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Research Article

The effects of mercury stress on the photosynthesis and chlorophyll fluorescence of wheat (*Triticum aestivum* L.) genotypes

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Abstract

Mercury is one of the toxic and heavy metals that causes pollution in agricultural fields. Mercury aggregation disturbs cellular behaviors and stops plant growth. In order to examine the effects of mercury on wheat growth indexes, a study with 4 commercial genotypes of Iran, Sirvan, Sivand, Parsi and Sepahan in various levels of this element (0, 30, 70, 100 mg/kg soil) was conducted. This experiment has been conducted in a random factorial design with 3 replications and indices including CO_2 assimilation rate (A), transpiration rate (E), stomatal conductance (g_s), water use efficiency (WUE), PSII photochemical efficiency (FV/FM), photosynthesis quantum yield (Y), electron transfer rate (ETR), intra-leaf CO_2 concentration (Ci), chlorophyll a, chlorophyll b, total chlorophyll, chlorophyll a/b, chlorophyll stability index and carotenoids were measured after 60 days. The result indicated that all measured parameters except carotenoid were decreased as mercury concentration increased. In 100 mg/kg stress level, Sirvan genotype had the highest transpiration rate, chlorophyll a, b, total chlorophyll, carotenoid, PSII efficiency and Ci as compared to other investigated genotypes. Also, Sepahan genotype showed the highest increase in chlorophyll stability index, stomatal conductance, ETR and quantum yield of photosynthesis at a stress level of 100 mg/kg as compared with control plants. Parsi genotype had the lowest transpiration rate, stomatal conductance, PSII efficiency, ETR and Ci at a stress level of 100 mg/kg as compared to other investigated genotypes. Based on these results, Parsi genotypes can be introduced as sensitive genotypes, and Sivand and Sirvan genotypes can be introduced as tolerant genotypes to mercury treatment.

Keyword: Chlorophyll fluorescence, Mercury, Photosynthesis, *Triticum aestivum*

Introduction

Because of its high toxicity and lengthy persistence in the environment, mercury (Hg) is a major source of concerned (Pirrone *et al*., 2010). Hg in the ecosystem can be released by both natural and anthropogenic factors. Volcanic activity, lithosphere weathering, and forest fires are primary producers of Hg are related to natural sources (Fitzgerald *et al*., 1998). Whilst human impacts are coal-fired power stations (Yoshimoto *et al*., 2016), mineral extraction, industrial emissions, medical waste incineration, and municipal waste combustion (Pirrone *et al*., 2010; Sloss, 2016).

It is clear that the chemical properties and movement of mercury in the environment are affected by biological components and their properties. Therefore, special attention should be paid to the source of mercury inlets and the paths it takes in nature, including the intense amount of it by sources such as domestic and hospital sewage (Wang *et al*., 2017). Recently, researchers have shown that agricultural pesticides can be an important factor in the release of mercury into nature (Teimoory, 2019).

Wheat is the world's second most popular crop after rice, covering one-third of the world's protein and caloric needs (Skovmand and Reynolds, 2000). By 2050, the FAO estimates that the world would require approximately 840 million tonnes of wheat. Wheat is expected to expand at a rate of 1.6 percent per year by 2020, owing to rising human consumption demand (Ortiz *et al*., 2008). Only a 2.5 percent increase in world wheat production annually would be enough to meet this goal (Singh *et al*., 2011). Notwithstanding, responses to various abiotic factors, such as drought, salinity, low soil fertility, and heavy metals, are responsible for inappropriate wheat growth and yield (up to 50%) (Khan and Ashraf, 2008, Rahaie *et al*., 2013). It remains vital to monitor heavy metal levels in soils due to increased industrial activity and use of pesticides and fertilizers (Parviz *et al*., 2015).

Mercury predominantly exists in the solid phase, and the ionic form (Hg^{2+}) is the most common form of Hg in soils (Han *et al*., 2006). Considering Hg is widely used

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as disinfectants for seed, fertilizers, and herbicides, understanding how it interacts with plant systems is critical (McLaughlin *et al*., 1996). Due to the interaction between plants and mercury, scientists have found that Hg plays a key role in the production of ROS in plant cells, including H_2O_2, superoxide, and hydroxyl radicals (Israr *et al*., 2006; Patra and Sharma, 2000). In comparison to other heavy metals, Hg seems to have the most significant inhibitory and toxic effects on seed germination, root elongation, hypocotyl, and coleoptile growth in wheat (Munzuroglu and Geckil, 2002). According to the mentioned cases, the researchers analyzed the effects of mercury on the chlorophyll content of winter wheat (var. jinan no. 17). In addition, the Ca level and Hg bioaccumulation in wheat leaves were measured using an inductively coupled plasma sector field mass spectrometer (ICP-SF-MS). The results showed that both minimum and maximum Hg concentrations enhanced chlorophyll production in the beginning phases of wheat growth, whereas they restricted chlorophyll production in the later phases. Also, as proven by ICP-SF-MS, the concentrations of Hg and Ca in leaves and stems increased with regularly rising Hg concentration with enhancing plant age (Liu *et al*., 2010).

Increased chloroplasts in young leaves and chlorophyll content, β-carotene content and Chla/b ratio are also affected by heavy metals ions. Pigment-protein complexes depend on thylakoid chloroplast membranes, which are organized into two large collections: photosystem I and photosystem II. Electron transfer in photosystem II connects to electron transport chain that has driven in photosystem I. The link for electron transfer from water oxidantion in the photosystem Π, ferredoxin and ultimately $NADP^+$ reducing the photosystem I is necessary (Pazireh *et al*., 2022). Much of the fluorescence chlorophyll, which has resulted from the photosystem Π (Krause and Weise, 1991), can be used as a tool to measure the photosynthetic apparatus. According to the photosynthetic efficiency, the reflection of chlorophyll fluorescence occurs (Kromkamp and Forster, 2003). High relative fluorescence means slowing of electron stream from the chlorophyll reaction centers of PS II as an effect of lower activity of PS Π oxygen evolving complex and slower water splitting (Toth *et al*., 2007). Electron transfer rate (ETR) is affected by the quantum efficiency of the photosystem II. These parameters show PS Π /LHC (Baker, 2008; Roseqvist and Kooten, 2003). A significant change of Fv/Fm reflects the poor performance of photosystem II. Patsikka and coworkers (2002) showed that the oxidative damage caused by heavy metals resulted in a significant decrease in the performance of photosystem II (Patsikka *et al*., 2002). Limiting factors of photosynthesis are divided into two kinds of stomatal and non-stomatal factors (Siosemardeh *et al*., 2006). Stomatal factors point to a lack of water and abiotic stress in the plant. Siddique and Coworkers (1999) found mercury stress in wheat plants caused a significant reduction in the rate of photosynthesis (Siddique *et al*., 1999) and stomatal conductance, reducing the development and growth of leaves. This led to the reduction of plant production. Non-stomatal factors limit carbon emissions, using the direct impact of water shortages on the biochemical processes (Siosemardeh *et al*., 2006). Since, the part of the photosynthetic activity during abiotic stress can be attributed to the non-stomatal limitation to metabolic processes (Lawlor and Cornic, 2002).

Heavy metals, causing many problems for the plant that will lead to a change in the cellular level, the size and shape of the chloroplasts, an increase in the size of vacuoles, and lipid per oxidation, are also at the level of physiology; dysfunctional openings led to a decrease in water content in the cells; photosynthesis and respiration were affected (Islam *et al*., 2008). The increase in the stress of heavy metals causes a decrease in the efficiency index of the oxygen-releasing complex Fv/F0 (Ebrahimi Nokandeh *et al*., 2023). Nicolas *et al*. (1985) compared two varieties of wheat under abiotic stress conditions and showed that the rate of photosynthesis in tolerant genotypes was higher about 60% than the sensitive genotypes at the end of the stress period was critical (Nicolas *et al*., 1985; Austin, 1989). Furthermore, closing stomatal reduced the photosynthesis and stomatal conductance (Del Blanco *et al*., 2000; Condon *et al*., 2002; Koc *et al*., 2003). Water use efficiency equals dry matter production per unit of water (Pessarkli, 1999) and it increased with increasing yield or reducing water consumption (Entesari *et al*., 2007). Photosynthetic water use efficiency shows the efficiency as an indicator of the rate of photosynthesis, stomatal conductance per unit, and transpiration (Larcher, 1995).

With the rising concerns over human/ecosystem health and very limited information about heavy metal pollution spatial Hg, the present study examined the cultivars that show acceptable performance and appearance in heavy metal mercury environment with maximum use of environmental facilities. The objectives are (i) to find the effect of Hg on the photosynthesis parameters (ii) to observe Hg uptake at various concentrations in sensitive and resistant wheat genotypes and, (iii) to identify the resistance of four wheat cultivars, including Parsi, Sepahan, Sivand, and Sirvan and activation of similar mechanisms to heavy metal stress of mercury at four levels 0, 30, 70, 100 mg in kg soil.

Materials and methods

Plant material: In order to examine and identify effective indicators in improving tolerance traits to mercury, four genotypes including Sirvan (ws-85-10), Sivand (M-84-18), Parsi (84 M-17) and Sepahan (M-73-18), in four levels of mercury treatments (0, 30, 70, 100 mg per kg of soil) under controlled conditions in a factorial experiment design with three replications were evaluated. Growth chamber temperatures were

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Table 1. Physicochemical characteristics of the soil before starting the experiment

Soil texture	pH	EC ($dS.m^{-1}$)	Nutrients elements (ppm)			
			Hg	P	N	K
Silty- loam	7.75	1.14	3.47	7.1	298	118

maintained at 25°C during the day and 12°C during the night for 60 days, light intensity was 200 µmol m^{-2} s^{-1} similar to normal field situations of wheat growth region. Sirvan, Sivand and Sepahan genotypes are resistant genotype to drought stress and Parsi genotype is sensitive to drought stress (Abdoli *et al.*, 2013; Najafian *et al*., 2012).

In this experiment, 2-liter containers/pots were filled with sand, gravel, and vermiculite campus with equal proportions. Soil moisture was set by measuring the weight and adding water in each pot. A pot of wheat was kept in the greenhouse until the seed filling stage due to seasonal varieties. The mercury polluted soil was prepared by adding different dilutions of $HgCl_2$ in an aqueous solution to reach heavy metal concentrations of 30 mg/kg, 70 mg/kg, and 100 mg/kg in the dry soil (EscobarVargaset *et al*., 2022).

Gas exchange measurement: Photosynthetic gas exchange was measured fromnon-detached young and fully expanded leaves using a portable infrared gas analyzer (IRGA, LCA4, ADC Bio. Scientific Ltd., Herefordshire, UK): leaf surface area 1 cm^2, ambient CO_2 concentration 370 µmol mol^{-1}, and PPFD 200 µmol $m^{-2}s^{-1}$. The leaf internal CO_2 concentration (Ci), CO_2 assimilation rate (A), transpiration rate (E) and stomatal conductance (gs) were recorded between 09.00 and 11.00 a.m. Water use efficiency (WUE) was calculated from the A/E ratio (Piper *et al*., 2007).

Chlorophyll fluorescence: Photosystem II photochemical efficiency (Fv/Fm) was measured using a porTable chlorophyll fluorometer (OS5-FL modulated chlorophyll fluorometer, ADC Bio Scientific Ltd.). Hoddesdon, Hert, EN11 0DB, England). Minimal fluorescence (Fo) was determined by applying weak modulated light (0.4 µmol $m^{-2}s^{-1}$) and maximal fluorescence (Fm) was induced by a short pulse (0.8 s) of saturating light (8000 µmol $m^{-2}s^{-1}$). Measurements were made from the same leaf used for gas exchange determination, after 20 min of dark adaptation (Maxwell and Johnson, 2000). All physiological measurements were obtained by examining plants that were being treated under drought stress and controlled conditions in four replications.

For measurement of photosynthetic quantum yield (Y) and electron transport rate (ETR), the instrument was set on Kinetic Mode and adjusted so that the initial Ft (instantaneous fluorescence signal) value in the control samples was approximately 210. The instrument detector gain was set between 75 and 85. Quantum yield was determined by the following light treatment: each cycle consisted of a 0.8 s pulse of saturating light generated with a laser diode actinic source to saturate PSII, followed by a 4 s far-red light pulse used to reoxidize PSII, and a 10 s delay allowing PSII to regain steady-state conditions. A total of seven cycles were performed for each sample. ETR values were expressed as percents of the ETR average values observed in control treatments (Dayan and Zaccaro, 2012).

Chlorophyll content: Fresh leaves (0.1 g) were extracted with 15 ml 80% acetone and centrifuged at 5000×g for 10 min. The absorbance of the supernatant was read at 663, 647 and 470 nm and calculated for chlorophyll a, chlorophyll b, total chlorophyll and carotenoid content according to Arnon (1949). The chlorophyll stability index (CSI) was determined according to Sairam and Srivastava. (2002) and calculated as follows:

CSI = (total chlorophyll under stress/total chlorophyll under control) × 100

Data measurement and recording data were analyzed using JMP software. Comparison was done by software MSTAT-C and figures were drawn by Microsoft Excel 22 (2019).

Results

CO_2 assimilation rate (A): The increase in mercury concentration has significantly decreased the CO_2 assimilation rate in all examined genotypes ($P<0.01$) (Table 6). In a zero/no-stress condition, the highest and lowest assimilation rates were related to the Sepahan and Sirvan genotypes, respectively ($P<0.05$) (Table 6). No significant difference was shown in the treatment level of 30 mg/kg among Sirvan, Sivand, and Parsi genotypes, but Sepahan showed the highest CO_2 assimilation rate significantly ($P<0.05$) (Table 6). In stress levels of 70 and 100, no significant difference has been observed among all examined genotypes (Table 6). The interaction effect of genotype/mercury in all studied genotypes was statistically significant ($P<0.01$) (Table 2).

Stomatal conductance (g_s): The effect of mercury treatment on the stomatal conductance was significant ($P<0.01$) (Table 2). In the control condition, Sivand had the highest and Sirvan the lowest stomatal conductance ($P<0.05$) (Table 6). In stress levels 30 70 and 100, Sepahan genotype had the highest stomatal conductance; also, this difference was not significant (Table 6). The interaction effect of genotype/mercury was not significant (Table 2). The stress level of 100 stomatal conductance in Sivand, Parsi, Sepahan and Sirvan genotypes, decreased by 90.99%, 90.18%, 85.8% and 82.66%, compared with the control, respectively ($P<0.05$) (Table 6).

Transpiration rate (E): The transpiration rate was decreased significantly in all examined genotypes as a result of an increase in mercury concentration ($P<0.01$) (Table 2). In the control condition, the rate of transpiration in Sepahan and Sirvan genotypes was

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Table 2. Analysis of variance CO_2 assimilation rate (A), transpiration rate (E), stomata conductance (gs), water use efficiency (WUE), internal CO_2 concentration (C_i), quantum yield of photosynthesis (Y), electron transfer rate (ETR) and PSll photochemical efficiency (Fv/Fm) in different levels of Hg in wheat genotypes.

Source	DF	Mean square							
		A ($\mu mol\ m^{-2}s^{-1}$)	E ($mmol\ m^{-2}s^{-1}$)	gs ($m^{-2}s^{-1}$)	WUE	Ci (Vpm)	Y	ETR ($\mu mol\ m^{-2}s^{-1}$)	PSII Fv/Fm
Mercury	3	76.73^{**}	1.304^{**}	0.284^{**}	37.810^{ns}	237001.2^{**}	0.000069^{**}	146.99^{**}	0.057^{**}
Genotype	3	6.15^{**}	0.095^{**}	0.038^{ns}	142.007^{*}	192394.9^{**}	0.000025^{**}	95.47^{**}	0.062^{**}
Mercury×Genotype	9	10.66^{**}	0.148^{*}	0.069^{ns}	255.65^{*}	146172.8^{**}	0.253^{ns}	195.64^{**}	0.009^{ns}
cv		13%	26%	31%	27%	3%	0.1%	0.1%	26.3%
Total	15								

***, ** and ns significant at 0.05 and 0.01 levels and non significant, respectively.**

Table 3. Analysis of variance chlorophyll a, chlorophyll b, chlorophyll a/b ratio, total chlorophyll and carotinoids in different levels of Hg in wheat genotypes.

Source	DF	Mean square				
		Chlorophyll a ($mg\ g^{-1}$ FW)	Chlorophyll b ($mg\ g^{-1}$ FW)	Chlorophyll a/b	Total chlorophyll ($mg\ g^{-1}$ FW)	Carotinoids ($mg\ g^{-1}$ FW)
Mercury	3	28.816^{**}	4.8^{**}	12.865^{ns}	47.378^{**}	2.027^{**}
Genotype	3	37.006^{**}	3.459^{**}	14.841^{ns}	54.999^{**}	1.851^{**}
Mercury×Genotype	9	2.603^{ns}	2.354^{ns}	21.57^{ns}	0.951^{ns}	0.463^{ns}
cv		16%	25%	43%	11%	18%
total	15					

***, ** and ns significant at 0.05 and 0.01 levels and nonsignificant, respectively.**

higher than Parsi and Sivand genotypes (Table 6). At the stress level of 30, the highest transpiration rate was for Sepahan and parsi and the lowest for Sivand genotypes ($P<0.05$) (Table 6). There was no significant difference between Parsi and Sepahan genotypes in 30 mg/kg mercury concentration (Table 6). There was no significant difference between all examined genotypes in 70 mg/kg mercury concentration (figure 1). Maximum transpiration rate at the stress level of 100 was for Sirvan genotype by 0.146, and the minimum was for Parsi genotype by 0.056 (Table 6), but the difference was not statistically significant (Table 6). The interaction effect of genotype/mercury was significant ($P<0.05$) (Table 1).

Water use efficiency (WUE): The effect of different levels of mercury on water use efficiency was not significant (Table 2). Mercury treatment decreased water use efficiency in all genotypes, but it was only significant in Sivand genotype ($P<0.05$). Under control and 30 mg/kg mercury concentration, the most water use efficiency was assigned to Sirvand genotype, and the least amount was devoted to Sirvan genotype ($P<0.05$) (Table 4). In stress level 70, the highest water use efficiency was shown in Sepahan genotype, and the lowest was assigned to Sirvan genotype (Table 4). In Sirvan, Sivand, Parsi and Sepahan genotypes, WUS showed a decrease of 45%, 52%, 31% and 35% in 100 treatment levels compared with the controlled condition, respectively (Table 4). The interaction effect of genotype/mercury was statistically significant ($P<0.05$) (Table 2).

PSll photochemical efficiency (Fv/Fm): Theresults of the analysis of variance showed that the mercury and genotype effects on Fv/Fm were significant ($P<0.01$), but the interaction effect of genotype/mercury was not significant (Table 2). In no stress condition, Sirvan and Sepahan genotypes, with a similar rate of 0.97, had more Fv/Fm than Parsi and Sivand genotypes, and there was no significant difference between Sirvan and Sepahan genotypes (Figure 1). At a stress level of 30, the maximum Fv/Fm was for Sirvan genotype, and the minimum was for Parsi and Sivand genotypes ($P<0.05$) (Figure 1). In stress levels 70 and 100 of mercury, Sirvan and Sepahan genotypes had the maximum Fv/Fm and Parsi and Sivand genotypes had the minimum Fv/Fm (Figure 1).

Electron transfer rate (ETR): The results of analyze of variance showed that mercury, genotype effects andthe interaction effect of genotype/mercury on ETR were significant ($P<0.01$) (Table 2). By the increasing of mercury stress ETR decreased in all genotypes ($P<0.01$) (figure 2). In no stress condition, ETR in Sivand genotype was significantly more than other genotypes ($P<0.05$) (figure 2) also no significant difference was seen between other genotypes (figure 2). The highest electron transfer rate belonged to Sivand genotype at the stress level of 30 but this difference was not significant (figure 2). In the stress level of 70, Sivand genotype showed the higher ETR than Sirvan ($P<0.05$), also there was no significant difference between Sepahan and Parsi genotypes (figure 1). At the stress level of 100, Sepahan genotype showed the higher ETR than Sivand, Sirvan and Parsi ($P<0.05$) (figure 2).

Quantum yield of photosynthesis (Y): The results of analyze of variance showed that mercury, genotype effects in all genotypes on the quantum yield of photosynthesis were significant ($P<0.01$) (Table 2) but interaction effect of mercury/genotype was not significant (Table 2). The quantum yield of photosynthesis decreased significantly in Sirvan, Sivand

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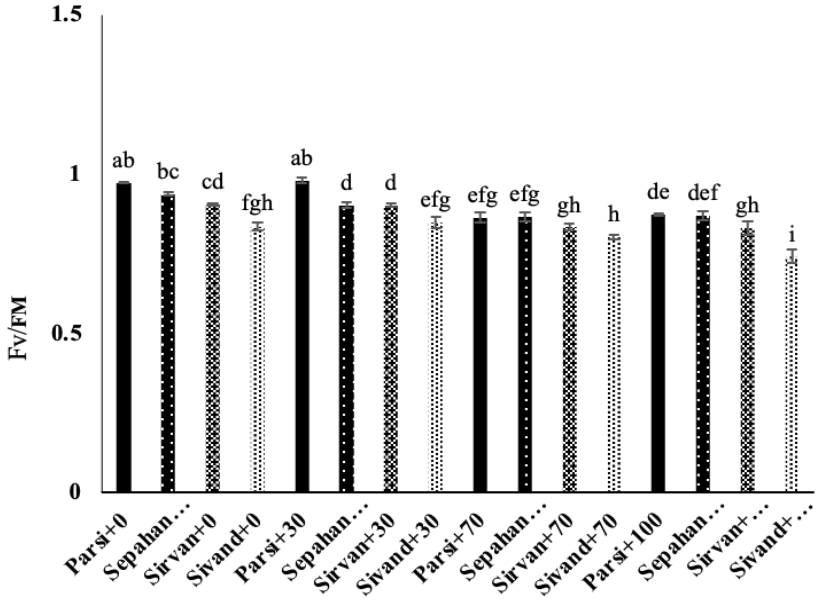


Figure 1. Effects of Mercury stress on PSII photochemical efficiency (Fv/Fm) in different levels of Hg in wheat genotypes

Table 4. Mean of chlorophyll a, chlorophyll b, chlorophyll a/b ratio, total chlorophyll, carotinoids, chlorophyll stability index (CSI), leaf internal CO_2 concentration (Ci) and water use efficiency (WUE) in different levels of Hg in wheat genotypes.

Genotype×Mercury (Mg in soil)	Chl a	Chl b	Chl a/b	Total chl	Car	Chlorophyll stability index	Leaf internal CO_2 concentration (Vpm)	Water use efficiency
	(mg g^{-1} FW)							
Sirvan+0	7.194 ^{a}	1.55 ^{ac}	4.77 ^{ab}	8.733 ^{a}	1.248 $^{d-g}$	100 ^{a}	814.73 ^{a}	8.533 $^{b-d}$
Sirvan+30	7.179 ^{a}	1.436 $^{b-d}$	5.84 ^{ab}	8.6 ^{a}	1.709 $^{a-c}$	98.4 ^{b}	804.33 ^{ab}	7.223 $^{b-d}$
Sirvan+70	6.518^{a-c}	1.3 $^{b-e}$	5.56 ^{ab}	7.81 ^{ab}	1.953 ^{ab}	89.4 ^{f}	796.96 $^{a-c}$	4.753 ^{d}
Sirvan+100	5.411 $^{b-e}$	1.063 $^{c-f}$	5.15 ^{ab}	6.356 $^{b-e}$	1.989 ^{a}	72.7^{i}	764.8 ^{cd}	4.67 ^{d}
Sivand+0	6.873 ^{ab}	2.157 ^{a}	3.4 ^{b}	9.056 ^{a}	1.518 $^{b-f}$	100 ^{a}	832.86 ^{a}	12.44 ^{a}
Sivand+30	6.741 $^{a-c}$	1.834 ^{ab}	3.71 ^{ab}	8.56 ^{a}	1.578 $^{a-d}$	94.5 ^{c}	800.93 $^{a-c}$	9.203 $^{b-d}$
Sivand+70	5.93 $^{a-d}$	1.566 $^{a-c}$	3.78 ^{ab}	7.486 ^{ab}	1.723 $^{a-c}$	82.6 ^{f}	738.46 ^{de}	6.956 $^{b-d}$
Sivand+100	5.214 $^{c-f}$	0.375 ^{g}	6.46 ^{ab}	5.58 ^{de}	1.795 $^{d-g}$	61.6 ^{k}	635.6 ^{f}	5.9 $^{c-d}$
Parsi+0	5.978 $^{a-d}$	1.44 $^{b-d}$	4.18 ^{ab}	7.406 $^{a-c}$	1.089 ^{fg}	100 ^{a}	753.33 ^{de}	10.356^{bc}
Parsi+30	5.271 $^{c-f}$	1.027 $^{c-g}$	5.17 ^{ab}	6.293 $^{b-e}$	1.107 $^{e-g}$	84.9 ^{e}	753 ^{de}	9.13 $^{b-d}$
Parsi+70	3.978 $^{e-g}$	0.837 $^{d-g}$	6.83 ^{a}	4.896 $^{e-f}$	1.55 $^{a-e}$	66.1 ^{h}	635.93 ^{f}	7.126 $^{b-d}$
Parsi+100	3.853 $^{f-g}$	0.656 $^{e-g}$	5.65 ^{ab}	5.696 $^{c-e}$	1.432 $^{c-f}$	76.91 ^{i}	373.06 ^{g}	7.092 $^{b-d}$
Sepahan+0	5.667 $^{a-d}$	1.022 $^{c-g}$	5.61 ^{ab}	6.683 $^{b-d}$	0.879 ^{g}	100 ^{a}	796.4 $^{a-c}$	10.933 ^{ab}
Sepahan+30	4.847 $^{d-f}$	0.897 $^{c-g}$	5.41 ^{ab}	5.73 $^{c-e}$	1.353 $^{c-f}$	85.7 ^{e}	775.76 $^{b-d}$	7.906 $^{b-d}$
Sepahan+70	4.535 $^{d-g}$	0.69 $^{e-g}$	6.74 ^{a}	5.22 $^{d-f}$	1.408 $^{c-f}$	78.1 ^{j}	738.30 ^{de}	7.243 $^{b-d}$
Sepahan+100	3.113 ^{g}	0.624 ^{fg}	5.62 ^{ab}	3.733 ^{f}	1.579 $^{a-d}$	55.8 ^{g}	721.3 ^{e}	7.085 $^{b-d}$
LCD	1.553	0.674	3.301	0.886	0.459	2.03	37.81	3.089

Values with the same letter within column do not differ significantly (P<0.05).

and and Parsi genotypes in stress level 100 as compared to control condition (P<0.05) (figure 3). There was no significant difference in Sepahan genotype at all stress levels as compared to control condition (figure 3). Maximum quantum yield of photosynthesis belonged to Sivand genotype in control condition and 30 and 70 Hg stress levels (figure 3)

Leaf internal CO_2 concentration (Ci): Mercury stress decreased significantly leaf internal CO_2 concentration in all genotypes (P<0.05) (Table 4). Parsi genotype decreased significantly in all stress levels and reached from 753.33 vpm in control condition to 373.06 vpm in100 stress level, which was the highest decline rate among the genotypes (P<0.05) (Table 4). In control condition and all stress levels, the highest Ci belonged to Sivad genotype and the lowest Ci belonged to Parsi genotype (P<0.05) (Table 4). In the stress level of 100, Ci decreased in Parsi, Sivand, Sepahan, and Sirvan genotypes respectively by 50.5, 23.6, 9.41, and 6.1 percent as compared with control condition (P<0.05) (Table 4). The interaction effect of mercury/genotype was significant (P<0.01) (Table 2).

Chlorophyll a: The increase of mercury concentration had significantly decreased the chlorophyll a content in all genotypes (P<0.01) (Table 4). The interaction effect of mercury/genotype was not

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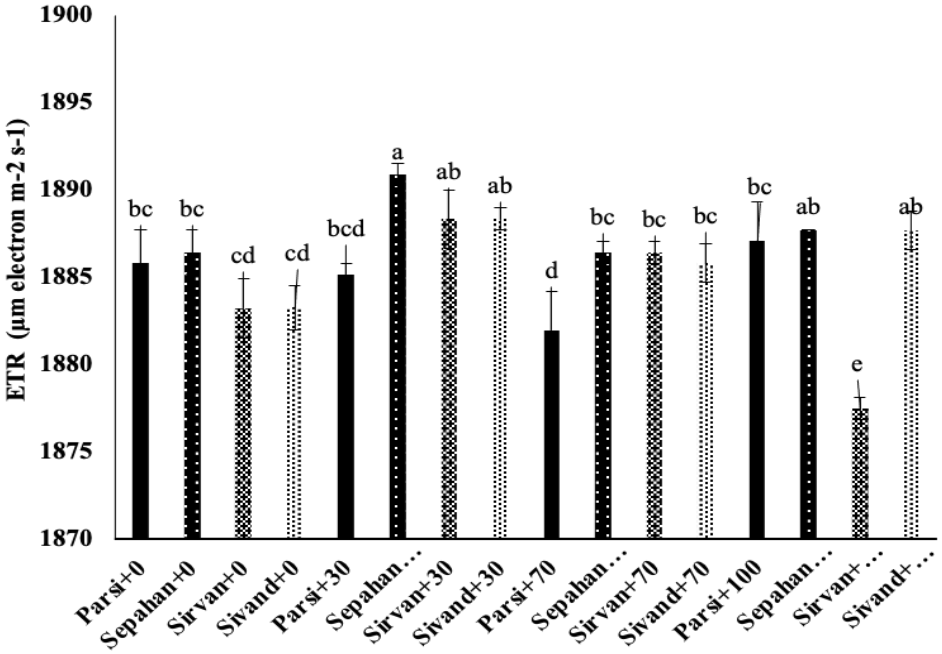


Figure 2. Effects of Mercury stress on electron transfer rate (ETR) in different levels of Hg in wheat genotypes.

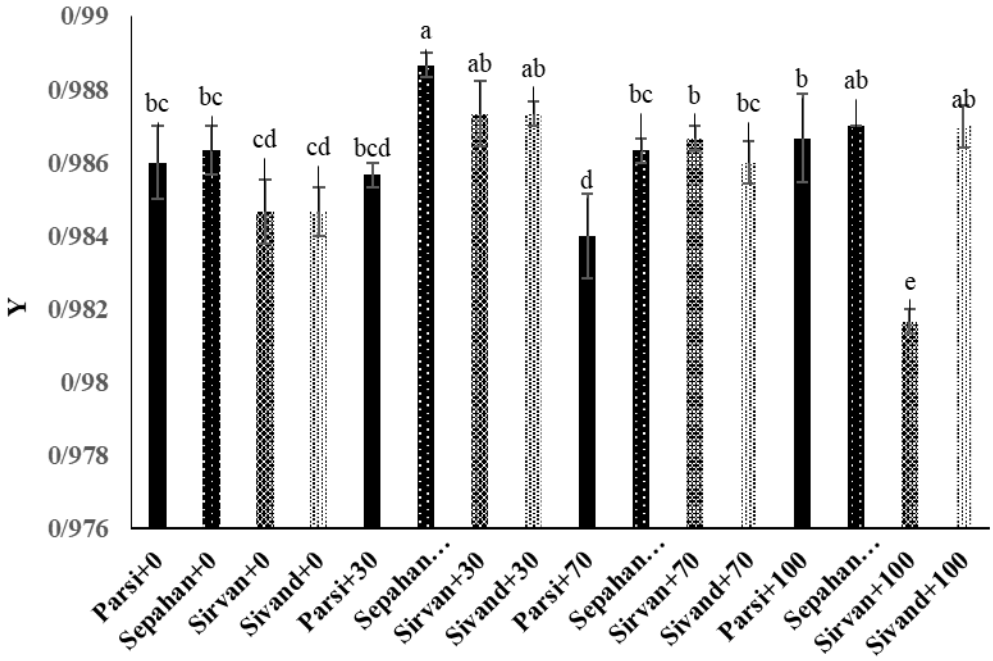


Figure 3. Effects of Mercury stress quantum yield of photosynthesis (Y) in different levels of Hg in wheat genotypes.

significant (Table 2). In the no-stress condition, the highest content of chlorophyll a was shown in Sirvan genotype and the lowest in Sepahan genotype ($P<0.05$) (Table 4). Sirvan genotype at the stress level of 30 and no-stress condition indicated no significant difference (Table 4). In all stress levels, the highest chlorophyll a was shown for Sirvan and Sivand genotypes, respectively (Table 4). The lowest chlorophyll a observed at a stress level of 70 mg per kg of soil was in Parasi genotype, and at the stress level of 100 it was for Sepahan genotype ($P<0.05$) (Table 4).

Chlorophyll b: Chlorophyll b content has dropped significantly in Sivand genotype and reached from 2.157 µg/g in the control treatment to 0.375 µg/gfw at the stress level of 100, which was the highest decline rate in all studied genotypes ($P<0.01$) (Table 4). Mercury stress in other genotypes caused a decrease in terms of chlorophyll b content, as well (Table 4). The interaction effect of mercury/genotype was not significant (Table 3). In the no-stress condition and in mercury stress at the levels of 30 and 70, the highest chlorophyll b content was for Sivand genotype, while Sirvan genotype with 1.063 µg/gfw had the highest chlorophyll b content at the stress level of 100 ($P<0.05$)

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(Table 4). The lowest chlorophyll b content in the stress condition at levels 30 and 70 and in the no-stress condition was for Sepahan genotype (P<0.05) (Table 4).

Chlorophyll a/b ratio: In the no-stress condition, the highest and lowest chlorophyll a/b ratios were for Sepahan and Sivand genotypes, respectively (Table 4). In Sirvan, Sivand, and Parsi genotypes, by increasing the stress of mercury up to 70, the chlorophyll a/b ratio has increased initially, but it declined at the stress level of 100 (Table 4). At the stress level of 30, the highest chlorophyll a/b ratio was for Sirvan genotype, and the lowest rate was for Sivand genotype (P<0.05) (Table 4). No significant difference was noted in all investigated genotypes at all stress levels as compared to the control condition (Table 4). The highest ratio of chlorophyll a/b ratio at the stress level of 70 was for Parsi genotype, and the lowest rate was for Sivand genotype (Table 4). The effect of mercury, genotype, and the interaction effect of mercury/genotype on the chlorophyll a/b ratio was not significant (P<0.01) (Table 3).

Carotenoid: In contrast to chlorophyll a and b, the content of carotenoids increased significantly in all genotypes by the increase of the mercury stress (P<0.05) (Table 4). The interaction effect of mercury/genotype was not significant (Table 3). The highest content of carotenoids in the no-stress condition was for Sivand and Sirvan genotypes (P<0.05) (Table 4). Sirvan genotype had the highest carotenoids in all stress conditions (Table 4). At the stress level of 100, the carotenoids content, in Sepahan, Sirvan, Parsi, and Sivand genotypes has been increased by 79, 59, 31, and 18 percent compared with control treatment, respectively (Table 4). The increase of carotenoid content in Sepahan and Sirvan genotypes was more than Parsi and Sivand genotypes (P<0.05) (Table 4). Sepahan genotype at the stress levels of 30 and 70 showed no significant difference (Table 4).

Total chlorophyll: In the no-stress condition, Sivand and Sirvan genotypes with 9.056 and 8.733 µg/gfw higher chlorophyll content had higher chlorophyll content than Parsi and Sepahan genotypes with 7.406 and 6.683 µgr/gr content (Table 4). No significant difference has been noted in Sirvan and Sivand genotypes in the no-stress condition and in all stress levels (Table 4). At the stress level of 70, the highest chlorophyll content was for Sirvan genotype, and the lowest amount was for Parsi genotype (P<0.05) (Table 3). At the stress level of 100, Sivand and Sepahan genotypes had the highest and lowest, respectively, total chlorophyll content (P<0.05) (Table 4). The interaction effect of mercury/genotype was not significant (Table 3).

Chlorophyll stability index: The increase in mercury concentration significantly decreased the chlorophyll stability index in all genotypes (P<0.05) (Table 4). In stress conditions of 30 and 70, the highest chlorophyll stability index was for Sirvan genotype, and the lowest rate in concentration of 30 was for Parsi and Sepahan genotypes by 78 percent and in concentration of 70 for Parsi genotype by 66 percent (P<0.05) (Table 4). In stress conditions at all levels, the highest chlorophyll stability index was for Sirvan genotype (P<0.05) (Table 4). The stress level of the 100 chlorophyll stability index in Sepahan, Sivand, Sirvan and Parsi genotypes, decreased by 44.2, 38.4, 27.3 and 23.1 percent, compared with the control treatment, respectively (P<0.05) (Table 4).

Hg concentration in soil: Hg concentration in soil has picked up significantly in Sepahan genotype and reached from 0.29 ppm in control treatment to 101.6 ppm at the stress level of 100, which was the highest increase rate in all studied genotypes (P<0.05) (Table 6). Mercury stress in other genotypes made an increase in terms of Hg concentration in soil, as well (P<0.01) (Table 6). The interaction effect of mercury/genotype was significant (P<0.01) (Table 5). No significant difference was noted in all investigated genotypes in the no-stress condition (Table 5). At the stress level of 30, the highest Hg concentration in soil was for Parsi genotype with a rate of 41.7 ppm, which is much more than other genotypes, and a significant difference has been noted in other genotypes (Table 6). Also, at the stress levels of 70 and 100, the highest Hg concentration in soil was for Parsi genotype (P<0.05) (Table 6).

Hg concentration in root: The increase in mercury concentration significantly climbed up the Hg concentration in root in all genotypes (P<0.01) (Table 6). In the no-stress condition and at all stress levels, the lowest Hg concentration in the root belonged to Sirvan genotype (P<0.05) (Table 6). In the no-stress condition and stress levels of 30 and 70, no significant difference has been observed in Sirvan, Parsi and Sepahan genotypes (Table 6). The highest rate in concentrations of 30, 70 and 100 was for Sivand genotype (P<0.05) (Table 6). The interaction effect of genotype/mercury was significant (P<0.01) (Table 5).

Hg concentration in leaf: The increase in mercury concentration has significantly increased the Hg concentration in leaf in all genotypes (P<0.01) (Table 6). In no stress condition, the highest and the lowest Hg concentration in leaf were for Sepahan and Sivand genotypes, respectively (P<0.05) (Table 6). In stress levels of 70 and 100, no significant difference has been observed between Sirvan, Sirvand and Sepahan genotypes (Table 6). Maximum and minimum Hg concentration in leaf at all stress levels was devoted to Sivand genotype (P<0.05) (Table 6). In no-stress and stress levels of 30, 70 and 100, no significant difference has been observed in Sirvan genotype (Table 6). The interaction effect of genotype/mercury was significant (P<0.05) (Table 4).

Hg concentration in seed: Mercury stress increases Hg concentration in seed in all genotypes (Table 6). Under control and stress condition in all stress levels and the least amount of Hg concentration in seed assigned to Sivand genotype (P<0.05) (Table 6). In the no-stress condition, the highest amount of Hg concentration in the seed was assigned to Sepahan

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Table 5. Analysis of variance Hg concentration in soil, root, leaf and seed in different levels of Hg in wheat genotypes

Source	DF	Mean square			
		Hg concentration in Soil (ppm)	Hg concentration in Root (ppm)	Hg concentration in leaf (ppm)	Hg concentration in seed (ppm)
Mercury	3	2407.3**	41210.2**	0.269**	0.0306**
Genotype	3	84.84^{ns}	13962.2**	0.095**	0.0147**
Mercury×Genotype	9	450.78**	7209.77**	0.11*	0.0202**
cv		27%	17%	26%	28%
total	15				

***, ** and ns significant at 0.05 and 0.01 levels and nonsignificant, respectively.**

Table 6. Mean concentration of Hg in soil, root, leaf and seed and CO_2 assimilation rate (A), stomatal conductance (gs) and transpiration rate (E) in different levels of Hg stress in wheat genotypes.

enotype	Hg (mg in soil)	Hg in soil (ppm)	Hg in root (ppm)	Hg in leaf (ppm)	Hg in seed (ppm)	Stomatal conductance (gs)	assimilation rate (A)	Transpiration rate (E)
Sirvan	0	0.14^{i}	0.07^{ef}	0.081 $^{c-e}$	0.081 $^{c-e}$	0.150^{bc}	2.596^{d}	0.573^{b}
	30	0.51^{i}	0.13^{ef}	0.094 $^{b-e}$	0.094 $^{b-e}$	0.093^{c}	2.393^{d}	0.3.6^{de}
	70	19.5^{i}	0.13^{ef}	0.12^{bc}	0.12^{bc}	0.073^{c}	1.643^{e}	0.230^{ef}
	100	24.6 ^{h}	0.16 ^{de}	0.137 ^{b}	0.137 ^{b}	0.026^{c}	0.673^{g}	0.146^{f-h}
Sivand	0	0.32^{i}	0.04 ^{f}	0.058 ^{e}	0.058 ^{e}	0.333^{a}	3.636^{g}	0.413^{cd}
	30	37.3^{fg}	0.25 $^{b-d}$	0.075 $^{c-e}$	0.075 $^{c-e}$	0.056^{c}	2.656^{d}	0.213^{ef}
	70	76.6 ^{d}	0.33 ^{ab}	0.088 $^{c-e}$	0.088 $^{c-e}$	0.060^{c}	1.313^{ef}	0.193^{e-g}
	100	88.3^{bc}	0.4 ^{a}	0.95 $^{b-e}$	0.95 $^{b-e}$	0.030^{c}	0.610^{g}	0.103^{f-h}
Parsi	0	028 ^{i}	0.08^{ef}	0.072 ^{de}	0.072 ^{de}	0.163^{a-c}	4.496^{b}	0.453^{bc}
	30	41.7 ^{f}	0.12^{ef}	0.08 $^{c-e}$	0.08 $^{c-e}$	0.120^{c}	2.576^{d}	0.366^{cd}
	70	79.9 ^{cd}	0.17 ^{de}	0.094 $^{b-e}$	0.094 $^{b-e}$	0.066^{c}	1.520^{e}	0.186^{e-h}
	100	92.5 ^{ab}	0.28 ^{ab}	0.106 $^{b-d}$	0.106 $^{b-d}$	0.016^{c}	0.836^{fg}	0.056^{h}
Sepahan	0	0.29^{i}	0.1^{ef}	0.088 $^{c-e}$	0.088 $^{c-e}$	0.303^{ab}	5.136^{a}	0.723^{a}
	30	26.6^{gh}	0.17 ^{de}	0.086 $^{c-e}$	0.086 $^{c-e}$	0.176^{a-c}	4.096^{bc}	0.376^{cd}
	70	55.4 ^{e}	0.18 $^{c-e}$	0.098 $^{b-e}$	0.098 $^{b-e}$	0.106^{c}	1.386^{e}	0.220^{ef}
	100	101.6 ^{a}	0.34 $^{a-c}$	0.225 ^{a}	0.225 ^{a}	0.043^{c}	0.510^{g}	0.070^{gh}

Values with the same letter within column do not differ significantly (P<0.05).

genotype (P<0.05) (Table 6). In stress level 30, there was no significant difference among all genotypes (Table 6). In stress levels 70 and 100, the highest Hg concentration in seed was shown in Sirvan and Sepahan genotypes, respectively (P<0.05) (Table 6). The interaction effect of genotype/mercury was significant (P<0.01) (Table 5). In stress level of 100 Hg concentration in seed in Sepahan, Sivand, Sirvan and Parsi genotypes, raised by 155, 98.2, 69.1 and 47.2 percent, compared with the control treatment, respectively (P<0.05).

Discussion

In the past few decades, breeders have been in search of new species of plants that sustain well in different habitats. But the interaction effect of environment and genotype has made choosing plants difficult with acceptable performance in all environmental conditions (including desirable and undesirable conditions). This issue has caused us to identify genotypes that present acceptable performance and demonstration by maximal usage of environmental facilities (Golabadi *et al*., 2007). In the considered study, Mercury stress caused to decrease transpiration level, stomatal conductance, carbon assimilation and photosystem II efficiency in all genotypes and their level was significantly higher in genotypes likely to suffer Sirvan, Sepahan and Sivand stress than Parsi sensitive genotypes. Chlorophyll decreasing and carotenoids increasing were observed in the conducted study. The probable Sirvan genotype allocated the highest chlorophyll a and b, total chlorophyll, chlorophyll stability index and carotenoid as compared with other studied genotypes. Toxic effects of Mercury on wheat photosynthesis are created by different mechanisms, which can be referred to as chlorophyll biosynthesis decreases via decreasing essential element concentrations of magnesium and iron in leaves, generating complexes by photosynthetic proteins, and increasing chlorophyllase activity to decompose chlorophyll (Sahu *et al*., 2012). Mirsa and Tandon with tests on wheat and corn showed that increasing mercury concentrations, reduced the amount of chlorophyll a and chlorophyll b and increased the levels of carotenoids (Misra and Tandon, 2009). The present study results indicated that chlorophyll number was decreased as mercury concentration was increasing in the growth environment. Decreasing photosynthetic pigments in plants subjected to mercury treatment can be because of oxidative damage. This decrease is also due to inhibitive effect of different stages of chlorophyll photosynthesis (Noorani and Kafilzadeh, 2012). Heavy metals disturb the formation of this complex via inhibiting complex LHC II proteins biosynthesis at the transcription level (Vajpayee *et al*., 2000). In the present

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study, decreasing chlorophyll has caused to growth and photosynthesis reduction. Also, an increase in mercury in the growth environment might limit nutrient elements absorption required for producing chlorophyll (Hegedus *et al*., 2001). In this study, chlorophyll a/b ratio influenced by reduced mercury concentration indicated that the mechanism influenced by heavy metals on photosynthetic pigments may be because: Penetrating heavy metals into chloroplasts and their high accumulation in this organelle, oxidative stress may occur that cause damages such as chloroplast peroxidation (Seregin and Kozhevnikova, 2006). Heavy metals can also influence chloroplasts morphology and functioning directly by bonding to sulfhydryl groups of disrupted enzymes and overall chlorophyll biosynthesis (Srivastava *et al*., 2006). Also, heavy metals restrain other essential elements absorption and transportation, such as Fe^{2+}, Mu^{2+}, and Zn^{2+} by means of antagonist effects, and thereby pigment synthesis capacity in plants is influenced (Gardea-Torresdey *et al.,* 2004). In addition, heavy metals have direct inhibitive impacts on one of the enzymatic stages, and furthermore, copper induces additional leaf chlorosis, which is due to cleave peroxidative pigments and membrane lipids and to decrease pigment contents (Meloni *et al.*, 2003).

Carotenoids increase in this study implies their protective role against oxidative stress. These pigments contribute to detoxifying chlorophyll and to decreasing the toxic effects of free radicals by accumulating in plant tissue during stress in soil (Sanitata *et al*., 1999). Chlorophyll analysis in the presence of heavy metals can also be regarded as important factors to decrease chlorophyll (Hegedus *et al.*, 2001). In stress conditions, chlorophyll molecule collapse is obvious (Barati and Bijanzadeh, 2022). Following green pigment collapse (chlorophyll), the plant appears chromatic because of increasing and being visible protective pigments like carotenoids and anthocyanins (Wojcik *et al*., 2006). Carotenoids play a protective role against oxidative stress. These pigments have a role in chlorophyll detoxification, and their aggregation in plant tissue during stress leads to decreased toxic effects of free radicals (Sanitata *et al*., 1999). The decrease in the performance of photosystem II and the decrease in the amount of chlorophyll caused a decrease in plant growth and a decrease in the production of insoluble carbohydrates. (Talebzadeh *et al*., 2022).

The studies of Raisi Sadati *et al*. (2015) showed that in two wheat cultivars, Gonbad and Tejn, mercury chloride treatment caused a significant decrease in the amount of soluble sugar and polyphenol oxidase enzyme, an increase in the amount of total protein, soluble sugar and lysine, and an increase in the amount of proline and methionine.

The studies of Balochi *et al*. (2016) on pinto beans (*Phaseolus vulgaris* L.) showed that heavy metals decreased the rate of photosynthesis compared to the control and caused an increase in ion leakage, proline, soluble sugar and malondialdehyde.

Gregersen and Holm (2007) stated that abiotic stress decreases chlorophyll content, and numbers with high chlorophyll content have more resistance in stress conditions. Also, chlorophyll can be associated with peroxidation of the chloroplast membrane by heavy metals. Antolin *et al*. (1995) found that leaf chlorophyll decreased as non-biotic stress was increasing, but chlorophyll a/b ratio was increased. Chlorophyll b showed a smaller reduction in stress conditions than chlorophyll a, so the a/b ratio increased. It seems that increased chlorophyll a/b ratio leads to leaves darkness and increased chlorophyll meter number. Also, other researchers confirmed chlorophyll a/b increase as a result of non-biotic stress (Ahmadi and Baker, 2000).

Mn-excess devastated the whole photosynthetic electron transport chain from the transferor side of PSII up to the reduction of end acceptors of PSI, thus limiting the production of reducing equivalents, and hence the rate of CO_2 assimilation (Li *et al*., 2010). Moreover, the decreased rates of CO_2 assimilation can also be partly attributed to the corresponding decrease of the photosynthetic unit concentration, i.e., the reduction in Chl content regulated by Cd (Chaneva *et al*., 2010). Pourrut *et al*. (2011) stated that Lead (Pb) inhibits transpiration, chlorophyll production, and water and protein content, causing alterations in chloroplast, obstructing electron transport chain, inhibition of Calvin cycle enzymes, impaired uptake of essential elements, Mg and Fe, and induced deficiency of CO_2 due to stomatal closure and Lead was negatively affected by the process of photosynthesis and carbon fixation reduces.

Chlorophyll fluorescence value and photosynthesis quantum performance indicate intact thylakoid membrane and electron relative efficiency from photosystem II to I (Moffatt *et al*., 1990). It was found that the dominant decrease of chlorophyll b by heavy metals potentially damages energy trapping efficiency through PSII and decreases electron transfer (Dube *et al*., 2003). Intracellular CO_2 concentration decreases because of closing stoma and also inhibiting ATP synthesis. Rubisco enzyme activity is considered one of the main reasons to decrease carbon assimilation in abiotic stress conditions (Asadi and Hatami, 2020). Mercury influences plant behavior in addition to changing carbon assimilation decrease via photosystem II structure collapse and fluorescence chlorophyll increase (Rahbarian *et al*., 2011).

Since it is assumed that dryness tension and heavy metals responses are the same, Sirvan and Sepahan genotypes as numbers likely to dryness stress, the resistant genotype to heavy metals stress, and Parsi genotype as sensitive number to dryness stress, are introduced as sensitive genotypes to heavy metals stress.

The interaction of heavy metals and soil chloride is a key factor in the absorption of heavy metals by plants of different groups: Agricultural, halophytes of salt marsh, desert, etc Blue macrophytes. Based on the results of studies by Rahbarian *et al*. (2019) on chromium ether on

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plants Loleracea Portulaca with increasing chromium concentration in the cultivation environment, concentration VI (Cr) in aerial parts and root increased. The results of the research showed that the highest amount of soil mercury under mercury stress is in Sepahan genotype, and the highest amount is under mercury stress in leaves and roots in Sivand, since the absorption of mercury in the plant is more, less amount of mercury remains in the soil.

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