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Effects of humic acid on photosynthetic efficiency of rapeseed plants growing under different watering conditions

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Abstract

Water is a limited resource and is likely to become even more restricted with climate change. The aim of this study was to evaluate the effect of humic acid (HA) applications on photosynthesis efficiency of rapeseed plants under different watering conditions. Water stress strongly increased electron transport flux, probability that trapped excitation can move an electron into the electron transport chain beyond Q_A, and quantum yield of reduction of end electron acceptors at the PSI acceptor side. Application of HA decreased the values of these parameters to be similar to those of non-stress conditions. We found that, the application of HA improved plants net photosynthesis under water stress *via* increasing the rate of gas exchange and electron transport flux in plants.

Additional key words: chlorophyll *a* fluorescence; photosynthesis; water stress.

Introduction

Productivity of plants depends on environmental conditions. Water stress is considered one of the most important environmental stresses limiting plant growth and crop productivity. Plants develop various strategies to eliminate the negative impacts of drought (Allakhverdiev and Murata 2004, Jithesh *et al.* 2006, Kalaji and Łoboda 2009), such as escape, dehydration avoidance, and development of physiological tolerance to drought (Berger *et al.* 2010). Water removal induces cell shrinkage, increasing the cytoplasm viscosity and cell wall folding, which results in membrane destruction and protein denaturation (Flexas *et al.* 2000). Drought resistance is typically based on an increased activity of antioxidant enzymes (Lu *et al.* 2007) and changes in plant morphology, anatomy, physiology, and biophysics (Jajoo and Kawamori 2006, Kreslavski *et al.* 2009). Understanding

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Abbreviations: ABS/RC – absorption flux per reaction center; CCI – chlorophyll content index; ChlF – chlorophyll *a* fluorescence; DI_0/RC – dissipation energy flux per RC; ETC – electron transfer chain; ET_0/RC – maximum electron transport flux per RC; $F_0 \sim F_{20\mu s}$ – minimum fluorescence intensity at 20 µs; F_m – maximum fluorescence intensity at P-step (~300 ms); F_v – variable fluorescence, F_v/F_0 – a value that is proportional to the activity of the water-splitting complex on the donor side of PSII, F_v/F_m – maximum quantum yield of PSII photochemistry, g_s – stomatal conductance; HA – humic acid; K_N – the nonphotochemical de-excitation rate constant in the excited antennae for non-photochemistry; K_P – the photochemical de-excitation rate constant in the excited antennae of energy fluxes for photochemistry; MWS – moderate water stress; OEC – oxygen-evolving complex; OJIP – transient-fluorescence induction defined by the names of its intermediate steps; PI_{total} – performance index; P_N – net photosynthetic rate; S_m – representing energy necessary for the closure of all reaction centers; SWS – severe water stress; TR_0/RC – trapped energy flux per RC; V_J – relative variable florescence at J-step (2 ms); V_I – relative variable florescence at I-step (30 ms); WWC – well watering condition; ψ_{E0} – probability that trapped excitation moves an electron into the electron transport chain beyond Q_A^-; φ_{R0} – quantum yield of reduction of end electron acceptors at the PSI acceptor side; δ_{R0} – probability with which an electron from the intersystem electron carriers move to reduce end electron acceptors at the PSI acceptor side.

the complex physiological, biochemical, and biophysical basis of stress tolerance in plants is an important way to resolve agricultural problems and increasing crop yield (Kreslavski *et al*. 2008).

Water stress may result in photoinhibition, including photodamage to the photosynthetic apparatus causing an irreversible inactivation of PSII (Singh and Reddy 2011). Plants convert light energy into ATP and NADPH through the photosynthetic electron transport chain on the thylakoid membranes in chloroplast. This energy can be used for the fixation of CO_2 in the Calvin cycle. However, variable environmental conditions such as water stress inevitably cause imbalances in the supply and demand of energy at different levels. The excess of energy can result in damage to the molecular structures in chloroplast, as in photoinhibition (Allakhverdiev *et al*. 2008, Vass 2012). The main direct effect of drought is associated with a decrease in the CO_2 supply for carboxylation caused by stomatal and mesophyll limitation (Lal *et al*. 1996; Flexas *et al*. 2012) or with direct negative nonstomatal effects on the metabolic pathways (Lawlor and Cornic 2002). It has been shown that the drought stress response of plants is closely related to changes in photosynthetic activity (Loreto *et al*. 2004), which is monitored by the JIP test. JIP-test equations are based on the theory of energy fluxes in biomembranes (Strasser 1978).

Photosynthesis is considered one of the most important metabolic processes in plants and its performance is greatly affected by stress conditions (Baker 2004). Photosynthetically active radiation (PAR) energy absorption by photosynthetic pigment molecules occurs in the antenna complexes located in the thylakoid membranes (Kalaji *et al*. 2012). The absorbed energy is then transferred as excitation energy to the PSI and PSII reaction center, where it is used to initiate photochemical reactions. A part of this energy is lost as heat and chlorophyll fluorescence (ChlF). The ChlF process can provide information on the functioning and structure of the photosynthetic apparatus (Kalaji 2007, Goltsev *et al*. 2009, Kalaji *et al*. 2016). All steps in the ChlF rise can be clearly revealed only when logarithmic presentation of time axis is used. The J and I steps usually appear at 2–3 ms and 30–50 ms, respectively. The O step stands for F_0 ($\sim F_{20\mu s}$) and the P-step represents F_m usually reached at ~200–500 ms (Lazár 2006). As the initial O–J transient reflects primary photochemical reactions, it was called the photochemical phase of the ChlF rise (Strasser *et al*. 1995). The main feature of this phase is that the initial slope and relative height of the phase strongly depends on the intensity of the exciting light (Strasser *et al*. 1995; Tomek *et al*. 2001). In contrast, subsequent J–I–P transient cannot be speeded up by further increase in the intensity of exciting light (Strasser *et al*. 1995) and it was called the thermal phase of the ChlF rise (Neubauer and Schreiber 1987). ChlF measurements allow evaluating the physiological status of plants based on the detection of changes in some PSII components, electron transport chain components, and light-dependent photochemical reactions. It is a very exact tool that senses changes in the general bioenergetic status of the photosynthetic apparatus of plants (Borawska-Jarmułowicz *et al*. 2014). Moreover, methods based on records of ChlF are simple, reliable, noninvasive, and powerful tools for assessing photosynthetic electron transport (Zivcak *et al*. 2013) and related photosynthetic processes and allow detection of stress in plants (Baker 2004, Borawska-Jarmułowicz *et al*. 2014).

Rapeseed (*Brassica napus*) is an important agricultural crop grown primarily for its edible oil. The meal, which remains after oil extraction, is valuable as a source of protein for the livestock feed industry. In Iran, the production of the rapeseed plants is limited by water shortage. A new plan for ameliorating water stress is to overcome the irregularities in plant physiological and biochemical mechanisms by some of the natural organic materials, such as humic acid (Lotfi *et al*. 2015a,b).

Plant growth-promoting rhizobacteria (PGPR) are the soil bacteria which are directly or indirectly involved in promoting plant growth and development *via* production and secretion of various regulatory chemicals in the vicinity of rhizosphere (Ahemad and Kibret 2013). Some examples of these bacteria are *Azotobacter chroococcum* and *Pseudomonas fluorescens* (Bhattacharyya and Jha 2012). Somers *et al*. (2004) classified PGPR based on their functional activities as biofertilizers, phytostimulators, rhizoremediators, and biopesticides.

Soil organic matter is recognized as one of the most important factors influencing soil fertility, crop production, and land protection against pollution, degradation, erosion, and desertification, especially in semiarid and arid zones (Peuravouri *et al*. 2004). During the process of vermicomposting, a large part of organic matter is mineralized into carbon dioxide, ammonia, and water, leaving what remains as stabilized, mature organic matter that chemically resembles humic substances found in the indigenous soil (Benitez *et al*. 1999). The application of humic substances such as humic acid (HA) to plants affects cell membranes and leads to the enhanced transport of minerals, improved protein synthesis, plant hormone-like activity, the solubilization of micro and macro elements, the reduction of active concentrations of toxic minerals, and to increased microbial populations (Peuravouri *et al*. 2004). We previously (Lotfi *et al*. 2015a,b) investigated the humic and fulvic acid effects on the basic parameters of ChlF and its relation to reactive oxygen species (ROS) production and antioxidant enzyme activities. But there is no detailed study on the effect of humic acid on the photosynthetic activity of plants under water stress, hence, our research aimed to investigate this by the JIP-test in rapeseed.

Materials and methods

Plant material and treatments: A factorial experiment (using the RCB design) with three replications was conducted in 2015 at Maragheh, East Azarbaijan, Iran, to evaluate the effects of a foliar application of humic acid (HA) on the photosynthesis and ChlF of rapeseed (*Brassica napus* L.) under water stress. Three irrigation treatments, including well-watered conditions (WWC), moderate water stress (MWS), and severe water stress (SWS) (50, 80, and 110 mm evaporation from a class A pan, respectively), and two concentrations of HA, including 0 (control) and 500 mg L^{-1}, were used in this study. Soil samples were taken from depths of 0–20 cm, using a soil auger, and were collected from 5 points of the experimental area. All samples were air-dried for 72 h, and then followed by oven drying at 60°C for 72 h, until the mass of the samples remained constant. The samples were then crushed and sieved through a 2-mm sieve (Santín-Montanyá *et al.* 2013).

Physical and chemical properties of the soils at the experimental fields. K – potassium; P – phosphorus; TN – total nitrogen; OC – organic carbon; TNV – total neutralizing value; EC – electrical conductivity; SP – saturation percentage.

Soil Texture	Clay [%]	Silt [%]	Sand [%]	TN [%]	OC [%]	TNV [%]	SP [%]	K [ppm]	P [ppm]	pH	EC × 10^3
Sandy loam	24	35	41	0.16	0.44	15.78	0.45	266	3.46	7.45	0.43

The sowing date of rapeseed was 26 April, 2015. The seeding density was 70 seeds m^{-2}. Each plot consisted of 4 rows of 4 m in length, spaced 25 cm apart. All plots were irrigated immediately after sowing, and, after seedling establishment, the plants were thinned to 40 plants m^{-2}. Subsequent irrigations were applied according to treatments specifications. The HA was sprayed on plants at the vegetative growth stage (40 d after emergence).

Bacterial strain inoculation: Bacterial isolates were obtained from the Soil Science Department at Tehran University. The Ps 59 strain of *Pseudomonas* genus was selected due to its P-solubilizing ability, and strain Az 21 was selected from the *Azotobacter* genus, for its N_2-fixing ability. *Azotobacter* and *Pseudomonas* bacteria belonged to *Azotobacter chroococcum* and *Pseudomonas florescense* species, respectively. After renewing bacterial cultures, populations of fresh inoculant were adjusted to about 4 × 109 colony forming units ml^{-1}, based on dilution factor, and then 25 mL of each inoculant were applied per kg of vermicompost (wet base) (Busato *et al.* 2012).

Extraction of HA: The organic material used for HA isolation was vermicompost, obtained from cow manure and crop residues in a ratio of 1:1, which we enriched with *Azotobacter chroococcum* and *Pseudomonas fluorescens* in an incubator at 28°C for 60 d. The samples of enriched vermicompost were mixed with 1 mol(urea) L^{-1} under N_2 at a ratio of 1:10 (w/v) (vermicompost/extract). After leaving the treated slurry to stand for 24 h in the dark and at room temperature, the supernatant was separated by centrifugation at 10,000 rpm. The suspension obtained after filtration was diluted with distilled water and precipitated slowly at a pH of 1.17–1.50 with HCl. The precipitate was purified with HCl:HF at 0.1:0.3 mol L^{-1} and then washed with distilled water until a negative AgCl test was recorded, and finally dried at temperatures below 50°C (Qi *et al.* 2004). The elemental composition was determined by C, H, N, and S in an analyzer instrument (*Vario EL*, *Elementar Analysen System GmbH,* Germany). Oxygen was calculated by difference as following: O [%] = 100 – (C [%] + H [%] + N [%] + S [%]) (ash and moisture-free basis). The ash content was determined as the percentage of dry solid mass after combustion in the air at 660°C for 6 h. Total acidity, carboxylic groups (COOH) and total hydroxyl content was determined according to conventional methods described by Page (1982) and phenolic-OH group content was calculated by their difference.

Elemental composition (ash and moisture-free basis), H/C and C/N ratio of humic acid (HA).

Sample	C [%]	H [%]	N [%]	S [%]	O [%]	Ash [%]	H/C	N/C
HA	53.30	4.30	5.90	0.50	36.00	1.80	1.00	6.12

Acidic functional groups contents of humic acid (HA).

Sample	Total acidity	Carboxylic groups [mmol g^{-1}]	Phenolic-OH groups [mmol g^{-1}]	Total hydroxyls [mmol g^{-1}]
HA	7.75	3.61	4.14	2.74

Chl fluorescence: Induction of ChlF (OJIP transient) was monitored using the *Handy-PEA* (*Hansatech Instruments Ltd*., UK). Before measuring the experimental signals, the plants were kept in the dark at least for 30 min. Measurements were carried out on the upper surface of fully developed leaves at 60 d after the emergence of rapeseed plants. From the OJIP transient measured during the 1 s, several expressions and fluorescence parameters were calculated that described the physiological state of the photosynthetic sample.

Stomatal conductance, Chl content index, and net photosynthesis: After 60 d from rapeseed plant emergence, the stomatal conductance (g_s) and Chl content index (CCI) were monitored with an *AP4* leaf porometer (*ADC*, UK), and a Chl meter (*SPAD 502*, *Minolta*, Japan), respectively. At the same time the net photosynthesis (P_N) was recorded by an infrared gas analyzer (*LCA4-*, *ADC*, UK).

Statistical analysis: All the data of g_s, CCI, and P_N were analyzed on the bases of experimental design, using *SAS 9.1* software. The means of each trait were compared according to the *Duncan*'s multiple range test at $p \leq 0.05$.

Results

ChlF transients of dark-adapted rapeseed leaves were plotted on a logarithmic time scale from 0.01 up to 1,000 ms (Fig. 1). The prompt fluorescence transient of rapeseed with and without the HA application under WWC and MWS had a typical shape with the polyphasic rise OJIP reaching the maximum value F_p in less than 1,000 ms. The application of HA under WWC caused an increase in J-I (3–30 ms) and I-P (30–300 ms) steps as compared samples without HA (Fig, 1*A*). Under MWS conditions, the application of HA only increased the J-step of treated plants (Fig. 1*B*). The J-step of the ChlF transients curve under SWS conditions strongly increased and then the J-I phase of the OJIP curve was completely lost, while in the HA-treated plants, under the same conditions, the shape of the ChlF transient curve was typical, with HA decreasing the levels of the O (0.02 ms)-step and increasing the P (300 ms)-step of ChlF, as compared with those without HA application (Fig. 1*C*).

The effect of HA on photosynthetic electron transport was estimated from several JIP-test parameters, and presented as a spider plot graph in Fig. 2. The spider plot graphically represents how water stress strongly increased the maximum electron transport flux per reaction center (ET_0/RC) with the probability that trapped excitation moves an electron into the electron transport chain beyond Q_A^- (ψ_{E0}) and the quantum yield of reduction of end electron acceptors at the PSI acceptor side (φ_{R0}). However, the application of HA under both MWS (Fig. 2*A*) and SWS (Fig. 2*B*) improved the JIP parameters, similar to those the levels of parameters under non-water stress conditions (control).

The application of HA increased the maximum quantum yield of PSII photochemistry (F_v/F_m), the activity of the water-splitting complex on the donor side of the PSII (F_v/F_0), and trapped energy flux per reaction center (TR_0/RC), ET_0/RC, the probability with which an electron from the intersystem electron carriers move to reduce end electron acceptors at the PSI acceptor side (δ_{R0}), and ψ_{E0} of rapeseed plants under both WWC and limited-water conditions. The performance index (PI_{total}), the photochemical de-excitation rate constant in the excited

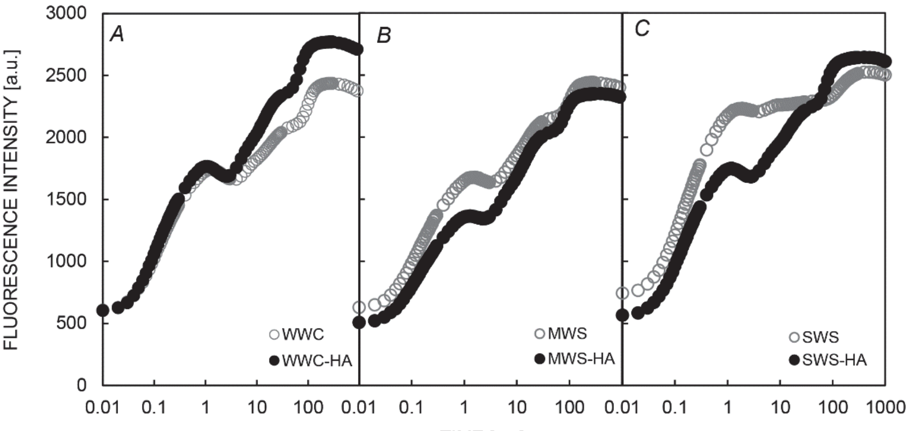

Fig. 1. Chlorophyll *a* fluorescence transients plotted on a logarithmic time scale and measured in response to humic acid under well-watered and water-stress conditions: Well-watered conditions (WWC, *A*), moderate water stress (MWS, *B*), and severe water stress (SWS, *C*).

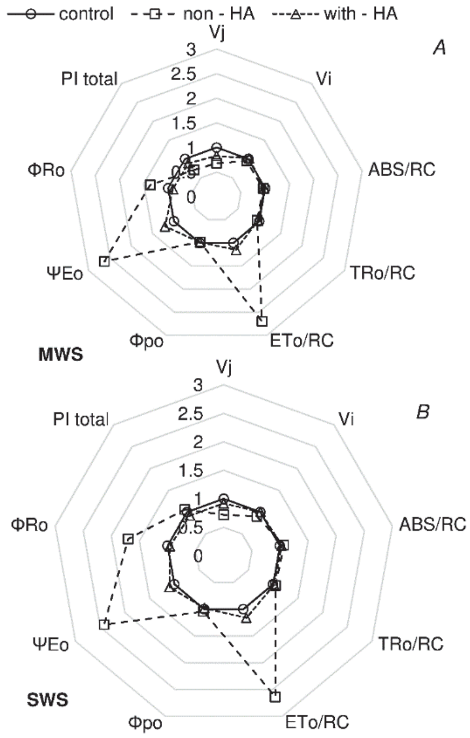

Fig. 2. Spider plot presenting the JIP-test parameters calculated from humic acid treated plants under moderate MWS (*A*) and severe SWS (*B*) water stress conditions.

antennae of energy fluxes for photochemistry (K_P), and φ_{R0} only under water stress, and absorption flux per reaction center (ABS/RC) under WWC all positively improved as a result of the HA treatment. HA had no effect on the nonphotochemical de-excitation rate constant in the excited antennae for non-photochemistry (K_N) under all conditions. The energy necessary for the closure of all reaction centers (S_m) and dissipation energy flux per reaction center (DI_0/RC) decreased with the application of HA. The effects of HA on the enhancement and reduction of ChlF parameters were more evident under SWS conditions as compared with WWC and MWS (Table 1).

ANOVA of the data indicated that water stress, HA, and also interactions between them significantly affected CCI, g_s, and P_N (Table 2). CCI, g_s, and P_N significantly decreased with increasing water stress levels. But, the application of HA strongly improved those traits. The effect of HA on CCI, g_s, and P_N was more evident under SWS. In most cases, the differences between HA-treated and HA-nontreated plants were not significant under WWC (Fig. 3*A*–*C*).

Discussion

Water stress affects photosynthesis typically starting with mostly stomatal effects at moderate water stress, and culminates in metabolic and structural changes caused by SWS (Jedmowski *et al.* 2013). It has been shown that PSII has high resistance to water deficit (compared to PSI) and negative impacts therefore only occur under conditions of extreme drought (Lauriano *et al.* 2006). Evaluation of fast ChlF transient in dark-adapted samples represents an efficient tool for monitoring many adverse environmental effects on photosynthetic activities. As illustrated in Fig. 1, the typical polyphasic transient was sensitive to water stress only under SWS conditions and the J-I phase was lost under these conditions. However, the application of HA caused plants to have a typical transient curve under SWS (Fig. 1*C*). The J-I of the curve corresponds to the reduction of the secondary electron acceptor Q_B, plastoquinone (PQ), cytochrome b_6f (Cyt b_6f), and plastocyanin (PC) (Kalaji *et al.* 2016). Our study indicated that the application of HA strongly increased the J-I and I-P stages under WWC (Fig. 1*A*). The increase in ChlF in the J-I phase is attributed to the reduction of Q_B, PQ, Cyt b_6f, and PC, while the increase in ChlF in the I-P part of the induction curve is associated with the reduction of the electron transporters (ferredoxin, intermediary acceptors and NADP) of the PSI acceptor side (Kalaji *et al.* 2016). The I–P phase seems to be related to the content of the PSI reaction centers (Ceppi *et al.* 2012) or to the availability of linear electron transport as determined by 820-nm transmission measurements (Zivcak *et al.* 2014). For example, the extent of the I–P loss in barley varieties depends on their drought tolerance (Oukarroum *et al.* 2009, Ceppi *et al.* 2012). The OJIP transient was divided into two phases, a photochemical phase O–J and a thermal phase J–I–P (Strasser *et al.* 1995). These phases reflect three different reduction processes of the electron transport chain (Strasser *et al.* 2004, Stirbet and Govindjee 2012). The O–J phase is strongly light-dependent (Schansker *et al.* 2006) and contains information on the antenna size and connectivity between the PSII reaction centers. The J to P rise reflects a reduction of the rest of the electron transport

Table 1. Some chlorophyll *a* fluorescence parameters of rapeseed plants in response to humic acid under good watering and water-stress conditions. All of the data are percentage of control without HA under well-watered conditions (WWC), moderate water stress (MWS), and severe water stress (SWS).

Treatment	F_v/F_m	F_v/F_0	S_m	ABS/RC	DI_0/RC	TR_0/RC	ET_0/RC	PI	K_P	K_N	δ_{R0}	ψ_{E0}	φ_{R0}
WWC	4	20	-21	2	-10	6	30	0	7	0	15	23	0
MWS	6	28	-5	0	-20	3	30	58	30	0	34	26	13
SWS	11	58	-2	0	-30	10	200	26	60	0	170	174	70

Table 2. Analysis of variance of the chlorophyll content index (CCI), stomatal conductance (g_s), and the net photosynthetic rate (P_N) of rapeseed plants. ** – significant at $p \leq 0.01$.

Source	df	CCI	g_s	P_N
Replication	2	0.7	0.2	0.1
Water stress (W)	2	18.1^{**}	2.9^{**}	5.3^{**}
Humic acid (H)	1	396.5^{**}	81^{**}	147.6^{**}
W × H	5	4.3^{**}	0.6^{**}	1.1^{**}
Error	10	1.4	0.1	0.02

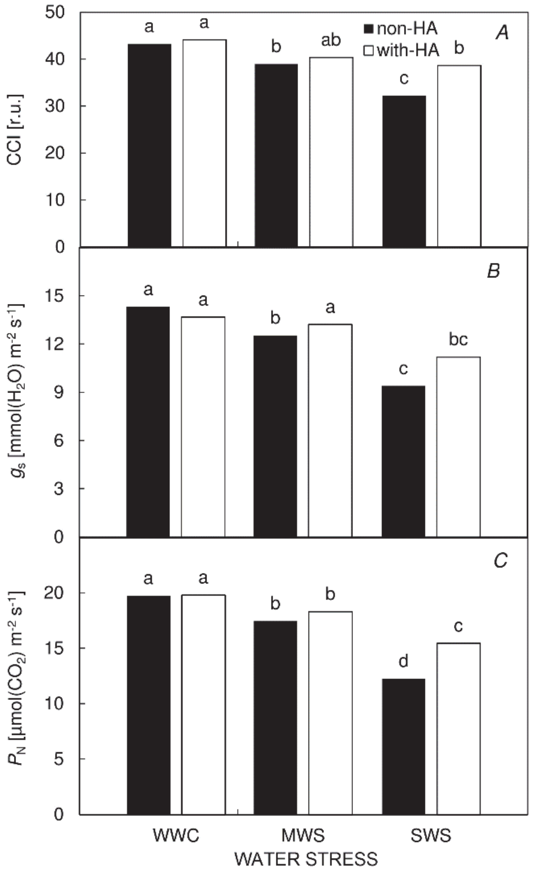

Fig. 3. Changes in the chlorophyll content index (*A*), stomatal conductance (*B*), and net photosynthesis (*C*) of rapeseed plants affected by humic acid under well-watered (WWC) and water stress conditions: moderate water stress (MWS) and severe water stress (SWS). Results are the mean of three replications ±SE, *Duncan*'s multiple range test at $p \leq 0.05$.

chain (Schansker *et al.* 2005). Our study shows that the effect of HA was greater on the reduction of the rest of the electron transport chain than on the light-dependent phase under WWC and SWS conditions.

We also found that under both MWS and SWS conditions the application of HA decreased F_0 and increased F_m (Fig. 1*B*,*C*). F_0 represents the fluorescence level when the PQ electron acceptor pool is fully oxidized, and it may change when exposed to stress (Fracheboud *et al.* 2004). An increase in F_0 under water stress can be interpreted as a reduction in the rate constant of energy trapping by the PSII centers, which could be the result of a physical dissociation of the LHC from the PSII core observed in several plant species under environmental stress (Armond *et al.* 1980). Similarly Mathur *et al.* (2011) reported that the increase in F_0 is as a result of the release of LHC II from the PSII complex, the inactivation of the PSII photochemical reaction and inhibition of electron flow due to the reduced transfer of Q_A to Q_B. The reduction in F_m under water limiting conditions may be caused by the inhibition of electron transport at the donor side of the PSII or may be related to denaturation of Chl proteins (Yamane *et al.* 1997). There are several possible interpretations of such a decrease in F_0 as a result of the HA application. First, the F_0 is an indicator of the water stress effects on the PSII acceptor side as it corresponds to a shift in the Q_A/Q_B^- equilibrium toward Q_A^- (Kouřil *et al.* 2004) with a decreased back electron transfer from Q_B to Q_A (Ducruet 1999, Kouřil *et al.* 2004). An alternative explanation of the exponential F_0 decrease is the greater connection of PSII light-harvesting antennae from the PSII core complex (Yamane *et al.* 1997). The F_m increase reflects the decreasing number of Q_A nonreducing reaction centers. The F_0 rise and F_m decrease indicate a block in the electron transport to Q_A^- (Krause and Weis 1991) and a development of the radiative less dissipation of the excited states of PSII antennae Chls.

The spider plot graphically showed that water stress strongly increased ET_0/RC, ψ_{E0}, and φ_{R0}, but the application of HA decreased those parameters similar to control conditions (Fig. 2). As the ψ_{E0} values of water-stressed samples were high and the electron supply from the PSII donor side (OEC) was low, the electron carriers efficiently transferred electrons to the next step of the electron transport chain. After the application of HA, the values of ψ_{E0} substantially decreased below the values of the non-stressed samples as the PSII donor side supplied many more electrons. Hence, the PSII acceptor side limitation became more serious than the limitation on the PSII donor side. This suggests that the application of HA had a greater effect on the donor side than that on the acceptor side of PSII. This assumption is supported by the ψ_{E0}, which reflects the reduction of the acceptor side of PSII (Tóth *et al.* 2007, Joshi *et al.* 1995). Indeed, the ψ_{E0} parameter is linked to the balance between the efficiency and inefficiency of the dark reactions after Q_A^- significantly increased as a result of the HA treatment. This result indicated that HA induced the redox reaction after Q_A due to the greater connectivity of electron flow from Q_A to Q_B.

Parameter φ_{R0} had a trend similar that of ψ_{E0} characterizing the PSII acceptor side. The PSI accepted almost all electrons when the donor-side electron supply was limited, but efficiency decreased after the electron transport from OEC was restored. HA affects two fluorescence parameters related to PSI activity. The φ_{R0} and δ_{R0} were shown in Table 1. These two fluorescence parameters increased with the application of HA. Such results indicated that HA affected electron flow on the PSI acceptor side. The increase observed in δ_{R0} suggests that the increase in the PSI number was reduced in the linear electron flow and its inclusion in the cyclic electron flow. The mechanism promoting such a switch is still unknown, but probably PSI or Cyt b_6f is moved further from PSII. With regards to ChlF parameters in light, an increase in the F_v/F_m and ψ_{E0} indicates that HA induced the redox reaction after Q_A and enhanced the electron transfer between Q_A^- and Q_B^-. A greater δ_{R0} level indicated an enhancement into a traffic jam of electrons at the acceptor side of PSI caused by the activation of ferredoxin NADP$^+$-reductase (Schansker *et al.* 2005).

PSII is one of the most susceptible components of photosynthetic machinery. Abiotic stress, such as drought or high light, results in an over-reduction of the electron transport chain (Zhang and Sharkey 2009). Detailed analysis, based on the selected parameters (S_m), suggests that there was a decreased size of the pool of PSII and PSI electron carriers (from Q_A to ferredoxin) as well as a decrease in the number of Q_A turnovers between F_0 and F_m. The specific energy fluxes in one of the active reaction centers ABS/RC, TR_0/RC, ET_0/RC were visibly increased, but DI_0/RC decreased with the application of HA. This change is especially expressed for parameters correlating to the electron transfer site within the PSI, from the PQH_2 to the PSI end acceptors φ_{R0} and δ_{R0}. These could be mechanisms by which the PSII of plants after the HA treatment enhanced the rate of the electron transport chain. This assumption was supported by increasing the photochemical de-excitation rate constant in the excited antennae of energy fluxes for photochemistry (K_P), while the nonphotochemical de-excitation rate constant in the excited antennae for non-photochemistry (K_N) was not affected by HA.

Among the ChlF parameters, the performance index (PI) provides the information on the general state of plants and their vitality (Oukarroum *et al.* 2009). It combines the information about the concentration of the fully active reaction center per Chl, primary photochemistry, and electron transport (Strasser *et al.* 2004). Changes in PI are susceptible to changes in antenna properties, electron trapping efficiency, and transport beyond Q_A (Oukarroum *et al.* 2009). Zivcak *et al.* (2013) pointed PI as a very sensitive index to prolonged drought stress in winter wheat. Moreover, the drought tolerance of wheat genotypes estimated from PI values recorded in drought stress also correlated well with the drought tolerance assessed by grain yield (Zivcak *et al.* 2008). In this study, significantly higher values of PIs were found with the application of HA under water stress. Our study clearly showed that the application of HA, especially under water stress conditions, improved the CCI in a positive way. PSII activity therefore increased the gas exchange and photosynthetic activity of rapeseed plants. These results were supported by improving of g_s of plants under different watering conditions especially under water stress with application of HA (Fig. 3*B*). Activation of the H$^+$-ATPase pump is a key step in g_s in response to a variety of stimuli including auxin (Schroeder *et al.* 2001). Since this enzyme has been shown to be activated by humic substances (Quaggiotti *et al.* 2004) and this material have been suggested to have auxin-like activities in a number of systems, it seemed likely that humic substances would stimulate stomatal opening. Similar result were reported by Russell *et al.* (2006) on *Pisum sativum* L.

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