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Growth and photochemical responses of three crop species treated with textile azo dyes

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Abstract: The present study was conducted to investigate the impact of textile dye on the early seedling stage of 3 crops: barley, maize, and wheat. Seeds were imbibed in different concentrations of textile dye for 12 h and were then grown in a controlled growth room for 8 days with a related dye solution. Some growth and polyphasic chlorophyll fluorescence parameters were measured and analysed to appraise the effect of textile dye on the 3 crops. Although different crop species showed differences in response to different concentrations, textile dye generally affected almost all radicle growth parameters more adversely than coleoptile growth parameters. While photosynthetic performance indexes of wheat increased significantly in all textile dye treatments, those same parameters in barley strongly decreased in all concentrations. While performance indexes of maize increased up to 250 ppm, indexes decreased with the highest dye concentration. Analysis of polyphasic chlorophyll fluorescence and growth parameters allowed for the separation of wheat, which exhibited a better performance than the other crops under textile dye treatment. The results point out that the impact of textile dye is dependent on its concentration and that it is crop-specific. In addition, textile dye may not be detrimental for these 3 crops.

Key words: Chlorophyll, fluorescence, crops, growth, JIP test, textile dye

Introduction

Azo dyes, or reactive dyestuffs, are widely used in the different textile industries all over the world. They are characterised by their typical –N=N-nature, and this is the most common chromophore of reactive dyes (Manu & Chaudhari, 2002). About 1000 mg/L of dye is present in a typical dye bath (Ince & Tezcanli, 1999). Coloured effluents that are discharged into receiver water decrease the light permeability of the aquatic environment and negatively affect photosynthetic activity. Furthermore, accumulation of dyestuffs in aquatic organisms creates the risk of toxicity and forms carcinogenic products (Levine, 1991). As a result, the process of removing dyes from the effluents of textile industries has become environmentally important. However, removal of dyestuffs is a considerably difficult process because of the complex chemical structures and synthetic origins of dyes (Sharma et al., 2007). In addition, different physical, chemical, and biological techniques have

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been applied to remove dyes from wastewater, and each has its own technical and economic limitations (Davies et al., 2005).

Dyes are the most difficult constituent of textile wastewater to treat (Şen & Demirer, 2003). Among the chemical classes of dyes, azo dyes, which are most widely used industrially (Fitzgerald & Bishop, 1995), are considered recalcitrant, non-biodegradable, and persistent (Mohan et al., 2005). Some researchers have reported that textile effluents have adverse effects on different plant species (Nirmala Rani & Janardhanan, 1988; Kaushik et al., 2005; Sharma et al., 2009), while others have proposed that there is no effect up to certain concentrations. Diluted textile effluent has even increased some growth parameters (Mohammad & Khan, 1985; Swaminathan & Vaidheeswarn, 1991; Pathak et al., 1999; Hati et al., 2007). Therefore, it is necessary to study the effect of industrial effluents, especially textile dyes, on the growth and development of crops. Plant growth parameters such as germination percentage, seedling survival, and seedling height have been taken as criteria to assess plant response to specific pollutants (Puvaneswari et al., 2006).

Although photosystem II (PSII) is reported to play an important role in the photosynthetic response of higher plants to environmental stress (Baker, 1991), little is known about the effect of textile dye on the PSII photochemistry of plants. It has been suggested that the kinetics of the chlorophyll *a* fluorescence transient is sensitive to stress (Bussotti et al., 2007; van Heerden et al., 2007). Illuminating dark-adapted leaves shows characteristic variable fluorescence kinetics known as the Kautsky effect (Kautsky & Hirsch, 1931). The variable fluorescence kinetics exhibit a sequence of steps named O (at 20 μs), J (at 2 ms), I (at 30 ms), and P (at about 300 ms) (Strasser & Govindjee, 1992; Strasser et al., 1995; Strasser et al., 2000; Tsimilli-Michael & Strasser, 2008). An analysis of the fast OJIP fluorescence kinetics has been developed, and it is called JIP test (Strasser & Strasser, 1995; Strasser et al., 2000). The JIP test describes the different steps and phases of the transient reflecting the redox states of PSII concomitantly with the efficiencies and fluxes of electron transfer (ET) in the intersystem chain between PSII and PSI and to the end electron acceptors (RE) up to the reduction of end at the PSI acceptor side (Tsimilli-Michael & Strasser, 2008). A number of parameters are derived from the fluorescence transients to quantify the flow of energy through the reaction centre (RC) of PSII (Strasser et al., 2004; Strasser et al., 2010).

The effects of industrial activity, including the textile industry, on the environment and the development of alternative technologies that can minimise the deleterious effects of industrial pollutants have been extensively documented (Veglio & Beolchini, 1997; Manu & Chaudhari, 2002; Melo et al., 2006; Körbahti & Tanyolac, 2008). However, there have been few studies related to the effect on plant growth and development (Moawad et al., 2003; Davies et al., 2005; Davies et al., 2009; Kaushik et al., 2005). Therefore, the aim of the present work was to estimate the impact of textile dye (Levafix CA blue, a type of azo dye) in vivo on 3 different crops (early seedling stage barley, maize, and wheat) by some growth parameters and the polyphasic chlorophyll *a* fluorescence OJIP transient, which is widely used for monitoring and screening for stress tolerance of different species and genotypes.

Materials and methods

Plant materials, growth, and treatment conditions

Three different crop species (*Hordeum vulgare* L., Bülbül-89; *Zea mays* L., Vero; and *Triticum aestivum* L., Bezostaya-1) were used. The seeds of barley and wheat cultivars were obtained from the Republic of Turkey Food, Agriculture, and Livestock Ministry; maize seeds were supplied by Pan Seed Improvement and Production Company. Seeds were imbibed in the different concentrations [0 (distilled water, control), 100, 250, and 1000 ppm)] of textile dye (Levafix CA Blue) for 12 h and then were transferred onto filter papers wetted with related dye solutions in germination cups. The experiment was conducted in a controlled growth room at 25 ± 1 °C, with 16 h day length, 50 ± 5% relative humidity and at 200 μmol m^{-2} s^{-1} light intensity for 8 days. Seedlings were irrigated regularly every 2 days with a related dye solution during the experiment.

Growth parameters

Coleoptiles and radicle lengths were measured at days 4 and 8. From these values, relative growth rates (RGR) of the coleoptile and radicle were calculated according to Costa França et al. (2000) with little modification, as follows:

$$RGR = (\ln W_2 - \ln W_1)/(t_2 - t_1),$$

where W is the length of the coleoptile or radicle, t is the time, and 1 and 2 correspond to data collections, respectively.

The dry weights of the coleoptile and radicle were measured by drying the fresh parts in an oven at 80 °C for 48 h.

Polyphasic chlorophyll a fluorescence measurements

The polyphasic OJIP fluorescence transient was performed with a Handy PEA (Hansatech Instruments Ltd., King's Lynn, Norfolk, UK) fluorimeter. Samples dark-adapted for at least 30 min were illuminated with continuous light (650 nm peak wavelength, 3000 μmol m^{-2} s^{-1} maximum light intensity, for 1 s) provided by 3 LEDs, and the Chl a fluorescence signals were recorded according to Strasser and Strasser (1995). The OJIP transient was analysed by JIP test based on the energy flux theory for biomembranes in a photosynthetic sample, which leads to the equations and calculations for the efficiencies of the whole energy cascade from absorption to the reduction of end electron acceptors at the PSI acceptor side and the performance indexes (Strasser & Tsimilli-Michael, 2001; Strasser et al., 2004; Strasser et al., 2010). The fluorescence parameters (Table) were calculated using the Biolyzer software package.

Table. Summary of the JIP test formulae using data extracted from the polyphasic chlorophyll *a* fluorescence (OJIP) transient in this study (Tsimilli-Michael & Strasser, 2008; Strasser et al., 2010).

Extracted and technical fluorescence parameters	Description
F_O	Initial fluorescence intensity, when all PSII RCs are open
F_J	Fluorescence intensity at the J-step (at 2 ms)
F_I	Fluorescence intensity at the I-step (at 30 ms)
F_M	Maximal fluorescence intensity, when all PSII RCs are closed
V_J	$(F_{2ms} - F_O)/(F_M - F_O)$, relative variable fluorescence at the J-step (2 ms)
V_I	$(F_{30ms} - F_O)/(F_M - F_O)$, relative variable fluorescence at the I-step (30 ms)
Quantum yields and efficiencies/probabilities	
$\varphi_{PO} = TR_O/ABS$	$(1 - F_O)/F_M$ or Fv/F_M, maximum quantum yield of primary photochemistry at t = 0
$\varphi_{EO} = ET_O/ABS$	$(1 - F_O/F_M) \times \Psi o$, quantum yield for electron transport at t = 0
$\Psi o = ET_O/TR_O$	$1 - V_J$, probability (at time 0) that a trapped exciton moves an electron into the electron transport chain beyond Q_A^-
$\delta Ro = RE_O/ET_O$	$(1 - V_I)/(1 - V_J)$, the efficiency with which an electron can move from the reduced intersystem electron acceptors to the PSI end electron acceptors
$\varphi_{RO} = RE_O/ABS$	$\varphi_{PO} \times \Psi_O \times \delta R_O$, the quantum yield of electron transport from Q_A^- to the PSI end electron acceptors
RC/ABS	$\varphi_{PO} \times (V_J/M_O)$, the concentration of reaction centres per chlorophyll
Performance indexes	
PI_{ABS}	$(RC/ABS) \times [\varphi_{PO}/(1 - \varphi_{PO})] \times [\Psi_O/(1 - \Psi_O)]$, performance index (potential) for energy conservation from photons absorbed by PSII to the reduction of intersystem electron acceptors
PI_{total}	$PI_{abs} \times [\delta R_O/(1 - \delta R_O)]$, total PI, measuring the performance up to the PSI end electron acceptors

Statistical analysis

The experiments were arranged in a completely randomised design with 3 replicates. Differences among treatments and crops were tested using SPSS. Statistical variance analysis of the data was performed using ANOVA and was compared with least significant differences (LSD) at the 5% level.

Results

Growth parameters

The length of coleoptiles and radicle showed differences among crops and textile dye (Levafix CA Blue) treatments (Figure 1). It was found that while the length of the coleoptile in maize and wheat decreased significantly with dye treatments above 100 ppm, the highest dye concentration (1000 ppm) did not change the length in barley compared to their controls. On the other hand, the radicle lengths of crops increased at 100 ppm dye concentration; they decreased significantly with increasing dye concentrations in maize and wheat (Figure 1).

The relative growth rate (RGR) of coleoptiles in barley was unaffected in all dye treatments, whereas the RGR increased significantly in wheat and decreased in maize for all dye concentrations (Figure 2). Although there were some differences between crop responses to treatments, the highest concentration significantly decreased the RGR of radicle in all examined crops (Figure 2).

All dye concentrations did not significantly affect dry weights of the coleoptiles and radicle in wheat and barley (Figures 3). On the other hand, dry weights of coleoptiles and radicle of maize decreased after 100 ppm; in maize, the response was more pronounced in radicle than in coleoptiles.

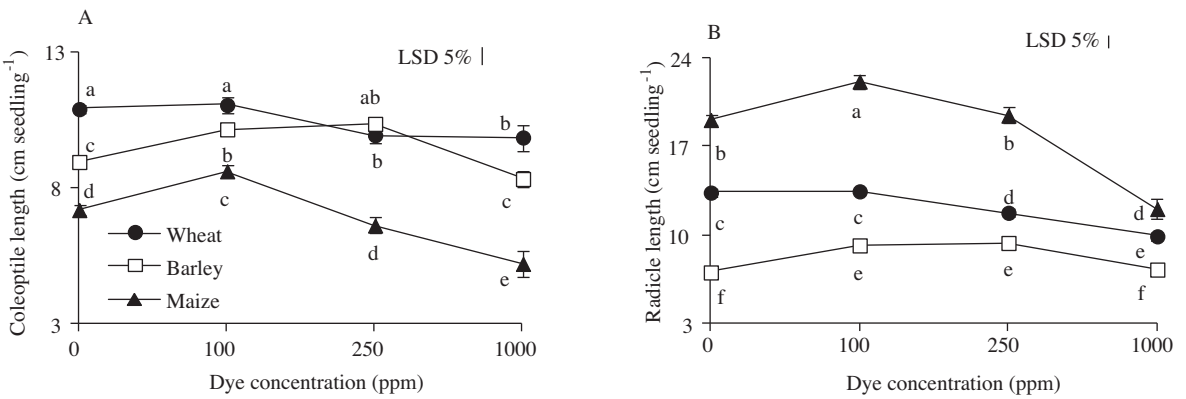

Figure 1. Effect of dye treatment on coleoptile (A) and radicle (B) lengths of 3 crops. Data are the mean of 3 replicates ± SE (n = 12).

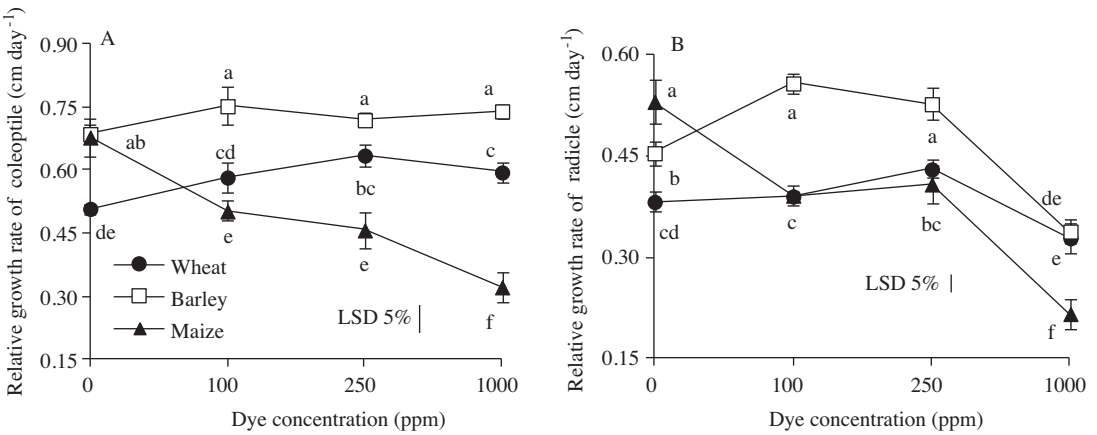

Figure 2. Effect of textile dye on relative growth rates of coleoptile (A) and radicle (B) in 3 crops. Data are the mean of 3 replicates ± SE (n = 12).

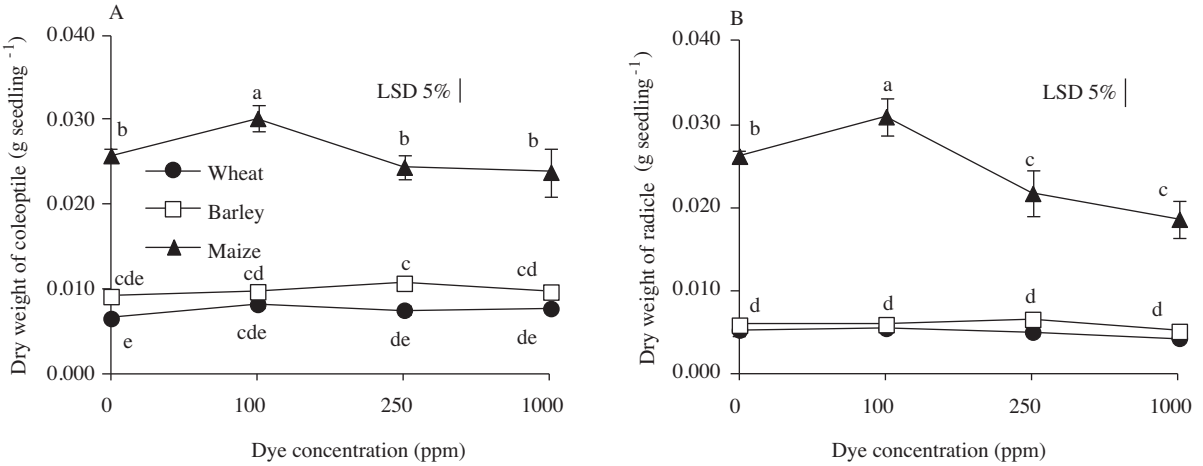

Figure 3. Changes in dry weights of coleoptile (A) and radicle (B) in 3 crops treated with different textile dye concentrations. Data are the mean of 3 replicates ± SE (n = 6).

Polyphasic chlorophyll a fluorescence measurements

Figure 4 displays a representative behaviour pattern of PSII with 8 biophysical parameters, which are the efficiencies (or yields or probabilities), the reaction centre density in the chlorophyll bed (RC/ABS), and the performance indexes (PI_{abs} and PI_{total}). The parameters obtained from dye treated seedlings are plotted relative to the parameters of the control plants. All dye treatments caused significantly lower values of PI_{abs}, PI_{total}, ET_O/TR_O, ET_O/ABS, and RE_O/ABS in barley (Figure 4A). The maximum quantum efficiency of PSII photochemistry (TR_O/ABS, F_V/F_M) parameter was almost unaffected in all dye treatments and in all crops with the exception of the 1000 ppm treatment in maize (Figure 4B). Only the highest dye treatment (1000 ppm) significantly decreased PI_{abs}, PI_{total}, RE_O/ABS, and RE_O/ET_O in maize (Figure 4B). Almost all dye concentrations caused an increase in PI_{abs}, PI_{total}, RC/ABS, RE_O/ABS, and RE_O/ET_O in wheat (Figure 4C).

Discussion

Plant growth and development are adversely affected by many environmental stress factors (Aksoy & Dane, 2011; Atik et al., 2011; Makbul et al., 2011) including textile dyes (Kaushik et al., 2005; Patil et al., 2009). Azo textile dyes are usually highly structured organic substances that are rather difficult to degrade. Although it is known that azo dyes in textile effluents affect many physiological and metabolic processes within aquatic organisms, few studies have reported on its effect on higher plants. In the present study, 3 crops-barley, maize, and wheat-were subjected to textile dye (Levafix CA Blue) throughout the experiment to reveal the responses of these plants. In general, textile dye enhanced or had no effect on growth and photochemical parameters depending on the tissue and crop.

Azo dyes did not significantly affect length and dry weights of the coleoptile in almost all crops, whereas radicle values showed differences among crops (Figures 1 and 3). The effect of textile dye was more pronounced in the relative growth rate of the radicle than in other parameters; all concentrations negatively affected all crops studied (Figure 2). The present findings show similarities with those reported by Bhati and Singh (2003) and Swaminathan and Vaidheeswarn (1991), who stated that irrigation with a mix of municipal and textile effluents improved the growth, biomass, and nutritional status of *Eucalyptus camaldulensis* seedlings, and 50% diluted textile effluent increased the seed germination, total sugars, starch, reducing sugars, and chlorophyll compared to distilled water (control) in *Arachis hypogea* seedlings, respectively. Moreover, Moawad et al. (2003) reported that wheat was resistant to the toxic effects of several textile dyes compared to other crops such as tomato, lettuce, and clover. Meanwhile, Kaushik et al. (2005) proposed that the effect of textile effluent is cultivar specific, and care should be taken before using textile effluent for irrigation purposes. A possible

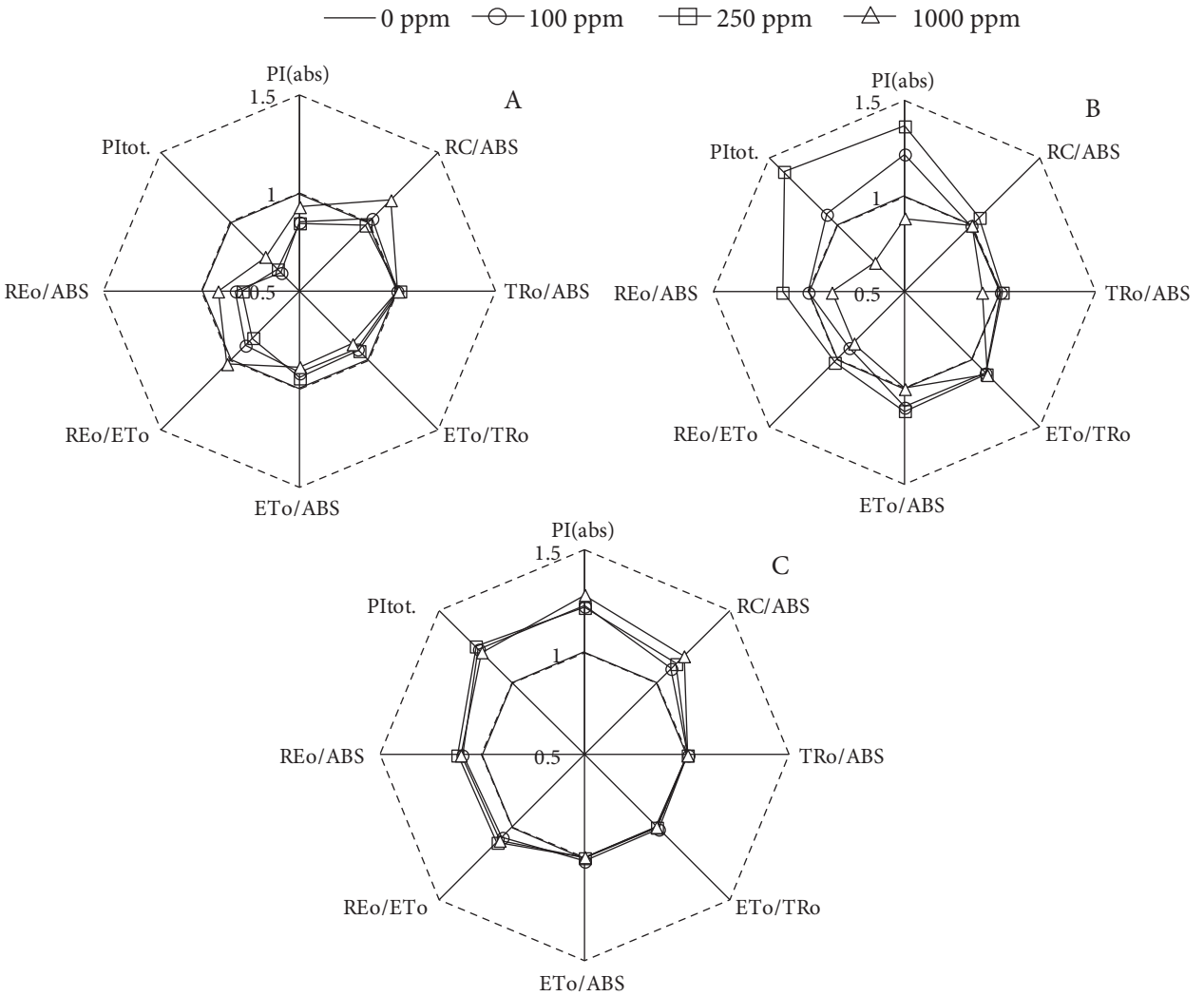

Figure 4. A radar-plot presentation of selected JIP test parameters quantifying the photosynthetic efficiencies of dark-adapted barley (A), maize (B), and wheat (C) leaves treated with textile dyes (Levafix CA blue). Parameters were plotted relative to their respective controls. Data are the mean of 3 replicates ± SE (n = 6).

reason for azo dyes' stimulating growth under low dye concentration in the early seedling stage of these crops is that some biodegradation (also called biotransformation) enzymes, especially azoreductase, a key enzyme used to degrade azo bonds in textile dye (Dhanve et al., 2008; Patil et al., 2009), may be activated by different dye concentrations. Induction of the activity of biotransformation enzymes such as lignin peroxidase, laccase, tyrosinase, Mn peroxidase, DCIP reductase, and/or azoreductase plays a crucial role in dye metabolism (Patil et al., 2009).

The impact of textile dye (Levafix CA Blue) on chlorophyll *a* fluorescence transients was analysed by JIP test to quantify the PSII behaviour/activity of 3 crops (Figure 4). PI_{abs}, as a measure of plant performance, revealed differences in response to dye treatments in the studied crops. PI_{abs} is created from 3 independent expressions: the concentration of reaction centres per chlorophyll (RC/ABS), a performance expression related to primary photochemistry ($TR_O/ABS = F_V/F_M$), and a performance expression related to electron transport (ET_O/TR_O) (Strasser & Tsimilli-Michael,

2001; Oukarroum et al., 2007). The maximum quantum efficiency of PSII photochemistry was almost unaffected in all treatments and crops. It is suggested that TR_O/ABS is not sensitive to the influence of textile dye treatment, as reported previously for other treatments (Oukarroum et al., 2007; Tsimilli-Michael & Strasser, 2008). In contrast, ET_O/TR_O (showing that a trapped exciton moves an electron into the electron transport chain beyond Q_A^-) displayed variability among crops; it did not change in wheat, increased in maize, and decreased in barley. Evidently the quantum yield for electron transport $ET_O/ABS = TR_O/ABS \times ET_O/TR_O$ showed a response similar to ET_O/TR_O. It has been claimed that lower ET_O/TR_O reflects suppression of electron transport enzyme reactions in functional PSII (Mehta et al., 2010; Mohapatra et al., 2010). RC/ABS, Q_A^- reducing reaction centres per PSII antenna chlorophyll (reciprocal of ABS/RC, absorption flux per RC, or measure for an average antenna size), did not significantly change in maize or barley, except for high dye concentrations; it increased in wheat under all dye treatments (Figure 4). Increase in RC/ABS value might reveal an enhancement of Q_A^- reoxidation capacity in agreement with inhibited electron transport at the acceptor side of PSII in wheat. The efficiency with which an electron can move from the reduced intersystem electron acceptors to the PSI end electron acceptors (RE_O/ET_O) decreased in barley and maize, while it increased in wheat under almost all dye treatments (Figure 4). The quantum yield of electron transport from water to the PSI end electron acceptors ($RE_O/ABS = TR_O/ABS \times ET_O/TR_O \times RE_O/ET_O$) showed responses similar to RE_O/ET_O, except for 250 ppm in maize.

PI_{total}, measuring performance up to the PSI end electron acceptors, is the combination of all parameters mentioned above. Therefore, changes in all these OJIP parameters are reflected in the expression PI_{total}. Textile dye treatment greatly enhanced the photosynthetic performance indexes (PI_{abs} and PI_{total}) in wheat compared to other crops (Figure 4).

In conclusion, performance indexes (PI_{abs}, PI_{total}) are very sensitive parameters used to screen crop response to textile dye treatments, as in many other environmental situations. Growth and polyphasic chlorophyll fluorescence parameters have showed that the effects of industrial effluents such as textile dyes may vary from one plant species to another. Levafix CA blue concentrations used in the present experiment have no detrimental effect on growth of the 3 crops investigated in the early seedling stage, although there were different responses. Generally, while low concentrations promote plant growth, more elevated amounts have negative effects. It can be speculated that the use of a low concentration of effluent may serve as an additional source of nutrients beneficial for the fertility and productivity of agricultural soils. It should be noted that only chemically pure textile dye was used in this study. Land application of low concentrations of textile effluent containing azo dyes is a common practice that can provide a cost effective utilisation strategy for recycling an essential plant nutrient (nitrogen). Analysis and evaluation of PI and growth parameter values against textile dye treatment allowed for classification of the crops as tolerant (wheat), followed by barley and maize. Additionally, textile azo dye (Levafix CA Blue) may not be toxic for the 3 crops investigated up to 250 ppm. The present study provides novel information on the effects of textile dye in different concentrations (covering the realistic range of dye concentrations found in effluents) on growth as well as photosynthesis in some crop plants. Hence, further detailed studies should be done to elucidate the effects of industrial effluents on whole plant growth and development as a function of effluent mixtures as well as dye concentrations in respect to different crops.

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