant can play a major role in refining wastewater through harboring phytochelatin synthase (PCS) gene a potential accumulator of heavy metals. The study represents molecular investigation of *C. demersum* L.affected by UV-B irradiation and wastewater.The results indicated that chlorophyll content is sensitive to UV-B irradiation through increased or decreased in relation to the control.The reduction of chlorophyll and carotenoids in plants irradiated with 50 minutes UV-irradiation resulting in photosynthesis breakdown and growth changes. Irradiated *C. demersum* L. consumed lower quantities of Nile water than non-irradiated plants.Irradiated plants at the same dose of UV-B consumed higher values of wastewater than that consumed from Nile water.

That greatest consumption of wastewater was achieved by 40 minutes UV-irradiated plants. The plants irradiated with 50 and 60 minutes of UV-irradiation revealed the greatest value of Nile water consumed.The plants irradiated with the same dose were better grown in Nile water than in wastewater. This is a good indicator on water quality.Therefore,*C. demersum* L. was beneficial as bioremediation agent of wastewater before using for irrigation of crops or gardening purposes. This study leads to test some other aquatic plants for bioremediation treatment of industrial effluents and municipal wastewater for removing pollutants and improving the characteristics of wastewater. The harmful effects of climate change as enhanced by temperature changes on *C. demersum* L. is the further reduce of genetic diversity.The effect of the latter is altering reproductive systemsvia inducing fewer flowers leading to reduce sexual reproduction.That is imparing the adaptability of aquatic plants and endangering their ecosystem functions.The greatest genetic polymorphism more than 80% was detected by SCoT-2 and SCoT-6 primer sets.Meanwhile, the high number of rare bands was achieved by SCoT-2 followed by SCoT-10 primer sets.The decreased genetic polymorphism reached to 31.25% was detected by SCoT-14primer followed by SCoT-4 which achieved polymorphism percentage reached to 35.71%.The greatest removal of mercury, titanium and manganese from wastewater was obtained by the plants irradiated with 60 minutes. Meanwhile, the highest removal of strontium, barium, iron and cobalt was achievedby the plants irradiated with 10 minutes.In addition, the greatest removal of nickel was obtained by the plants irradiated with 30 minutes.Further studies in this direction will be needed to reveal other molecular components affected by wastewater involved in metal detoxification mechanism of *Ceratophyllum demersum* L. This is order to provide molecular understanding about phytoremediation potential of *C. demersum* L.

Acknowledgments

The authors express in their sincere gratitude to Prof. Dr. Mohammed A. El-Wakil, Professor of Plant Pathology, Faculty of Agriculture, Mansoura University, Egypt, for providing financial support in the Experimental Farm of Plant Pathology Department to carry out phytoremediation studies.

Conflict of interest: The authors declare that this study was conducted in the absence of any commercial or financial relationships that could be construed as a potential with no conflict of interest.

Authors contributions

MIK: Conceptualization, formal analysis, investigation, data availability, data curation, methodology, visualization, collect the aquatic plant, planned the experiments, writing original drats. KAZ:

revised the manuscript, technical assistance. ASA: methodology, data collection. AHA: technical assistance, revised the manuscript.

Funding statement: This work was not supported by an internal or external grant or other external funding sources.

Ethical approval: This study does not involve any human or animal testing or feeding.

REFERENCES

Abbasi, S. and Afsharzadeh, S. 2016. An efficient and simple CTAB based method for total genomic DNA isolation from low amounts of aquatic plants leaves with a high level of secondary metabolites. *Progress in Biological Science*, 95-106.

Abdulwahida, K.D. 2023. Phytoremediation of cadmium pollutants in wastewater by using *Ceratophyllumdemersum* l. as an aquatic macrophytes. *Water Conservation & Management* (WCM), 7 (2): 83-88.

Abu Almaaty, A.H., Abd-Alaty, H.E., Abbas, O.A. and Hassan, M.K. 2020. Genetic relationship between two species of genus *Dicentrarchus* based on SCoT markers and SDS-PAGE. Egyptian *Journal of Aquatic Biology & Fisheries*., 24 (5): 393-402.

Afaj, A.H., Jassim, A.J., Noori, M.M. and Schüth, C. 2016. Effects of lead toxicity on the total chlorophyll content and growth changes of the aquatic plant *Ceratophyllumdemersum* L. *International Journal of Environmental Studies,* 6 (33): 1-11.

Allendorf, F.W. and Luikart, G. 2007. Conservation and the genetics of populations 2nd ed. Blackwell publishing. Oxford U. K. 642pp.

Al-Nabhan, E.A.M. and Al-Abbawy, D.A.H. 2021. Improving wastewater quality by using *Ceratophyllumdemersum* L. IOP Conf. Series: Earth and Environmental Science, 910: 1-9.

Alotaibi, M.O. and Abd-Elgawad, M.E. 2022. ISSR and SCoT for evaluation of hereditary differences of 29 wild plants in Al Jubail Saudi Arabian. *Saudi J. Biol. Sci.,* 29: 3223-3231.

Al-Yasi, H.M. and Al-Qthanin, R. 2024. Comparing genetic differentiation and variation using ISSR and SCoT among Juniper plant markers in Saudi Arabia. *Frontiers in Plant Science.,* 15: 1-9.

Amirmoradi, B., Talebi, R. and Karami, E. 2012. Comparison of genetic variation and differentiation among annual *Cicer* species using start codon targeted (SCoT) polymorphism, DAMD-PCR, and ISSR markers. *Plant Systematics and Evolution,* 298 (9): 1679-1689.

APHA. 1995. Standard methods for the examination of water and wastewater. 19th Edition, *American Public Health Association* Inc., New York.

Atienzar, F.A., Conradi, M., Evenden, A.J., Jha, A.N. and Depledge, M.H. 1999. Qualitative assessment of genotoxicity using random amplified polymorphic DNA: comparison of genomic template stability with key fitness parameters in Daphnia magna exposed to benzo [a] pyrene. *Environmental Toxicology and Chemistry,* 18 (10): 2275-2282.

Bae, W., Mehra, R.K., Mulchandani, A. and Chen, W. 2001. Genetic engineering of *Escherichia coli* for enhanced uptake and bioaccumulation of mercury. *Appl. Environ. Microbiol.,* 67: 5335-5338.

Baraki, S.G. and Siahkolaee, S.N. 2018. Assessment of SCoT and DAMD molecular markers in genetic diversity and species delimitation of three moss species grown in Iran. *Iranian Journal of Genetics and Plant Breeding,* 7 (2): 33-41.

Barceló, D., Martí, G.A., Acuña, V., Batalla, R.J., Elosegi, A., Guasch, H. and Sabater, S. 2011. Combined scenarios of chemical and ecological quality under water scarcity in Mediterranean rivers. *TrAC Trends in Analytical Chemistry,* 30 (8): 1269-1278.

Barrat-Segretain, M.H. 1996. Strategies of reproduction, dispersion, and competition in river plants: a review. Vegetatio, 123: 13–37.

Barta, C., Kalai, T., Hideg, K., Vass, I. and Hideg, E. 2004. Differences in the ROS-generating efficacy of various ultraviolet wavelengths in detached spinach leaves. Functional Plant Biology, 31 (1): 23–28.

Beheary, M., Sheta, B.M., Hussein, M., Nawareg, M., El-Matary, F.A. and Hyder, A. 2019. Environmental remediation of tilapia aquaculture wastewater Using *Ceratophyllumdemersum* and *Lemna minor*. *Egyptian Journal of Aquatic Biology & Fisheries*, 23 (2): 379-396.

Berti, W.R. and Cunningham, S.D. 2000. In phytoremediation of toxic metals. using plants to clean up the environment. (Ed. Raskin, I.). Wiley-Interscience, John Wiley and Sons, Inc. New York, NY, 71-88.

Boonmee, S., Neeratanaphan, L., Tanee, T. and Khamon, P. 2015. The genetic differentiation of *Colocasia esculenta* growing in gold mining areas with arsenic contamination. *Environmental Monitoring and Assessment,* 187 (227): 1-8.

Brunetti, P, Zanella, L., Proia, A., De Paolis, A., Falasca, G., Altamura, M.M., Di Toppi S.L., Costantino, P. and Cardarelli, M. 2011. Cadmium tolerance and phytochelatin content of Arabidopsis seedlings over-expressing the phytochelatin synthase gene AtPCS1. J. Exp. Bot., 62: 5509-5519.

Cambier, S., Gonzalez, P., Durrieu, G. and Bourdineaud, J.P. 2010. Cadmium-induced genotoxicity in zebra fish at environmentally relevant doses. *Ecotoxicology and Environmental Safety*, 73: 312-319.

Cao, Q., Liu, B. and Hu, F. 2021. Effects of hydrological connection and human disturbance on genetic variation of submerged *Vallisneria natans* populations in four lakes in China. *J. Oceanol. Limnol.,* 39: 1403-1416.

Cao, Q.J., Mei, F.F. and Wang, L. 2017. Population genetic structure in six sympatric and widespread aquatic plants inhabiting diverse lake environments in China. *Ecol. Evol.,* 7: 5713-5723.

Capers, R.S. 2003. Macrophyte colonization in a freshwater tidal wetland (Lyme, CT, USA). Aquat. Bot., 77: 325-338.

Collard, B.C.Y. and Mackill, D.J. 2009. Start codon targeted (SCoT) polymorphism: A simple, novel DNA marker technique for generating gene-targeted markers in plants. *Plant Mol. Biol. Rep.,* 27: 86-93.

Correia, S., Matos, M., Ferreira, V., Martins, N., Gonçalves, S., Romano, A. and Pinto-Carnide, O. 2014. Molecular instability induced by aluminum stress in *Plantago* species. *Mutation Research/Genetic Toxicology and Environmental Mutagenesis,* 770: 105-111.

Crespo, P.D., Terracciano, S., Giordano, S. and Spagnuolo, V. 2014. Molecular markers based on PCR methods: A guideline for mosses. Cryptogamie, Bryologie, 35 (3): 229-246.

Davis, C.D., Epps, C.W., Flitcroft, R.L. and Banks, M.A. 2018. Refining and defining riverscape genetics: How rivers influence population genetic structure. *Wiley Interdiscip. Rev. Water,* 5: 1-12.

Devi M.B., Yaikhom, V. and Chingangbam, D.S. 2019. Evaluation of genetic variability in wild population of *Chironomids* of Manipur using SCoT marker. *Journal of Entomology and Zoology Studies,* 7 (1): 1192-1195.

Di Tomaso, J.M., Kyser, G.B., Oneto, S.R., Wilson, R.G., Orloff, S.B., Anderson, L.W. and Mann, J.J. 2013. Weed control in natural areas in the western United States. Weed Research and Information Center, University of California. 544.

Djingova, R. and Kuleff, I. 2000. Instrumental techniques for trace analysis. In: B.H., Markert and K. Friese (Eds.). Trace Elements: Their Distribution and Effects in the Environment (Sofia: Elsevier Science), pp 596.

Duarte, C.M., Planas, D. and Pen˜uelas, J. 1994. Macrophytes, taking control of an ancestral home. In Margalef, R. (ed.), Limnology now: paradigm of planetary problems. *Else vier Science, Amsterdam,* 59-79.

Dutta, S., Mitra, M., Agarwal, P., Mahapatra, K., De, S., Sett, U. and Roy, S. 2018. Oxidative and genotoxic damages in plants in response to heavy metal stress and maintenance of genome stability. *Plant Signaling and Behavior,* 13 (8): 1-17.

Eckert, G.C., Dorken, M.E. and Barrett, S.C.H. 2016. Ecological and evolutionary consequences of sexual and clonal reproduction in aquatic plants. *Aquat. Bot.,* 135: 46-61.

Elshemy, M. 2017. Review of technologies and practices for improving agriculture drainage water quality in Egypt. In

Unconventional water resources and agriculture in Egypt. Springer Cham, 163-188.

Engloner, A.I., Németh, K., Kós, P.B., Meglécz, E. and Bereczki J. 2023. Genetic diversity of the submerged macrophyte *Ceratophyllumdemersum* depends on habitat hydrology and habitat fragmentation. *Front. Plant Sci.,* 14 (9): 1-10.

Fan, Y.X., Rong, D.Q., Chen, X.G. and He, K.Y. 2009. Effects of free ammonia on growth and physiological properties of *Ceratophyllumdemersum*. *Journal of Hebei North University*, (in Chinese). 25: 36-41.

Gaberščik, A., Voncina, M., Trost, T., Germ, M. and Björn, L.O. 2002. Growth and production of buckwheat (*Fagopyrum esculentum*) treated with reduced, ambient and enhanced UV-B radiation. *Journal of Photochemistry and Photobiology B Biology,* 66: 30-36.

Gajera, H.P., Bambharolia, R.P., Domadiya, R.K., Patel, S.V. and Golakiya, B.A. 2014. Molecular characterization and genetic variability studies associated with fruit quality of indigenous mango (*Mangifera indica* L.) cultivars. *Plant Syst. Evol.,* 300: 1011-1020.

Gao, J.Q., Ma, N., Zhou, J., Wang, W.L., Xiong, Z.T., Mba, F.O. and Chen, N. 2012. Peroxidation damage and antioxidative capability of *Ceratophyllumdemersum* under NH4 þ-N stress. *J Freshw. Ecol.,* 27: 539-549.

Gao, Y.H., Zhu, Y.Q., Tong, Z.K., Xu, Z.Y., Jiang, X.F. and Huang, C.H. 2014. Analysis of genetic diversity and relationships among genus *Lycoris* based on start codon targeted (SCoT) marker. *Biochemical Systematics and Ecology*, 57: 221-226.

Gargiulo, G.M., El-Bakkouri, B., Crisafulli, A., Donato, M. and Picone, R. 2022.Polysomaty and chromosome number variation in a population of *Ceratophyllumdemersum* L. from Aquila Lake (Aspromonte Mountains, Calabria, Italy). *Aquat. Bot.,* 180: 103530.

Ge, J., Li, J.J., Zhang, J. and Yang, Z. 2012. Time-dependent oxidative stress responses of submerged macrophyte *Vallisneria natans* seedlings exposed to ammonia in combination with microcystin under laboratory conditions. *Bull Environ Contam. Toxicol.,* 89: 67-72.

Germ, M., Drmaz, D., Sisko, M. and Gaberscik, A. 2002. Effects of UV-B radiation on green alga *Scenedesmus quadricauda*: growth rate, UV-B absorbing compounds and potential respiration in phosporus rich and phosporus poor medium. Phyton (Horn Austria), 42: 25-37.

Ghorbanzadeh, A., Ghasemnezhad, A., Sarmast, M.K. and Ebrahimi, S.N. 2021. An analysis of variations in morphological characteristics, essential oil content, and genetic sequencing among and within major Iranian Juniper (*Juniperus* spp.) populations. Phytochemistry, 186: 1127-1137.

Gill, S.S. and Tuteja, N. 2010. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Plant Physiol. Biochem., 48: 909-930.

Gondek, M., Weindorf, D.C., Thiel, C. and Kleinheinz, G. 2020. Soluble salts in compost and their effects on soil and plants. A review Compost Science & Utilization, 28 (2): 59-75.

Gorji, A.M., Poczai, P., Polgar, Z. and Taller, J. 2011. Efficiency of arbitrarily amplified dominant markers (SCoT, ISSR and RAPD) for diagnostic fingerprinting in tetraploid potato. Am. J. Potato Res., 88: 226-237.

Green, A.J., Figuerola, J. and Sa´nchez, M.L. 2002. Implications of waterbird ecology for the dispersal of aquatic organ isms. Acta Oecologica, 23: 177-189.

Gupta, P. and Chandra, P. 1996. Response of cadmium to *Ceratophyllumdemersum* L., a rootless submerged plant. Waste Management, 16: 335-337.

Häder, D.P., Kumar, H.D., Smith, R.C. and Worrest, R.C. 1998. Effects on aquatic ecosystems. In Environmental Effects of Ozone Depletion. UNEP, Journal of Photochemistry and Photobiology B Biology, 46: 53–68.

Hammer, A.T., David, A.T.H. and Paul, D.R. 2001. PAST:Palaeontological statistics software package for education and data analysis. Palaeontologia Electronica. 4: 9.

Heidari, S.H., Azizinezhad, R. and Haghparast, R. 2017. Investigation of genetic diversity in *Triticum turgidum* L. var. durum using agro-morphological characters and molecular markers. Indian Journal of Genetics, 77 (2): 242-250.

Hu, S., Li, G., Yang, J. and Hou, H. 2017. Aquatic plant genomics: advances, applications and prospects. International Journal of Genomics, 1-9.

Huang, L., Huang, X., Yan, H., Yin, G., Zhang, X., Tian, Y., Zhang, Y., Jiang, X., Yan, Y., Ma, X., Peng, Y., Zhou, J. and Nie, G. 2014. Constructing DNA fingerprinting of *Hemarthria* cultivars using EST-SSR and SCoT markers. Genet Resour. Crop Evol., 61: 1047-1055.

Ibrahim, S.D., Abd El-Hakim, A.F., Ali, H.E. and Abd El-Maksoud, R.M. 2019. Genetic differentiation using ISSR, SCoT and DNA Barcoding for Quinoa genotypes. Arab J. Biotech., 22 (2): 103-118.

Jassim, A.J. 2009. Bioremediation of lead from water bodies using aquatic plant *Ceratophyllumdemersum*L. Msc. Thesis (unpubl.). Al-Mustansiriyah University, Baghdad.

Jawad, M.M., Abed, E.H. and Oudah, H.K. 2018. Using of *Ceratophyllumdemersum* L. for lead and cadmium pollution removal by columns technology. J Bacteriol Mycol Open Access, 6 (1): 19-21.

Jiang, L.F., Qi, X., Zhang, X.Q., Huang, L.K., Ma, X. and Xie, W.G. 2014. Analysis of diversity and relationships among orchardgrass (*Dactylis glomerata* L.) accessions using start codon-targeted markers. Genet. Mol. Res., 13 (2): 4406-4418.

Kadhim, N.F. and Abdul Kareem, N. 2023. Physiological responses in two aquatic plants (*Ceratophyllumdemersum* and *Lemna minor*) as indicator to phytoremediation of wastewater polluted by some nitrogen compound. Mesopotamia Environmental Journal. 7 (2): 24-35.

Kaur, L., Gadgil, K. and Sharma, S. 2010. Effect of pH and lead concentration on phytoremoval of lead from lead contaminated water by *Lemna minor*. American-Eurasian Journal of Agricultural and Environmental Sciences, 7: 542-550.

Khaleel, H.A., Jawad, H.J. and Alrufaye, Z.T.A. 2022. Genotoxic effect of heavy metals on *Ceratophyllumdemersum* L. using RAPD markers. Iran. J. Ichthyol., 9: 46-53.

Khaleel, H.A., Jawad, H.J. and Alrufaye, Z.T.A. 2022. Genotoxic effect of heavy metals on *Ceratophyllumdemersum* L. using RAPD markers. Iran. J. Ichthyol. 9: 46-53.

Kim, K.S. and Sappington, T.W. 2013. Microsatellite data analysis for population genetics, in Microsatellites: Methods and Protocols. Ed. S. K. Kantartzi (New York: Humana Press, New York), 271-295.

King, R.A., Gornall, R.J., Preston C.D. and Croft, J.M. 2002. Population differentiation of *Potamogetonpectinatus* in the Baltic Sea with reference to waterfowl dispersal. Molecular Ecology, 11: 1947–1956.

Kondrateva, N.P., Kasatkina, N.I., Kuryleva, A.G., Baturina, K.A., Ilyasov, I. R. and Korepanov. R.I. 2021. Effect of treatment of seeds of grain crops by ultraviolet radiation before sowing. IOP Conference Series: Earth and Environmental Science, 433 (1):1-8.

Kong, F., Wu, Z., Wang, H., Chen, J. and Xu, X. 2019. Population genetic structure of the whorl-leaf watermilfoil *Myriophyllumverticillatum* shaped by topography and geographic distance. Hydrobiologia, 838: 55-64.

Kong, F., Wu, Z., Wang, H., Chen, J. and Xu, X. 2019. Population genetic structure of the whorl-leaf watermilfoil *Myriophyllumverticillatum* shaped by topography and geographic distance. Hydrobiologia, 838: 55-64.

Kumar, H., Priya, P., Singh, N., Kumar, M., Choudhary, B.K., Kumar, L., Singh, I.S. and Kumar, N. 2016. RAPD and ISSR marker based comparative evaluation of genetic diversity among Indian germplasms of Euryale ferox: an aquatic food plant. Applied Biochemistry and Biotechnology, 180 (7): 1345-1360.

Kumar, S. and Deswal, S. 2020. Phytoremediation capabilities of *Salvinia molesta*, water hyacinth, water lettuce, and duckweed to reduce phosphorus in rice mill wastewater. International journal of phytoremediation, 22 (11): 1097-1109.

Kumari, N., Yadav, M. and Sharma, V. 2018. Differential response of *Brassica juncea* cultivars to Al; consequences for chlorophyll a fluorescence, antioxidants and *psb* A gene. Journal of Plant Interactions., 13 (1): 496-505.

Les, D.H. 1988. Breeding systems, population structure and evolution in hydrophilous angiosperms. Annals of the Missouri Botanical Garden, 75: 819-835.

Li, Y., Xia, M., Zhao, X., Yang, J., Li, G., Sun, Z., Xinzeng, W. and Hou, H. 2022. Temperature is a cryptic factor to shape the geographical pattern of genetic variation in *Ceratophyllumdemersum* across a subtropical freshwater lake. Authorea, 06: 1-12.

Li, Z., He, L., Zhang, H., Urrutia-Cordero, P., Ekvall, M.K., Hollander, J. and Hansson, L.A. 2017. Climate warming and heat waves affect reproductive strategies and interactions between submerged macrophytes. Glob. Change Biol., 23: 108-116.

Lichtenthaler, H.K. and Wellburn, A.R. 1983. Determinations of total carotenoids and chlorophylls a and b of leaf extracts in different solvents. Biochemical Society Transactions, 1: 591-592.

Lu, X., Kruatrachue, M., Pokethitiyook, P. and Homyok, K. 2004. Removal of cadmium and zinc by water hyacinth, *Eichhornia crassipes*. Science Asia, 30 (93): 93-103.

Miretzky, P., Saralegui, A. and Cirelli, A. F. 2006. Simultaneous heavy metal removal mechanism by dead macrophytes. Chemosphere, 62: 247-254.

Mitsch, W.J. and Gosselink, J.E. 2000. Wetlands, 3rd ed. John Wiley & Sons, Inc., New York, NY.

Mjelde, M. and Faafeng, B.A. 1997.*Ceratophyllumdemersum* Hampers phytoplankton development in some small Norwe Gian lakes over awide range of phosphorus concentrations and Geographic allatitude. Fresh Water Biol., 37: 355-365.

Mohan, J., Pandey, A. and Singh, V. 2023. Application of aquatic plant *Ceratophyllumdemersum* in phytoremediation of wastewater. Agricultural Science Digest, 43 (5): 655-660.

Molin, D., Coelho, C. J., Máximo, D.S., Ferreira, F.S., Gardingo, J.R. and Matiello, R.R. 2013. Genetic diversity in the germplasm of tropical maize landraces determined using molecular markers. Genet. Mol. Res., 12 (1): 99-114.

Monselise, B.E. and Kost, D. 1993. Different ammonium uptake, metabolism and detoxification efficiencies in two Lemnaceae. Planta, 189: 167-173.

Moore, L.D., Le, T. and Fan, G. 2013. DNA methylation and its basic function. Neuropsychopharmacology. 38: 23-38.

Mulpuri, S., Muddanuru, T. and Francis, G. 2013. Start codon targeted (SCoT) polymorphism in toxic and nontoxic accessions of *Jatropha curcas* L. and development of a codominant SCAR marker. Plan. Sci., 207: 117-127.

Mustafa, H.M. and Hayder, G. 2021. Recent studies on applications of aquatic weed plants in phytoremediation of wastewater. A review article Ain Shams Engineering Journal, 12 (1): 355-365.

Nagajyoti, P.C., Lee, K.D. and Sreekanth, T.V.M. 2010. Heavy metals, occurrence and toxicity for plants: a review. Environmental chemistry letters, 8 (3): 199-216.

Neeratanaphan, L., Boonmee, S., Srisamoot, N., Tanomtong, A. and Tengjaroenkul, B.2016. Analysis of genetic similarity of *Limnocharis flava* individuals growing around a gold mining area with arsenic contamination. Applied Ecology and Environmental Research, 14: 105-114.

Olafisoye, O.B., Adefioye, T. and Osibote, O.A. 2013. Heavy metals contamination of water, soil and plants around an electronic waste dumpsite. Polish Journal of Environmental Studies, 22: 1431-1439.

Oron, G., De-Vegt, A. and Porath, D. 1988. Nitrogen removal and conversion by duckweed grown on wastewater. Water Research, 22 (2): 179-184.

Osmolovskaya, N. and Kurilenko, V. 2005. Macrophytes in phytoremediation of heavy metal contaminated water and sediments in urban inland ponds. Geophysical Research Abstracts, 7: 105-110.

Pandey, A. and Verma, R.K. 2020. Assessment of the efficiency of *Ceratophyllumdemersum* in waste water treatment. Bulletin of Environment, Pharmacology and Life Sciences, 9 (4): 117-123.

Parkpoom, T., Chowrong, S., Intamat, S., Soulivongsa, L. and Neeratanaphan, L. 2023. Heavy metal contamination and genetic differentiation in two edible aquatic plants near an electronic waste dumpsite. Environment. Asia, 16 (2): 149-161.

Parthiban, S., Govindaraj, P. and Senthilkumar, S. 2018. Comparison of relative efficiency of genomic SSR and EST-SSR markers in estimating genetic diversity in sugarcane. 3 Biotech., 8: 1-12.

Patel, D.K. and Kanungo, V.K. 2010. Ecological efficiency of *Ceratophyllumdermesum*, L. in phytoremediation of nutrients from domestic waste water. The Ecoscan, 4 (4): 257-262.

Patterson, R.A. 2003. Nitrogen in wastewater and its role in constraining on site planning. Future directions for on-site systems: best management practice. proceedings of on-site ’03 conference. 313-320.

Pavlicek, A., Hrda, S. and Flegr, J. 1999. Free-tree–freeware program for construction of phylogenetic trees on the basis of distance data and bootstrap/jackknife analysis of the tree robustness. Application in the RAPD analysis of genus Frenkelia. Folia. Biol. (Krakow), 45 (3): 97-99.

Pollux, B.J.A., Jong, M.D.E., Steegh, A., Verbruggen, E., Van Groenendael J.M. and Ouborg, N.J. 2007. Reproductive strategy, clonal structure and genetic diversity in populations of the aquatic macrophyte *Sparganiumemer*sum in river systems. Molecular Ecology 16: 313-325.

Prasad, D.D.K. and Prasad, A.R.K. 1987. Altered d-aminolevulinic acid metabolism by lead and mercury in germinating seedlings of Bajra (*Pennisetum typhoideum*). J. Plant Physiol., 127: 241-249.

Que, Y., Pan, Y., Lu, Y., Yang, C., Yang, Y., Huang, N. and Xu, L. 2014. Genetic analysis of diversity within a Chinese local sugarcane germplasm based on start codon targeted polymorphism. BioMed Research International. 1-10.

Rai, P.K. 2009. Heavy metal phytoremediation from aquatic ecosystems with special reference to macrophytes. Critical Reviews in Environmental Science and Technology, 39 (9): 697-753.

Rai, U.N., Sinha, S., Tripathi, R.D. and Chandra, P. 1995. Wastewater treat-ability potential of some aquatic macrophytes: removal of heavy metals. Ecological Engineering, 5: 5-12.

Rajesh, M.K., Sabana, A.A., Rachana, K.E., Rahman, S., Jerard, B.A. and Karun, A. 2015. Genetic relationship and diversity among coconut (*Cocos nucifera* L.) accessions revealed through SCoT analysis. Biotech., 5: 999-1006.

Rebechini, H.M. and Hanzely, L. 1974. Lead-induced ultrastructural changes in chloroplasts of the hydrophyte *Ceratophyllumdemersum*. Z. Pflanzenphysiol. 73: 377-386.

Reddy, V.R. and Behera, B. 2006. Impact of water pollution on rural communities: An economic analysis. Ecological Economics, 58 (3): 520-537.

Reitsema, R.E., Wolters, J.W., Preiner, S., Meire, P., Hein, T., De Boeck, G., Blust, R. and Schoelynck, J. 2020. Response of submerged macrophyte growth, morphology, chlorophyll content and nutrient stoichiometry to increased flow velocity and elevated CO2 and dissolved organic carbon concentrations. Front. Environ. Sci., 11 (8): 1-13.

Rosengren, F., Cronberg, N., Reitalu, T. and Prentice, H.C. 2013. Genetic variation in the moss *Homalotheciumlutescens* in relation to habitat age and structure. Botany, 91: 431-441.

Salt, D.E., Smith, R.D. and Raskin, I. 1998. Phytoremediation. Annu. Rev. Plant Physiol. Plant Mol. Biol., 49: 643-668.

Santamaría, L. 2002. Why are most aquatic plants widely distributed? Dispersal, clonal growth and small- scale heterogeneity in a stressful environment. Acta Oecologica, 23: 137-154.

Sarmast, M.K., Mosavizadeh, S.J., and Sharifani, M. 2018. Evaluation of *Junipers* spp. genetic diversity in northern Iran using ISSR markers. Ecol. Iranian For., 6 (11): 14-20.

Sas-Nowosielska, A., Galimska-Stypa, R., Kucharski, R., Zielonka, U., Małkowski, E. and Gray, L. 2008. Remediation aspect of microbial changes of plant rhizosphere in mercury contaminated soil. Environmental monitoring and assessment, 137 (1): 101-109.

Sathapondecha, P., Boonsermsukchareon, L. and Whankeaw, S. 2021. Comparison of multiplex PCR kits for SCoT and SRAP genotyping in plants. 1-6.

Sawant, S.V., Singh, P.K., Gupta, S.K., Madnala, R. and Tuli, R. 1999. Conserved nucleotide sequences in highly expressed genes in plants. J. Genet., 78: 123-131.

Say, R., Yilmaz, N. and Denizli, A. 2003. Biosorption of cadmium, lead, mercury, and arsenic ions by the fungus *Penicillium purpurogenum*. Sep. Sci. Technol., 38: 2039-2053.

Sbordoni, V. 2010. Strength and limitations of DNA barcode under the multidimensional species perspective. Tools for Identifying Biodiversity: Progress and Problems, 275-280.

Shaban, A.S., Arab, S.A., Basuoni, M.M., Abozahra, M.S., Abdelkawy, A.M. and Mohamed, M.M. 2022.SCoT, ISSR, and SDS-PAGE investigation of genetic diversity in several Egyptian wheat genotypes under normal and drought conditions. Int. J. Agron., 1-14.

Sharma, P. and Dubey, R.S. 2005. Lead toxicity in plants. Brazilian journal of plant physiology, 17: 35-52.

Shelef, O.G.A. and Rachmilevitch, S. 2013. Role of plants in a constructed wetland: Current and New perspectives. Water journal, 5: 405-419.

Shukla, D., Kesari, R., Mishra, S., Dwivedi, S., Tripathi, R.D., Nath, P. and Trivedi P.K. 2012. Expression of phytochelatin synthase from aquatic macrophyte *Ceratophyllumdemersum* L. enhances cadmium and arsenic accumulation in tobacco. Plant Cell Rep., 31: 1687-1699.

Shukla, D., Tiwari, M., Tripathi, R.D., Nath, P. and Trivedi, P.K. 2013. Synthetic phytochelatins complement a phytochelatin-deficient Arabidopsis mutant and enhance the accumulation of heavy metal (loid) s. Biochem. Biophys. Res. Commun. 434: 664-669.

Singh, D., Tiwari, A. and Gupta, R. 2012. Phytoremediation of lead from wastewater using aquatic plants. Journal of Agricultural Technology, 8 (1): 1-11.

Singh, S., Kang, S.H., Lee, W., Mulchandani, A. and Chen, W. 2010. Systematic engineering of phytochelatin synthesis and arsenic transport for enhanced arsenic accumulation in E. coli. Biotechnol. Bioeng., 105: 780-785.

Sivaprakash, K.R., Prasanth, S.R., Mohanty, B.P. and Parida, A. 2004. Genetic diversity of black gram landraces as evaluated by AFLP markers. Current Science, 86: 1411-1415.

Sneath, P.H.A. and Sokal, R.R. 1973. Numerical taxonomy; the principles and practice of numerical classification. San Francisco: Freeman Medical Reseach Council Microbial Systematic Unit. Leicester, England and Dept. Ecology and Evolution State Univ. New York, Stony Brook, NY.

Spagnuolo, V., Muscariello, L., Cozzolino, S., Giordano, S., Castaldo. and Cobianchi, R. 2002.Polimorfismo di lunghezza di trnL (cpDNA) nelmuschioPleurochaetesquarrosa (Brid.) Lindb. Proceedings of Annual Congress of Societa` Botanica Italiana, Lecce (Italy), 24-26.

Steel, R.G.D. and Torrie, J.H. 1960. Principles and procedures of statistics. the Biological Sciences. McGraw Hill, New York, 187-287.

Terracciano, S., Giordano, S., Bonini, I., Miserere, L. and Spagnuolo, V. 2012. Genetic variation and structure in endangered populations of *Sphagnum palustre* L. in Italy: a molecular approach to evaluate threats and survival ability. Botany, 90: 966-975.

Vaillant, N., Monnet, F., Hitmi, A., Sallanon, H. and Coudret, A. 2005. Comparative study of responses in four Datura species to a zinc stress. Chemosphere, 59: 1005–1013.

Vieira, M.L.C., Santini, L., Diniz, A.L. and Munhoz, C.D.F. 2016. Microsatellite markers: what they mean and why they are so useful. Genet. Mol. Biol., 39: 312-328.

Vivodík, M., Balážová, Ž., Gálová, Z. and Petrovičová, L. 2017. Genetic diversity analysis of maize (*Zea mays* L.) using SCoT markers. J. Microbiol. Biotech. Food Sci., 6 (5): 1170-1173.

Vivodík, M., Gálová, Z., Balážová, Ž. and Petrovičová, L. 2016. Start codon targeted (SCoT) polymorphism reveals genetic diversity in European old maize (*Zea mays* L.) Genotypes. Potravinarstvo, 10 (1): 563-569.

Wahyudi, D., Hapsari, L. and Sundari, S. 2020. RAPD analysis for genetic variability detection of mutant soybean (*Glycine max* (L.) Merr). Journal of Tropical Biodiversity and Biotechnology, 5(1): 68-77.

Wang, H.Y., Ni, L.Y. and Xie, P. 2013. The mitigating effect of calcification dependent of utilization of inorganic carbon of *Chara vulgaris* Linn on NH4 þ-N toxicity. Chemosphere, 93: 373-379.

Wilkins, D.A. 1978. The measurement of tolerance to edaphic factors by means of root growth. New Phytologist, 80 (3): 623-633.

Xiong, F.Q., Zhong, R.C. and Han, Z.Q. 2011. Start codon targeted polymorphism for evaluation of functional genetic variation and relationships in cultivated peanut (*Arachis hypogaea* L.) genotypes. Mol Biol Rep., 38: 3487-3494.

Yang, Y., Sun, P., LV, L., Wang, D., Ru, D., Li, Y., Ma, T., Zhang, L., Shen, X., Meng, F., Jiao, B., Shan, L., Liu, M., Wang, Q., Qin, Z., Xi,Z., Wang, X., Davis, C.C. and Liu, J. 2020. Prickly waterlily and rigid hornwort genomes shed light on early angiosperm evolution. Nat. Plants, 6: 215-222.

Zhang, J., Xie, W., Wang, Y. and Zhao, X. 2015. Potential of start codon targeted (SCoT) markers to estimate genetic diversity and relationships among Chinese *Elymus sibiricus* accessions. Molecules, 20: 5987-6001.

****