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POLSKA AKADEMIA NAUK

ACTA BIOLOGICA CRACOVIENSIA Series Botanica 60/1: 65–71, 2018
DOI: 10.24425/118048

PAN POLSKA AKADEMIA NAUK ODDZIAŁ W KRAKOWIE

PHOTOSYNTHETIC RESPONSES OF *CHLORELLA VULGARIS* L. TO SHORT-TERM UV-B RADIATION EXPOSURE

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Received April 12, 2018; revision accepted June 14, 2018

Exposure of green algae *Chlorella vulgaris* to short-term UV-B radiation (280 nm – 315 nm) induced several changes in the function of photosystem II (PS II) studied by means of chlorophyll fluorescence (FL) and oxygen evolving. The intensity of photosynthetic oxygen evolving intensity of algae suspension decreased in a similar way to the FL parameter values in proportion to the applied dose of UV-B radiation (0.0, 3.2, 6.4, 12.8 kJ·m^{-2}). The correlation between photosynthetic oxygen evolving intensity and F_V/F_O ratio was better than that between photosynthetic oxygen evolving intensity and F_V/F_M. The vitality index (Rfd) in the UV-B irradiated algae strongly decreased, compared to the control, which indicates inhibition of potential CO_2 fixation and cooperation between light and dark reactions of photosynthesis. It may indicate damage of Rubisco.

Keywords: chlorophyll fluorescence, microalgae, oxygen evolving, photosystem II, phytoplankton, ultraviolet radiation

Abbreviations: Chl – chlorophyll; ETR – photosynthetic electron transport rate; FL – Chl fluorescence; F_O – initial Chl fluorescence in the dark-adapted state; F_M – maximal Chl fluorescence at a saturating radiation pulse in the dark-adapted state; F_S – steady state Chl fluorescence; $F_V = F_M - F_O$ – variable fluorescence in the dark-adapted state, F_V/F_M – maximal quantum yield of PSII; F_V/F_O – maximal quantum yield of water photolysis system of the donor side of PSII; PPFD – photosynthetic photon flux density; PS II – photosystem II; Rfd – vitality index, potential activity of photosynthesis process; Rubisco – ribulose-1,5-bisphosphate carboxylase; UV-B – ultraviolet radiation in the range of 280 nm – 315 nm.

INTRODUCTION

UV-B radiation is a ubiquitous component of solar radiation in the biosphere, but its level varies quite considerably, both spatially and temporally (Caldwell et al., 2007; Jansen et al., 2008). The UV-screening stratospheric ozone layer is relatively thin at low latitudes, which – in combination with a steep solar angle – results in relatively high UV-B levels in the tropics, compared to mid and high latitudes. UV-B measurements in Central Europe showed increase of 5% per decade (McKenzie et al., 2007; UNEP, 2016). Current global terrestrial UV-B radiation levels are somewhere between 0 and 12 kJ m^{-2} per day on the Earth's surface (Lidon et al., 2012). Phytoplankton is the most important biomass producer in aquatic ecosystems that produces more than half of biomass on our planet and incorporates at least the same amount of atmospheric carbon dioxide as terrestrial ecosystems (Gao et al., 2007; Häder et al., 2011). Exposure of phytoplankton cells to increased UV-B radiation may considerably inhibit the process of carbon dioxide assimilation and consequently decrease its total efficiency in the oceans as well as in the land water (Harrison and Smith, 2009; Häder et al., 2015). Studies of phytoplankton in water around Antarctica under the ozone hole conditions showed a reduction in primary productivity ranging from 4% to 13% as a result of elevated UV-B (UNEP, 2018). UV-B radiation damages phytoplankton by affecting growth, metabolism, orientation, reproduction, photosynthetic enzymes, photosynthetic pigments and photosynthesis process (Prasad et al., 1998; Garcia-Corral et al., 2015). There is a wide diversity of UV-B tolerances among phytoplankton species (Herrmann et al., 1996; Holzinger and Lütz,

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PL ISSN 0001-5296

2006). One of the most important phytoplankton species living both in seawater and freshwater is *Chlorella vulgaris* (Lewis and McCourt, 2004, Safi et al., 2014). Chlorophyll fluorescence is an increasingly popular method of assessing photosynthetic apparatus for stress factors of the environment (Kalaji et al., 2012; Porcar-Castell et al., 2014; Lazar, 2015). UV-B radiation is just one of these factors (Hollosy, 2002; Caldwell et al., 2007). The purpose of this paper was to investigate the response of this green algae suspensions to three values of UV-B radiation dose (0.0, 3.2, 6.4, 12.8 kJ m^{-2}) using chlorophyll fluorescence parameters and the corresponding photosynthetic oxygen decreasing concentration.

MATERIAL AND METHODS

Inoculum culture of *Chlorella vulgaris* obtained from the Department of Hydrobiology of Adam Mickiewicz University in Poznań was cultured photoautotrophically on growth medium L5m (Jankowski, 1964) at 22°C in white fluorescent light (PPFD 80 μmol $m^{-2}s^{-1}$), photoperiod 12 h/12 h (day/night) and continuously inflation of air. In the phase of logarithmic growth at chlorophyll content of 180 mg m^{-3} the suspension of *Chlorella vulgaris* it was used for measurements. A single sample was 100 cm^3 of suspension in a glass, and one series – six samples. All 24 samples were divided into four groups, one of which was the control, and three others were subjected to UV-B irradiation with the broadband lamp VL-115 M (emission spectrum presented by Skórska and Murkowski, 2012) for 20, 40 and 80 minutes respectively, and the equivalent values of UV-B doses were 3.2, 6.4 and 12.8 kJ m^{-2}. The measurements of UVB radiation were performed using an IL 1403 radiometer with a SEL 240-UVB1 calibrated detector (International Light Inc., USA). After irradiation the samples were incubated for 15 minutes in weak light of a tungsten lamp, PPFD 8 μmol $m^{-2}s^{-1}$. The intensity of oxygen evolving in each sample was measured using a LDO HQD40 Portable Luminescence Oxygen Meter (Hach LANGE, Dublin, Ireland). The measurements were carried out in a thermostatic (21±1.5)°C cylindrical cuvette with a magnetic stirrer. The intensity of photosynthetic flux density (PPFD) on the front wall of the cuvette was 1200 μmol $m^{-2}s^{-1}$, and on the back wall ca. 500 μmol $m^{-2}s^{-1}$. According to the described procedure, the samples, the control and UV-B irradiated ones, were prepared for FL measurements. Then all samples were infiltrated through a Whatman GF/A filter (12 mm diameter disks) and after 20 minutes of dark adaptation initial chlorophyll fluorescence (F_O) was recorded using a pulse-amplitude-modulated fluorescence-based method (PAM 200 fluorometer - Walz, Effeltrich, Germany), where variable fluorescence at 665 nm is monitored (Schreiber et al., 1994; Van Kooten, 1990). The maximum fluorescence (F_M) was performed after 0.8 s saturation pulse of PPFD 3200 μmol $m^{-2}s^{-1}$, then actinic light PPFD 120 μmol $m^{-2}s^{-1}$ was turned on. After 4 minutes of chlorophyll fluorescence recording (to the stationary level, F_S), the quenching coefficient, qP, and electron transport rate, ETR, were measured. The vitality index, Rfd, was calculated as a ratio of $(F_M - F_S)/F_S$, according to Lichtenthaler (2005). After the measurements, the chlorophyll was extracted from the biofilter with 90% acetone, and the absorbance of the clear extract was measured at 663.2 nm and 664.8 nm for total chlorophyll measurement in a spectrophotometer using the formula of Lichtenthaler (1987). All measurements were performed in 6 biological replications. The results are expressed as mean values ± standard deviations. The data were subjected to one way analysis of variance by ANOVA (Statistica 13 software). A post-hoc analysis allowed the separation of homogenous groups by means of Newman-Keuls test ($p < 0.05$), which are marked with the same letters. A regression line and a coefficient of determination, R^2, at significance level $a < 0.05$ were prepared using Excel software.

RESULTS

The applied UV-B radiation caused a decrease of the intensity of oxygen evolving in the algae suspension, from 3.83 mg dm^{-3} s^{-1} for the control (non irradiated) samples to 0.88 mg dm^{-3} s^{-1}, depending on the irradiation time corresponding to the applied dose (Fig. 1). Particularly at the medium dose of UV-B radiation (6.4 kJ m^{-2}) the concentration of diluted oxygen was reduced to 60% of the control value, and at the highest dose (12.8 kJ m^{-2}) – to 23% of the control value.

A similar pattern was observed in the case of chlorophyll fluorescence parameters (Fig. 2, Table 1). It should be noticed that UV-B radiation at a dose of 6.4 kJ m^{-2} moderately decreased F_V/F_M (by 29%) values, while F_V/F_O was reduced to 48%, in comparison with the control value (Fig. 2a). The observed decrease of both parameters was a result of increase of the initial fluorescence, F_O, more than decrease of maximal fluorescence, F_M. At the medium applied dose of UV-B radiation F_M was reduced by 11%, while F_O increased by 32%, compared to the control values. At the highest dose it was even more noticeable, because F_M was lower by 26% and F_O was higher by 58%, compared to the control values (Table 1). Electron transport

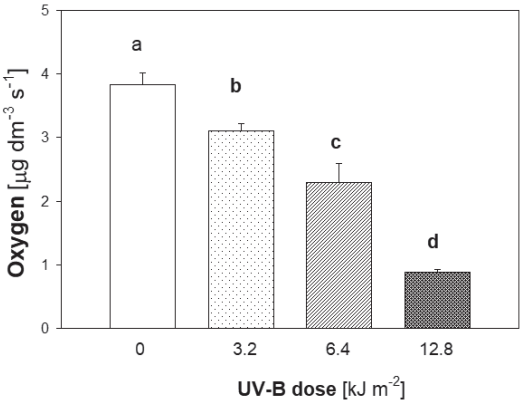

Fig. 1. Intensity of oxygen evolving in green algae *Chlorella vulgaris* suspension subjected to UV-B radiation at various doses; the columns marked with the same letters are not significantly different at $p \leq 0.05$ according to Newman-Keuls test, n = 6.

efficiency in the photosystems, ETR, at the dose of 6.4 kJ m^{-2} decreased by 55% compared to the control, and by as much as 80% at the highest applied dose of 12.8 kJ m^{-2} (Fig. 2c). The vitality index, Rfd, informing about the interaction of the light phase reactions with biochemical dark reactions of photosynthesis and considered also as an index of potential activity of all process of photosynthesis (Lichtenthaler et al., 2005), at the doses of 6.4 kJ m^{-2} and 12.8 kJ m^{-2} decreased by 60% and 95% respectively, in comparison to the control (Fig. 2d). A significant correlation between F_V/F_O parameter and the intensity of oxygen evolving in the investigated green algae suspension was observed (Fig. 3a). A similar correlation was found between F_V/F_M parameter and the intensity of oxygen evolving (Fig. 3b), but the determination coefficient was lower.

The quenching coefficient, qP, at the medium and highest doses of UV-B decreased by 25% and 50% respectively, compared to the control (Table 1). The chlorophyll content in the samples subjected to the highest dose (12.8 kJ m^{-2}) of UV-B irradiation decreased by 17%, compared to the control, while in the samples irradiated with smaller doses (3.2 and 6.4 kJ m^{-2}) the observed changes were not statistically significant (Table 1).

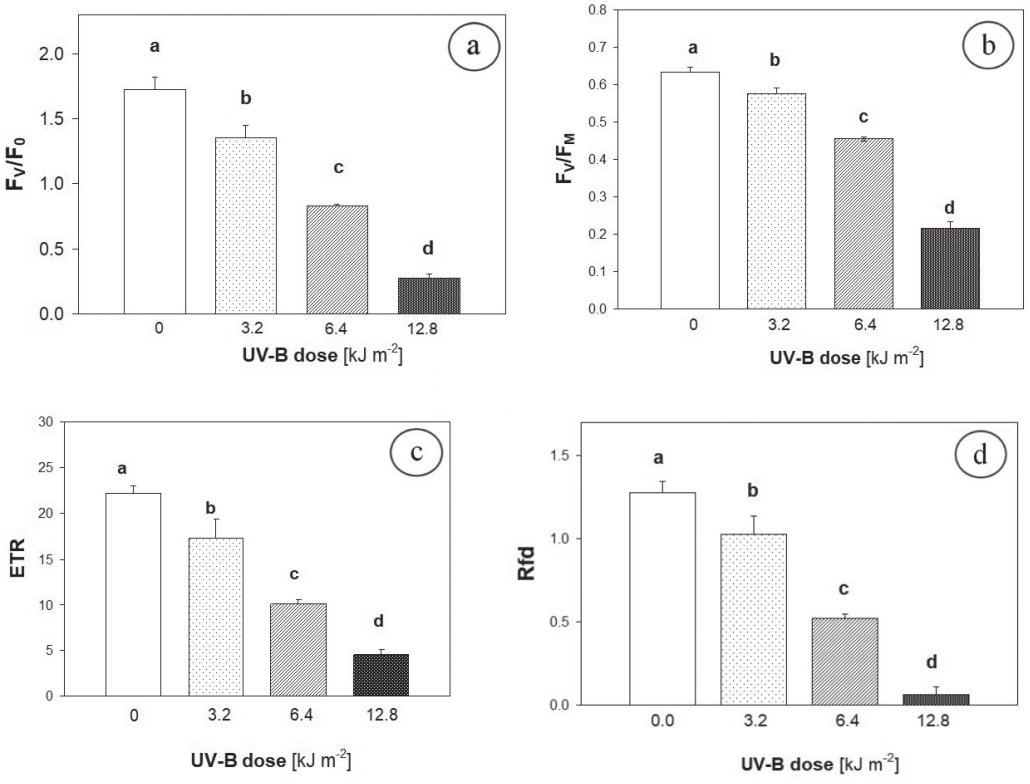

Fig. 2. *Chlorella vulgaris* suspension subjected to UV-B radiation at various doses. (**a**) Chlorophyll fluorescence F_V/F_O parameter. (**b**) Chlorophyll fluorescence F_V/F_M parameter. (**c**) Electron transport rate (ETR) in photosystem II. (**d**) Vitality index, Rfd. The columns marked with the same letters are not significantly different at $p \leq 0.05$ according to Newman-Keuls test, n = 6.

TABLE 1. The values of chlorophyll initial (F_O) and maximal fluorescence (F_M), quenching coefficient (qP), and chlorophyll content of *Chlorella vulgaris* suspension subjected to UV-B radiation at various doses. Values in the column marked with the same letters are not significantly different at $p \leq 0.05$ according to Newman-Keuls test, n = 6.

Parameter	UV-B dose [kJ m^{-2}]			
	0.0	3.2	6.4	12.8
F_O	224 ± 10 a	240 ± 15 b	295 ± 6 c	355 ± 16 d
F_M	611 ± 32 a	566 ± 43 b	542 ± 12 b	454 ± 12 c
qP	0.83 ± 0.03 a	0.80 ± 0.05 a	0.62 ± 0.02 b	0.41 ± 0.08 c
Chl [mg m^{-3}]	1.28 ± 0.15 a	1.25 ± 0.14 a	1.21 ± 0.14 a	1.05 ± 0.15 b

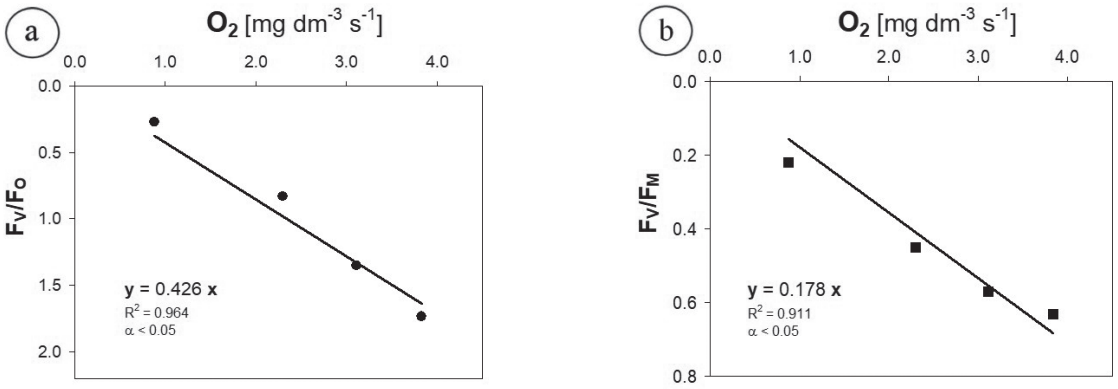

Fig. 3. *Chlorella vulgaris* suspension subjected to UV-B radiation at various doses. (**a**) Relationship between values of F_V/F_O parameter and intensity of oxygen evolving in *Chlorella* cells. (**b**) Relationship between values of F_V/F_M parameter and intensity of oxygen evolving in *Chlorella* cells. Each point is the mean of six values; R^2 denotes determination coefficient and a – significance level.

DISCUSSION

The results described above confirmed that UV-B radiation damages the oxygen evolving complex (OEC) on the PSII donor side (Hideg et al., 1993; Masi and Melis, 1997; Gao et al., 2007; Szilárd et al., 2007, Kantaria et al., 2014). Like other stress factors interfering with the flow of electrons from the manganese complex to the PSII reaction centre, UV-B radiation decreases F_M – the maximum chlorophyll fluorescence, caused by lower number of reduced primary acceptors Q_A (Govindjee, 1995; Maxwell and Johnson, 2000).

In our experiment on algae of *Chlorella vulgaris* under the influence of UV-B radiation in the doses used, we also observed a regular increase in the initial fluorescence (F_O) level due to the increase of losses when transferring excitation energy from energy antennas to the PSII reaction center (Baker and Rosenquist, 2004) and to the decrease of the number of reduced Q_B acceptors due to UV-B radiation (Van Rensen et al., 2007). Both the decrease in the F_M value and the increase in the F_O level result in a significant decrease in the value of the F_V/F_O parameter, and to a lesser extent also the F_V/F_M parameter defining the potential PSII efficiency (Govindjee, 1995; Maxwell and Johnson, 2000; Lichtenthaler et al., 2005). In our experiment at UV-B dose of 6.4 kJ m^{-2}, the F_V/F_M value decreased by 29%, and the F_V/F_O value by as much as 52%, compared to the control. The values of the F_V/F_M parameter are frequently determined in articles on the effects of UV-B radiation on PSII, although this parameter is less sensitive than the F_V/F_O quotient (Lichtenthaler et al., 2005). Unfortunately, the F_V/F_O parameter is rarely presented in articles, perhaps because it is not displayed on the screens of popular chlorophyll fluorescence measurement kits (Kalaji et al., 2017).

The reduction of F_V/F_M in various species of algae exposed to short-term UV-B radiation was demonstrated by Kristoffersen et al. (2016) on

Tetraselmis, Herrmann et al. (1996) on *Dunaliella salina*, Hughes (2006) on *Stichococcus bacillaris*. Similarly, Heraud and Beardall (2000) observed a reduction of F_V/F_M to 50% of the control in *Dunaliella tertiolecta* algae subjected to 40 min UV-B irradiation of 6.7 kJ m^{-2}.

Apostolova et al. (2014) showed that UV-B at 7.5 W m^{-2} within 30 minutes (equivalent to 13.5 kJ m^{-2}) resulted in a reduction of photosynthetic oxygen emission to 60%, and F_V/F_M to 76% of the control in mesophilic *Chlorella*. These values coincide with the results obtained in our research, and they are also in accordance with the ones described by El Khachia et al. (2008), who compared their results using a similar measuring instrument with the parameters of fluorescence induction of freshwater algae of *Chlorella emersonii*. The correlation between the values of F_V/F_O quotient and oxygen emission intensity (Fig. 3a), which we have demonstrated, allows us to use both measuring methods to assess the harmful effects of UV-B radiation on photosynthetic reactions in algae cells.

Using the direct fluorescence measurement method (after sample adaptation to the dark), the values of F_V, F_M and F_V/F_M, F_V/F_O and some other parameters were obtained after just 2 seconds of measurement, and the Rfd value after extending the measurement time to about 4 minutes, when fluorescence reached the stationary level F_S. After this time, the equilibrium in the production of ATP and NADPH reducing factor in the light phase was achieved in chloroplasts, with the demand of these important photoproducts in dark reactions (Murchi and Lawson, 2013; Lazar, 2015). In order to simultaneously measure ETR and qP parameters, chlorophyll fluorescence measurements should be performed using a PAM fluorimeter (Schreiber, 1994; Murkowski, 2002; Kalaji et al., 2017).

The ETR parameter determines the speed of electron flow through photosystems, which is often reduced due to stress factors, one of them being UV radiation (Murkowski and Skórska, 2010; Murchi and Lawson, 2013). In our experiment, the ETR values were significantly reduced at all UV-B doses used, like it was shown for plants (Skórska, 2011). On the other hand, the unchanged ETR level of freshwater green algae *Zygnema* subjected to UV-B radiation at the dose of 36.3 kJ m^{-2} was attributed to high tolerance of the photosynthetic apparatus of this species (Holzinger et al., 2008).

The photochemical quenching coefficient, qP, determines the proportion of light energy used in the photochemical reaction by PSII to the total light energy absorbed by this photosystem (Schreiber et al., 1994). Reduction in the value of qP indicates the increased use of excitation energy in photochemical reactions (Maxwell and Johnson, 2000; Lazar, 2015).

The Rfd parameter that specifies the potential ability of the photosynthetic apparatus to convert light energy into chemical energy in photosynthesis has also been called the index of vitality by Lichtenthaler et al. (1986). By measuring the relative reduction of the FL value from the maximum to the F_S level, the efficiency of cooperation of light reactions of photosynthesis with dark enzymatic reactions is determined. Under the influence of various stress factors, the Rfd value decreases (Lichtenthaler et al., 1986; Murkowski and Skórska, 1997; Lazar, 2015). The Rfd values in our experiment, under the influence of the applied doses, decreased significantly, and at the highest dose of 12.8 kJ m^{-2} the value of the vitality index dropped below 5% of the control value, which can be considered almost complete blocking of photosynthetic production. We believe that the measurement of the Rfd value can be used for the integral evaluation of the entire photosynthesis process, especially in the assessment of the effects of abiotic stress such as photoinhibition, UV-B, drought, frost, heat, heavy metals and others (Skórska, 2000; Murkowski, 2002; Murkowski and Skórska, 2010).

The slight reduction in the chlorophyll content, even under the influence of the highest UV-B dose, is consistent with the results of Thomas et al. (2009) regarding freshwater algae.

Our results indicate high sensitivity of *Chlorella* algae to the applied doses of UV-B radiation. This is consistent with the work of Prasad et al. (1998) who showed that UV-B dose of 9.0 kJ m^{-2} (2.5 W m^{-2} over 60 min) resulted in the loss of vital functions of *Chlorella vulgaris* algae.

The results of our experiment confirm the findings of other researchers that UV-B radiation causes damage to the photosynthetic apparatus of *Chlorella vulgaris* in the oxygen emission complex, D2 and D1 proteins associated with primary Q_A and Q_B acceptors and other PSII components (Szilárd et al., 2007; Lidon et al., 2012; Van Rensen et al., 2007; Dobrikova et al., 2013). It should be noted that a significant decrease in the Rfd parameter under the influence of UV-B may indicate that this short-wave radiation has a particularly strong effect on the photosynthetic reduction of carbon dioxide in chloroplasts. This can be explained by the direct effect of UV-B on reducing the activity of Rubisco, a key enzyme that controls the process of CO_2 assimilation (Takeuchi et al., 2002; Lidon et al., 2012; Kataria et al., 2014; Rastogi et al., 2014; Dotto and Casati, 2017).

CONCLUSIONS

UV-B radiation reduced photosynthetic oxygen evolving intensity of *Chlorella vulgaris* suspension inversely proportionally to the dose of radiation.

There is a high correlation between F_V/F_O parameter and the intensity of oxygen evolving in the investigated green algae cells. The vitality index – Rfd of the irradiated UV-B algae significantly decreased compared to the control, which indicates a strong inhibition of CO_2 assimilation process and cooperation between light and dark reactions of photosynthesis. These properties of Rfd should encourage researchers to use its measurements more often to assess the integral UV-B influence on the reduction of the efficiency of photosynthesis reactions, and the potential productivity of whole phytoplankton assemblies.

AUTHORS' CONTRIBUTIONS

The authors of this article have contributed equally, and declare that there are no conflicts of interest.

ACKNOWLEDGEMENTS

The authors are indebted to the members of the Department of Hydrobiology of Adam Mickiewicz University in Poznań for kindly providing inoculum culture of *Chlorella vulgaris* for the experiment. The authors are also grateful to the anonymous reviewers for useful and valuable advice in the received reviews.

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