Environmental pollution is reflected in the activity of the photosynthetic apparatus

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Abstract

The efficiency of chlorophyll (Chl) fluorescence for forecasting plant response to environmental pollution was examined in four wheat cultivars. The plants were irrigated with wastewater from Razi petrochemical complex. A forecasting model was designed based on the data of maximal quantum yield of photosystem II (Fv/Fm), efficiency of both photosystems (PIABS), and Chl contents of 60-d analysis. A comparison of the forecasting model results with the real data of Fv/Fm, PIABS, and Chl in response to wastewater suggested Fv/Fm as an accurate tool for stress forecasting. The high salt concentration in wastewater induced high accumulation of H2O2, lipid peroxidation, inhibition of photosynthesis, and reduction in contents of proteins and carbohydrates, which resulted in growth suppression of all cultivars. Taken together, the negative effect of environmental pollution on crop can be detected at an early stage of stress using Fv/Fm as a fast and accurate tool to prevent irrecoverable damage to plants.

Additional key words: antioxidant; early intervention; metabolites; reactive oxygen species; yield.

Introduction

The photosynthesis process is one of the main physiological processes in plants; its perturbation can directly or indirectly inhibit numerous physiological processes and metabolite production, followed by growth restriction (Baker and Rosenqvist 2004). Abiotic stress results in a reduction of the electron transport chain, which in turn leads to a decline in photosynthesis (Gururani et al. 2015). The absorbed light energy by Chl molecules can be used for one of three processes: photochemistry (photosynthesis), heat dissipation, or Chl fluorescence emission. These three processes are in competition, so that measuring the yield of one process can provide information about the changes in the other two processes (Maxwell and Johnson 2000).

The rapid evaluation of crop performance is an important feature to monitor plant responses throughout diverse environmental conditions. The Chl fluorescence measurement from intact plants has been widely used to characterize the physiological performance of plants. Although this technique has been used for many years, further studies are needed for understanding the complicated plant response to environmental stresses (Baker and Rosenqvist 2004). The measurement of Chl fluorescence reflects a plant’s photosynthetic performance and abnormal readings are a specific sign of abiotic stress. The fluorescence measurement can provide information about a plant ability to tolerate abiotic stress and the extent of damage to the photosystems. PSII is frequently referred as the most susceptible compartment to abiotic stresses in the photosynthetic apparatus, whereas very efficient mechanisms protect PSI from damage. The photodamage to PSI can happen when the electrons supply from PSII exceeds the PSI capacity for accepting electrons. The Fv/Fm measurement, as an indicator of maximum quantum efficiency of PSII photochemistry, has the potential to reveal stress-induced disorders in the photosynthetic apparatus (Baker and Rosenqvist 2004, Tuba et al. 2010, Živčák et al. 2014, Dąbrowski et al. 2015, 2016; Kalaji et al. 2017a,b). The efficiency of both PSI and
PSII can be characterized through the measurement of the performance index (PI$_{ABS}$) (Živčák et al. 2008). A decline in the photosynthetic capability of Chl because of stress damage can be followed by a reduction in the light absorption and excess excitation energy, with the aim of increasing of the energy dissipation, and lowering the PSII efficiency (Demmig-Adams and Adams 2006). The reduction of PI$_{ABS}$ indicates that the energy conservation ability and the photosynthetic apparatus activity were inhibited by abiotic stress and suggests a reduction in net photosynthetic rate ($P_N$) and water-use efficiency (WUE) (Adams et al. 2013, 2014; Cohu et al. 2014). The decline in photosynthesis is followed by a post-photosynthetic process of carbon metabolism (e.g., carbohydrates, proteins, amino acids), which can be evidenced in the reduction of plant growth (Gamage et al. 2018). Abiotic stress stimulates the production of reactive oxygen species (ROS), which results in damage of lipid membranes, proteins, and nucleic acids (Anjum et al. 2015).

The world population has quadrupled from 1.8 billion to 7.7 billion between 1915 and 2019. According to a UN estimate, the global population could reach 9.7 billion by 2050, along with an increased food demand. Farmers will need to produce more crops, along with increasing the water demand because crop production is one of the largest water-demanding industries. The problem of water scarcity encourages farmers to reuse wastewater in the agricultural industry in arid and semiarid areas. This study proposes a methodological framework for conducting rapid and efficient analysis of intact crops, which may be used by farmers for the case study of plants under unfavourable growth conditions, besides wastewater irrigation practice. With regard to the benefits derived from the reuse of wastewater, with the aim of preserving groundwater reserves, the evaluation of crop responses to wastewater irrigation at early growth stages is beneficial to manage the early decision-making on selecting the most appropriate crop.

Because of the significant demand for water, wastewater reuse in the agricultural industry has increased worldwide. Wastewater contains different amounts of beneficial nutrients (e.g., micro and macro essential elements) and toxic pollutants (heavy metals) depending on its source (Chen et al. 2013), reusing of the wastewater in agricultural practice can be considered as a challenge, which calls for further case studies to determine the most appropriate crops for wastewater irrigation. High amounts of wastewater produced by petrochemical operations makes this industry one of the centres of attention for reusing its output water. The Razi petrochemical complex located in Mahshahr, Iran, produces some chemical compounds, such as diammonium phosphate, ammonia, phosphoric acid, and sulphuric acid, which introduce different salts to the environment and surface water (Tehrani et al. 2012, Alavi et al. 2013). Using the industrial wastewater for crop irrigation is valuable to save water sources. Therefore, the tolerant crops to high salt concentrations should be introduced for irrigation with wastewater. Comparing the response of different Triticum aestivum cultivars to wastewater irrigation is important to introduce the most tolerant cultivar. The main aim of this paper was to determine whether measurements of Chl fluorescence and/or Chl contents in intact plants at the early stage of plant growth could help predict sensitivity of plants to high salt contents in wastewater. The decrease of Chl fluorescence and Chl contents, due to environmental stress, are usually followed by ROS accumulation, damages to lipid membranes, decline in photosynthesis process, reduction of carbohydrates biosynthesis, and growth inhibition. Accordingly, if the plant sensitivity to environmental stress can be detected early in intact plants, future damages to plants can be prevented. For this purpose, a continued monitoring of Chl fluorescence, Chls, and photosynthetic characteristics in intact plants from early plant growth stage was done to exemplify a rapid and accurate tool in agricultural practice. Up to our knowledge, numerous articles are available in this area, but none of them monitored $F_v/F_m$, PI$_{ABS}$, and Chl for 60 d in different genotypes to introduce the most accurate variable for crop tolerance to environmental stress. To confirm the accuracy of photosynthetic results, the physiological and morphological responses of plants were evaluated in the wastewater-irrigated plants.

Materials and methods

Plant culture and treatment: The elements present in waste water obtained from the discharge channel of the Razi Petrochemical Company, Mahshahr, Iran, was analysed using inductively coupled plasma-optical emission spectrometry (Varian 735 ICP-OES, Australia). The amounts of oil, total suspended solids (TSS), chemical oxygen demand (COD), NH$_4^+$, NO$_3^-$, SO$_4^{2-}$, and PO$_4^{3-}$, and pH were measured based on the standard methods for wastewater analysis (Williams 1984, Eaton et al. 2005, Patnaik 2017). The results showed a high content of suspended solids in the wastewater, whereas the amounts of heavy metals were lower than that of the standard limits (Hajishahemi et al. 2019). The wastewater from the Razi Petrochemical Company is discharged in Musa Bay, which results in wastewater dilution. As wastewater contains high amounts of nutrients and salts, the dilution of wastewater can minimize the adverse effects of high TSS (Hajishahemi et al. 2019). Accordingly, in the present study, the wastewater was diluted with distilled water to 25% and 50% (v/v).

Four cultivars of Triticum aestivum including Behrang, Chamran, Pishgam, and Mehregan were sterilized for 1 min in ethanol (70%, v/v), 20 min in sodium hypochlorite (20%, v/v), and washed three times with distilled water. Then, ten Petri dishes for every cultivar, each containing ten seeds, were prepared and incubated in a growth chamber with a photoperiod of 16/8 h light/dark and a temperature of 25 ± 1°C for two weeks. Ten similar seedlings with two true leaves were transplanted into each pot containing equal amounts of soil and perlite. They were transferred into a plant growth chamber with controlled conditions of a photoperiod of 16-h day and 8-h night; the air temperature was 25 ± 1°C during the day and 18 ± 1°C during the night, and humidity of 60 ± 2%. The wastewater irrigation of plants was performed at three concentrations of 0 (distilled...
water), 25, and 50% of wastewater in distilled water (v/v). Ten pots for each irrigation level were prepared for every cultivar. Ten days after sowing, the number of plants was reduced to four similar plants. The plants were irrigated with different concentrations of wastewater every 5 d for 3 months. The plants were harvested two times, i.e., at 30 and 60 d after sowing. The plant culture and treatment were repeated three times to have enough plant mass for physiological and morphological analysis.

Chl fluorescence and photosynthetic traits: The parameters of Chl fluorescence ($F_{v}/F_{m}$ and $PI_{ABS}$) and Chls were measured on the apical fully expanded leaves of ten different plants, per every treatment. They were measured continually every three days for 60 d after the first wastewater irrigation of plants. $F_{v}/F_{m}$ and $PI_{ABS}$ were measured with a portable fluorimeter ($PEA$, Hansatech, UK) early in the morning before the lights of the phytotron were on (Zivčák et al. 2008, Hajihashemi et al. 2018). The total amount of Chls was measured with a portable chlorophyll meter (CCM-200 plus Chlorophyll Content Meter, Thailand). The photosynthetic parameters including net photosynthetic rate ($P_{n}$) and water-use efficiency (WUE) were measured with a portable plant photosynthesis meter ($KR8700$ system, Korea Tech. Inc., Korea) on the apical fully expanded leaves of ten different plants, per every treatment (Hajihashemi et al. 2018). The photosynthetic traits were measured for 60 d at 12-d intervals.

Growth analysis: The area of ten apical, fully expanded leaves from different plants was measured for 60 d after the first wastewater irrigation at 12-d intervals. The leaf area was measured with a portable leaf area meter ($KR9700$, Korea Tech. Inc., Korea). After 30 and 60 d of wastewater irrigation, the fresh mass of ten fully expanded leaves from different plants was immediately measured after harvesting. The dry mass of leaves was measured after their incubation in an oven at 70°C for 48 h.

Physiological parameters: To measure the physiological parameters, the plants were harvested after 60 d. The amount of water-soluble carbohydrates (WSC) was measured in the dry mass of harvested leaves based on the Dubois et al. (1956) method, after 30 and 60 d of wastewater irrigation. The amount of anthocyanins (Wagner 1979), flavonoids (Jia et al. 1999), phenols (Singleton and Rossi 1965), protein (Bradford 1976), proline (Bates et al. 1973), and malondialdehyde (MDA) (Hajihashemi and Esfahianpour 2014), was measured in fresh leaves harvested after 60 d of wastewater irrigation. To measure $H_{2}O_{2}$, fresh leaves were extracted with trichloroacetic acid and assayed according to Velikova et al. (2000).

Forecasting evaluation: To develop a forecasting model for the $F_{v}/F_{m}$ ratio, $PI_{ABS}$, and Chls, the FORECAST.ETS function in Excel software was applied. Based on this model, a target date, timeline, and the values for the target timeline are necessary. In order to examine if the forecasting of the $F_{v}/F_{m}$ ratio, $PI_{ABS}$, and Chls traits was accurate, the parameters were measured for 60 d. A timeline of 1–30 d was set up and the target days were from the 31st to the 60th d, at three-day intervals. Accordingly, the responses of $F_{v}/F_{m}$ ratio, $PI_{ABS}$, and Chls from the 30th to 60th d were forecast based on the values obtained for the 1st to 30th d. Then, the real responses of $F_{v}/F_{m}$ ratio, $PI_{ABS}$, and Chls were compared with the results of forecasting.

Forecasting evaluation = FORECAST.ETS (target date, values, timeline, [seasonality], [data completion], [aggregation]).

Statistical analysis: The plant culture and treatment were repeated three times. All experiments were performed by using a randomized complete block design with cultivar as the main plot and treatments as sub-plot. Each experiment included a set of ten pots, with four plants per pot. Data were subjected to analysis of variance in $SPSS$ software (ver. 23) with the 0.05 probability level. The means of $F_{v}/F_{m}$, $PI_{ABS}$, Chls, $P_{n}$, $C$, WUE, and area, length, and fresh and dry mass of leaf were obtained from ten leaves from ten pots. The means of carbohydrates, MDA, proline, protein, phenol, anthocyanins, and flavonoids amounts are the average of four values for each treatment. Significant differences between data were characterized using analysis of variance ($ANOVA$) test. The means were compared using Duncan’s test ($p<0.05$).

Results

The diurnal changes in the $F_{v}/F_{m}$ ratio, $PI_{ABS}$, and Chls of plants irrigated with different concentrations of wastewater (0, 25, and 50%) were investigated for four wheat cultivars. The wheat plants, regardless of their cultivars, showed a significant gradual decline in $F_{v}/F_{m}$, $PI_{ABS}$, and Chls over the 60 d of analysis (Figs. 1–3). All of the cultivars showed the highest reduction in $F_{v}/F_{m}$ ratio, $PI_{ABS}$, and Chls on the 60th d of analysis, more so in 50% wastewater than in 25% wastewater. At 25 and 50% wastewater irrigation, the first significant decline in the $F_{v}/F_{m}$ ratio, $PI_{ABS}$, and Chls was observed between the 9th and 12th d, depending on the wheat cultivar. On the 60th d, 50% wastewater irrigation significantly reduced the $F_{v}/F_{m}$ ratio by 15, 22, 15, and 20% in the Behrang, Chamran, Mehregan, and Pishgam cultivars, respectively, relative to the controls (Fig. 1). The $PI_{ABS}$ value in Behrang, Chamran, Mehregan, and Pishgam cultivars showed the highest decline on the 60th d in 50% wastewater-irrigated plants, by 53, 73, 50, and 66%, respectively (Fig. 2). In comparison, the response to 50% wastewater irrigation showed that the highest drop of Chls occurred in Behrang, Chamran, Mehregan, and Pishgam on the 60th d, by 50, 68, 52, and 54%, respectively (Fig. 3).

A forecasting model was developed based on the data obtained from the measurement of the $F_{v}/F_{m}$ ratio, $PI_{ABS}$, and Chls in the first 30 d of wastewater irrigation (Figs. 1–3). The forecasting was performed for the period from 30–60 d. The results of Chls and $PI_{ABS}$ forecasting were very inconsistent depending on the cultivar and wastewater concentration, whereas the results of $F_{v}/F_{m}$ forecasting almost matched with the results obtained for the wastewater-irrigated plants. The data of Chls forecasting showed that in Behrang and Mehregan cultivars...
Fig. 1. Maximal quantum yield of PSII photochemistry ($F_v/F_m$) value of four wheat cultivars [Behrang (A), Chamran (B), Pishgam (C), and Mehregan (D)] irrigated with 0 (distilled water; Con), 25, and 50% of wastewater obtained from Razi petrochemical complex. The means are the average of ten plants. The error bars show standard deviation. The blue symbols are actual experimental results and the orange symbols are the results of forecasting (For.) evaluation.

Fig. 2. Performance indices (PI_ABS) value of four wheat cultivars [Behrang (A), Chamran (B), Pishgam (C), and Mehregan (D)] irrigated with 0 (distilled water; Con), 25, and 50% of wastewater obtained from Razi petrochemical complex. The means are the average of ten plants. The error bars show standard deviation. The blue symbols are actual experimental results and the orange symbols are the results of forecasting (For.) evaluation.
irrigated with 25% of wastewater, the forecasting matched the experimental results, whereas for the Chamran and Pishgam cultivar at 25% of wastewater and for all four wheat cultivars at 50% of wastewater, the forecast reduction in the Chls value was significantly higher than the real results obtained experimentally. The results of PIABS forecasting were almost matching to the real results of this experiment only in Behrang and Chamran irrigated with 25% of wastewater, whereas the results of forecasting of Mehregan and Pishgam irrigated with 25% of wastewater and all four cultivars irrigated with 50% of wastewater showed higher decline than that of the real experimental results. In contrast to Chls and PIABS forecasting results, the data obtained from F_v/F_m forecasting in four cultivars irrigated with both 25 and 50% of wastewater almost matched with the real results obtained experimentally.

Wastewater irrigation at both 25 and 50% resulted in a significant gradual reduction in P_N and WUE during 60 d of analysis, with 12-d intervals. The highest reduction in P_N and WUE were observed at 50% of wastewater irrigation on the 60th d of irrigation (Fig. 4). The highest reduction was in the P_N value at 50% of wastewater irrigation on the 60th d in Behrang, Chamran, Mehregan, and Pishgam, i.e., 59, 69, 53, and 69%, respectively. The WUE trait showed the greatest reduction at 50% of wastewater on the 60th d in Behrang, Chamran, Mehregan, and Pishgam cultivars, by

Fig. 3. Chlorophyll value of four wheat cultivars [Behrang (A), Chamran (B), Pishgam (C), and Mehregan (D)] irrigated with 0 (distilled water; Con), 25, and 50% of wastewater obtained from Razi petrochemical complex. The means are the average of ten plants. The error bars show standard deviation. The blue symbols are actual experimental results and the orange symbols are the results of forecasting (For.) evaluation.

Fig. 4. Net photosynthetic rate (P_N) and water-use efficiency (WUE) of four wheat cultivars [Behrang (A), Chamran (B), Pishgam (C), and Mehregan (D)] irrigated with 0 (distilled water; Con), 25, and 50% of wastewater obtained from Razi petrochemical complex. The means are the average of ten plants.
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61, 66, 56, and 63%, respectively.

The diurnal measurement of the leaf area, with a 12-d interval, showed a significant gradual decline over the 60 d of analysis (Fig. 5). The highest reduction of the leaf area was observed on the 60th d of wastewater irrigation at 50% wastewater concentration in Behrang, Chamran, Mehregan, and Pishgam cultivars, by 53, 59, 57, and 58%, respectively. Wastewater irrigation significantly reduced the fresh and dry mass of leaves on the 30th and 60th d of treatment (Fig. 5). The highest reduction of leaf fresh mass on the 60th d of irrigation was achieved with 50% of wastewater in Behrang, Chamran, Mehregan, and Pishgam cultivars, by 43, 69, 43, and 60%, respectively. The leaf dry mass showed the highest reduction at 50% of wastewater on the 60th d in Behrang, Chamran, Mehregan, and Pishgam cultivars, by 42, 62, 47, and 56%, respectively.

The amount of WSC was measured on the 30th and 60th d after wastewater irrigation (Fig. 6A). In accordance with photosynthetic traits, the amounts of WSC decreased significantly in all cultivars in response to wastewater irrigation, more so on the 60th d than on the 30th d. The rate of WSC content reduction in 50% wastewater-irrigated plants was higher than that in plants irrigated with 25% wastewater, regardless of the cultivar. On the 60th d, the WSC content in Behrang, Chamran, Mehregan, and Pishgam cultivars was reduced by 44, 53, 47, and 50%, respectively. Wastewater irrigation caused a significant increase in the amount of nonphotosynthetic pigments including anthocyanins (Fig. 6B), flavonoids (Fig. 6C), and phenols (Fig. 6D) in all wheat cultivars, with the highest amount in plants treated with 50% wastewater. In plants treated with 50% wastewater, the phenols content increased in Behrang, Chamran, Mehregan, and Pishgam cultivars, by 88, 39, 56, and 51%, respectively. The flavonoids content in Behrang, Chamran, Mehregan, and Pishgam cultivars showed the highest increase by 77, 50, 73, and 53%, respectively, in response to treatment with 50% wastewater. The amount of anthocyanins in Behrang, Chamran, Mehregan, and Pishgam cultivars irrigated with 50% of wastewater increased by 20, 14, 30, and 17%, respectively.

Wastewater irrigation significantly increased the \( H_2O_2 \) accumulation in all cultivars. The \( H_2O_2 \) content in plants treated with 50% wastewater was higher than that in those treated with 25% wastewater (Fig. 7A). The rate of \( H_2O_2 \) increase in Behrang, Chamran, Mehregan, and Pishgam cultivars treated with 50% wastewater was by 80, 158, 83, and 136%, respectively. Similar to the increase in \( H_2O_2 \), the amount of MDA significantly increased in wastewater-irrigated plants, with the highest increase in those treated with 50% wastewater (7B). The MDA in Behrang, Chamran, Mehregan, and Pishgam cultivars irrigated with 50% wastewater increased by 25, 40, 28, and 36%, respectively. The wastewater irrigation significantly increased the proline content (Fig. 7C), whereas significantly reduced the protein contents (Fig. 7D).
Proline content showed the highest increase in 50% of wastewater in Behrang, Chamran, Mehregan, and Pishgam cultivars, by 61, 23, 53, and 29%, respectively. The protein content decreased in 50% of wastewater more than that in 25% of wastewater in all cultivars. The amount of proteins declined in Behrang, Chamran, Mehregan, and Pishgam cultivars in 50% of wastewater by 17, 25, 19, and 24%, respectively.
Discussion

The wastewater from Razi petrochemical complex contained high amounts of suspended solids (325 mg L⁻¹; Ca, K, Mg, Na, P, S, Si, NH₄⁺, PO₄³⁻, and SO₄²⁻), which is approximately 7 times more than that of the allowed standard value (45 mg L⁻¹) (Williams 1984, Eaton et al. 2005, Patnaik 2017), which negatively affected the yield of *Brassica napus* (Hajihashemi et al. 2019). In our previous research, we studied the response of *B. napus* to wastewater irrigation at a later growth stage, too late to save plants from salt-induced damage (Hajihashemi et al. 2019). Understanding the plant's response to unfavourable growth conditions at the early stage of environmental stress would allow us to reduce the damage. Evaluation of the Chl fluorescence has been introduced as a rapid tool to study the negative effect of environmental stress on intact plants at an early stage of exposure under unfavourable conditions (Maxwell and Johnson 2000, Joshi and Mohanty 2004, Sharma et al. 2014, Guo and Tan 2015, Kalaji et al. 2018, Stirbet et al. 2018). The wastewater containing high amounts of salts induced the gradual decrease in Fᵥ/Fₘ, PI_ABS, and Chls in four wheat cultivars treated for 60 d and measured at 3-d intervals, with the highest reduction observed in Chamran cultivar. The radar chart illustrated the negative effect of wastewater irrigation on Fᵥ/Fₘ, PI_ABS, and Chls during 60-d wastewater irrigation (Fig. 8). The first reduction was observed after 6 d of wastewater irrigation and continued during 60-d analysis. Kalaji et al. (2011) reported the first obvious reduction of Fᵥ/Fₘ at around 7 d of salt treatment. Generally, the Fᵥ/Fₘ value in higher plants is almost 0.83 under controlled conditions (Kalaji et al. 2011), while it was about 0.8 in four wheat cultivars. The Fᵥ/Fₘ value, under 50% wastewater irrigation, was between 78 to 85% of that noted in control wheat cultivars. This observed reduction in the Fᵥ/Fₘ value can occur due to either the damages in the reaction centres (photochemically inactive) followed by reduced electron transport capacity in PSII, and nonphotochemical quenching (Maxwell and Johnson 2000, Kalaji et al. 2011). Kalaji et al. (2011) observed a significant reduction in PI_ABS in the salt-stressed barley cultivars similar to the observed reduction in the PI_ABS in the wastewater-irrigated wheat cultivars. PI_ABS is a complex parameter that is related to

![Fig. 8. Radar chart of chlorophylls (A–D), maximal quantum yield of PSII photochemistry (Fᵥ/Fₘ) (E–H), and performance indices (PI_ABS) (I–L) of four wheat cultivars Behrang, Chamran, Pishgam, and Mehregan.](image-url)
the maximum quantum yield of PSI, the ratio of reaction centres per light absorption flux, and the quantum yield of electron transport (Kalaji et al., 2011, 2017b). The adverse effect of 50% wastewater irrigation on PI_{ABS}, a reduction rate 27–50% lesser than that of controls, was greater than that for F_{ABS}/F_{m}. PI_{ABS} response to wastewater irrigation was very variable among four wheat cultivars, with the lowest and highest reduction of 27 and 50% in Mehregan and Chamran cultivars, respectively, less than that in control plants. One symptom of environmental stress in plants is the reduction of photosynthetic pigments, which is the result of pigment photooxidation and Chl degradation due to ROS accumulation and oxidative stress (Yildiz and Terzi 2008, Farooq et al. 2009). This may be ascertained by measurement of H_{2}O_{2} contents in leaves. The high accumulation of Zn in Brassica napus irrigated with wastewater from the Razi petrochemical complex can be the reason for the observed reduction in the Chl contents (Hajihashemi et al. 2019), due to Mg substitution with Zn in Chl molecules, inhibition of photosynthesis, and subsequently a growth restriction (Küpper and Andresen 2016). Accordingly, the observed reduction in the Chl contents in the wastewater-irrigated cultivars might be also due to toxic effect of high Zn concentration in the wastewater. Taken together, the reduction of F_{ABS}/F_{m}, PI_{ABS}, and the Chl contents in the wastewater-irrigated plants occurred due to the high salt concentrations, with the most observed reduction in PI_{ABS} in Chamran cultivar after 60 d of treatment.

The effect of wastewater irrigation on F_{ABS}/F_{m}, PI_{ABS}, and Chl content was significant, and to the best of our knowledge, there has been no similar study in which Chl fluorescence was measured in different genotypes of wastewater-irrigated crops (Misra et al. 2001, Strasser et al. 2004, Li et al. 2006, Kalaji et al. 2011, 2016, 2017b, 2018; Brešič and Živčák 2013, Guo and Tan 2015, Brešič et al. 2018, Hajihashemi et al. 2018, Stirbet et al. 2018, Baba et al. 2019). As crops are frequently exposed to the environmental stresses, the reliable and efficient tools in screening of the stress at early stage of crop growth are required (Brešič and Živčák 2013). Therefore, the main focus of this study was to determine which one of F_{ABS}/F_{m}, PI_{ABS}, and Chl can be used at the early stage of wastewater irrigation to predict the future function of plants in response to stress. As it is shown in Figs. 1, 2, and 3, the PI_{ABS} and Chl values are in the shape of logarithmic timescale curves, especially at 50% of wastewater, after 60-d wastewater irrigation, while F_{ABS}/F_{m} curve is more likely linear. The rate of reduction in PI_{ABS} and Chl, for 30-d and 50% wastewater irrigation, was greater than that during the second 30 d of wastewater irrigation. In contrast to the PI_{ABS} and Chl parameters, the rate of reduction in the F_{ABS}/F_{m} was almost stable during 60-d wastewater irrigation and no significant changes were observed between the first and second part of the treatment. Based on the forecasting model (Figs. 1–3), the plants’ predicted responses to 50% wastewater irrigation stress, based on PI_{ABS} and Chls, would be negative by 60 to 70 d after exposure. In contrast to the results of the forecasting model, the real response of plants showed that after 40 d of wastewater irrigation, the rate of reduction in PI_{ABS} and Chl values slowed down and their values were almost stable. In contrast to PI_{ABS} and Chl response to wastewater, the F_{ABS}/F_{m} showed almost a gradual and stable rate of reduction in response to wastewater irrigation for 60 d in all wheat cultivars, and it was the reason why the results of the forecasting model were similar to the real results of wastewater-irrigated plants. Accordingly, F_{ABS}/F_{m} could be introduced as an accurate tool for forecasting plant response to environmental stress, but PI_{ABS} and Chl were not accurate according to the forecasting model.

The Chl a fluorescence evaluation can provide the knowledge to estimate the photosynthetic performance and consequently the plant function (Baker 2008, Brešič and Živčák 2013, Brešič et al. 2018, Baba et al. 2019). In the present study, photosynthetic function was in accordance with the F_{ABS}/F_{m} response to wastewater irrigation. The diurnal measurement of the PI_{ABS} and WUE values, at 12-d intervals, showed a significant linear reduction in their response to wastewater irrigation, which was similar to the effect of wastewater on F_{ABS}/F_{m}. Accordingly, the measurement of F_{ABS}/F_{m} can be used to forecast the photosynthetic response of plant to stress. In accordance with Chl fluorescence response to wastewater, the highest reduction in PI_{ABS}, F_{ABS}/F_{m} and WUE was observed in Chamran cultivar. Oyiga et al. (2016) suggested that the reduction in photosynthesis may be due to the inhibition of PSII activity and the reduction of Chl in leaves, which confirms the results of present study. Overall, the mechanisms limiting photosynthesis in the wastewater-irrigated plants are complex and might include: (1) the reduction in the Chl content; (2) the stomatal closure to maintain the leaf water content; (3) the injury in the photosynthetic apparatus caused by ROS accumulation; (4) the reduced activity in the PSI and PSII and the reduction of photosynthetic electron transfer between the photosystems; (5) the ROS-induced damages to the proteins involved in the photosynthetic process (e.g., Rubisco activase, ATP-ase, chloroplast fructose-bisphosphate aldolase, oxygen-evolving enhancer protein, etc.); (6) the lipid peroxidation due to ROS accumulation which affects the composition and stoichiometry of the photosynthetic membrane including the ration of PSII to PSI, the ration of LHCII to PSII, the super complexes PSII-LHCII content, and the electron transport between photosystems (Misra et al. 2001, Oukarroum et al. 2007, Kalaji et al. 2011, 2014, 2016; Brešič and Živčák 2013, Brešič et al. 2018, Hajihashemi et al. 2018, 2019, Dąbrowski et al. 2019). Reduction of plant growth can be typically induced by disorder in the photosynthetic process or mineral nutrient imbalance, which is followed by a disorder in plant metabolism and decline in yield (Imtiaz et al. 2015, Kalaji et al. 2016). The reduction in photosynthesis was followed by a significant reduction in WSC content along with plant growth suppression. The value of Chl fluorescence measurement lies in its relationship to the photosynthesis process since the light absorbed by a plant, which does not drive the biosynthesis of carbohydrates, can be reemitted as florescence light or dissipated as heat energy (Brešič and Živčák 2013). Accordingly, the observed early reduction in F_{ABS}/F_{m} ratio could reflect the achieved decrease in carbohydrate biosynthesis in...
the wastewater-irrigated wheat cultivars. Similar to the measurement of the photosynthetic parameters, the leaf area was measured during 60 d at 12-d intervals, whereas the fresh and dry mass of the leaf was measured on the 1st, 30th, and 60th d after the first wastewater irrigation. Taken together, the effect of wastewater containing high salts on the leaf area, fresh and dry mass was in accordance to the photosynthetic parameters and the Fv/Fm response to wastewater treatment.

H2O2 acts as a signaling molecule in plants and involves in various biochemical and physiological processes including regulation of antioxidant system, growth and development, and photosynthetic capacity (Khan et al. 2018). The antioxidant systems play an important role in regulating the H2O2 content (Hajihashemi and Ehsanpour 2014, Nievola et al. 2017, Khan et al. 2018). The H2O2 content significantly increased in the wastewater-irrigated wheat cultivars with the highest increase in Chamran cultivar. The water-soluble pigments of anthocyanin belong to flavonoids family, with an antioxidant property (Larson 1988). The observed high accumulation of anthocyanin and flavonoids in wastewater-irrigated plants might increase antioxidant capacity of wheat against high accumulation of H2O2. The secondary metabolites of phenolic compounds constitute a large group of compounds with antioxidant property. Phenols with ROS-scavenging ability can protect cell compounds including lipid membrane, nucleic acid, and proteins under environmental stress. Irrigation of plants with wastewater induced high accumulation of phenols in all wheat cultivars. Regardless of high accumulation of anthocyanin, phenols, and flavonoids, high accumulation of H2O2 occurred in the wastewater-irrigated plants, followed by lipid peroxidation and high MDA accumulation and reduction of protein contents. The ROS accumulation induces damages to the proteins, lipid membranes, and nucleic acids (Anjum et al. 2015). The proline content, which serves as both osmoprotection and antioxidant in response to environmental stress (Hajihashemi and Ehsanpour 2014), significantly increased in the wastewater-irrigated plants. Based on the photosynthetic and biochemical parameters, Chamran cultivar was the most sensitive cultivar to the toxicity of high TSS in the wastewater from the Razi petrochemical complex than other wheat cultivars.

In conclusion, the results from analysis of Fv/Fm correspond to those of other parameters such as PIABS, Chls contents, Ps, C, WUE, WSC, growth, H2O2, antioxidants (phenols, flavonoids, and anthocyanins), proteins and proline. This confirmed that measuring Fv/Fm was an excellent method to obtain the timely information necessary to prevent irreversible damages to crops from environmental stress.

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