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Tables : 1 , Figs : 6, Photographs : 2

Effects of wheat intercropping on the senescence of cucumber leaves

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ABSTRACT

We investigated in greenhouse experiment, the effects of intercropping of wheat (*Triticum aestivum* L.) with cucumber (*Cucumis sativus* L.) on the leaf senescence of later crop. The leaves of the cucumber plants under wheat intercropping (CW) showed higher chlorophyll content and lower intercellular CO_2 concentration than in cucumber monocropping (CM). The CW also increased the leaf activity and expression of superoxide dismutase and peroxidase enzymes, but reduced the hydrogen peroxide and the superoxide anion in leaves. In intercropped cucumber leaves, increased the activities of enzymes [ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) and polyamine oxidase] and also the expression of Rubisco actives and transglutaminase were increased than cucumber grown in monoculture. Putrescine and spermidine contents were decreased more in CW than in CM. These results suggested that wheat intercropped with cucumber decreased the accumulation of reactive oxygen species in the cucumber leaves, which in turn improved the carbon assimilation and thereby, delayed the leaf senescence.

Key words: Cucumber, greenhouse, *Cucumis sativus* L., enzymes, greenhouse, intercropping, monoculture, leaf senescence, photosynthesis, polyamines, reactive oxygen species, ROS, *Triticum aestivum*, wheat.

INTRODUCTION

The physiological processes in plant cells always release reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2) and superoxide radical ($O_2^{\cdot-}$) (37,44). During the senescence or plant aging, the ROS production strongly increases, this causes harmful oxidative damage in the cells and metabolism, which leads to the death of leaves and other plant organs (6,41,50). Several plant defensive mechanisms prevents the ROS damage and delays the senescence of plant organs. These are (i). ROS scavenging enzymes [catalase (CAT) and superoxide dismutase (SOD) (30)] and (ii). Polyamines (PAs) [putrescine (Put), spermidine (Spd) and spermine (Spm)] act as free radical scavengers (26,35). Transglutaminase (TGase) a key enzyme transforms the free PAs into insoluble bound PAs. This enzyme covalently links PAs to endoglutamines of proteins to play an important role in the post-translational modifications of proteins (11). Polyamines can be oxidized by polyamine oxidases (PAO), to yield hydrogen peroxide, which induces the programmed cell death (46).

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High levels of ROS inhibit the photosynthesis during the leaf senescence (51). Photosynthetic CO_2 fixation via ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) is the primary input of carbon into plants biomass (25). Therefore, the reduction of Rubisco activity reduces the photosynthetic rate (40). Rubisco activity is inhibited by the accumulation of ribulose 1,5-bisphosphate (RuBP). Rubisco actives (RCA) (17), a protein member of the AAA+ ATPase family, uses the energy released by the ATP hydrolysis to activate the Rubisco (31). The RCA is not only related to the initial Rubisco activity but also to the net photosynthetic rate (Pn) in the leaves during senescence (10,32).

Cucumber (*Cucumis sativus* L.), a nutrients-rich vegetable crop, is grown in most parts of the world especially in China (40). The continuous monocropping problem is the main cause, inhibiting the growth and development of cucumber (7). Previous research indicated that its intercropping with wheat decreased the accumulation of ROS (13) and increased the activity of nitrogen metabolism in the cucumber leaves, thereby delaying the leaf senescence (23). In this study, we investigated the effects of wheat intercropping on the photosynthesis and antioxidant capacity in cucumber leaves, to develop strategies to reduce the cucumber leaves senescence.

MATERIALS AND METHODS

The experiments were conducted from June to September 2018 in our greenhouse, Northeast Agricultural University (45°41′N, 126°37′E; Altitude: 165 m; Annual rainfall: 529 nm; Maximum temperature: 32.6 °C; Minimum temperature: 22.1 °C). The experimental soil, contained ammonium nitrogen (47.65 $mg \cdot kg^{-1}$), nitrate nitrogen (149.66 $mg \cdot kg^{-1}$), available P (318.25 $mg \cdot kg^{-1}$), available K (305.81 $mg \cdot kg^{-1}$), total nitrogen (1.31 $g \cdot kg^{-1}$), total P (1.43 $g \cdot kg^{-1}$), organic matter (67.25 $g \cdot kg^{-1}$). The pH and EC were 7.8 and 1.04 $mS \cdot cm^{-1}$, respectively. The experimental treatments were: (i). Cucumber monoculture and (ii). Cucumber + wheat intercropping.

Seeds of cucumber (*Cucumis sativus* L.) cultivar ‘Jinzao-9’ were obtained from the Academy of Agricultural Sciences, Tianjin, China and wheat cultivar ‘PinziII-5’ from the Laboratory of Vegetables, Physiological Ecology, Northeast Agricultural University, Harbin, China.

Greenhouse experiment

Cucumbers seeds were soaked in distilled water at 55 ℃ for 30 min and then kept at room temperature for 6 h, before germinating in tray (30 cm × 17 cm × 6 cm) covered with moist gauze at 28 ℃. Two days later, the germinated cucumber seeds were sown in soil in trays (40 cm × 30 cm × 10 cm). The seedlings emerged from the soil (after 7-days). Ten days old seedlings were transplanted into pots (10 × 10 cm). At the fourth true leaf stage (35 days later), uniform cucumber seedlings were transplanted in greenhouse. There were two experimental treatments: (i). Cucumber monoculture (CM) and (ii). Cucumber + wheat intercropping (wheat row 5 cm away from cucumber plants) (CW) [Photographs 1 and 2]. As the wheat (sown 5 cm apart from cucumber) may affect the growth of cucumber seedlings, hence, wheat was sown 7 days after cucumber transplanting. The plot size was 5

m × 0.6 m, containing 2 ridges (60 cm apart), which included protected rows on both sides. The treatments were replicated thrice in Randomized Block Design. The cucumber plant to plant spacing was 30 cm and wheat was sown on the ridge 5-cm away from the cucumber plants. To prevent the contact between the cucumber and wheat plants, when 20 days old we started cutting them at 10 cm height every 4- days (11-times i.e. Till 64-days) and the biomass were added back to the soil. The experiment was irrigated daily, and 200 mL urea ($0.025g \cdot mL^{-1}$) solution was applied to each cucumber plant at day 35, to maintain normal growth. The wheat was not harvested for yield, it existed only as intercrop. The photosynthesis of cucumber leaves was measured at the fourth leaf stage. At the same time, the leaves were harvested and stores in liquid nitrogen at -80 °C to determine ROS, chlorophyll and PAs content, antioxidant enzymes, Rubisco, PAO activity and their related gene expression over the fourth leaf at days 30, 40 and 50.

Photograph 1.
Cucumber grown in monoculture (CM).

Photograph 2.
Cucumber + wheat intercropping (CW).

Cucumber yield : Six cucumber plants were selected in each treatment to determine fruit yield. The fruits were weighed to get fruits yield ($kg \cdot ha^{-1}$).

Chlorophyll contents and Rubisco activity: The chlorophyll contents were determined as per Carlos (5) Cucumber leaves (0.2 g) were ground in 3 mL of 80 % acetone and the homogenate was mixed in 25 mL of 80 % acetone. For chlorophyll content, the absorbance of mixture was recorded at 645 nm and 663 nm and chlorophyll contents was calculated.

Rubisco activity was determined as per Zhang (47) with minor modifications. Cucumber leaves (0.2 g) were grounded in liquid nitrogen with 1.5 mL Tris-HCl buffer (pH 7.6, 40 mM) containing $MgCl_2$ (10 mM), EDTA (0.25 mM) and glutathione (5 mM).

To collect the supernatant, the homogenate was centrifuged (12,000 g) at 4 °C for 20 min and then stored at 0 °C. Twenty μL enzyme extract was added into 220 μL Tris-HCl buffer (pH 7.8, 50 mM) that included $MgCl_2$ (12 mM), EDTA (0.4 mM), ATP (5 mM), phosphocreatine (Cr-P 5 mM), $NaHCO_3$ (10 mM), RuBP (2.5 mM), NADH (0.5mM), creatine phosphokinase, glyceraldehyde-3-phosphate dehydrogenase and 3-phosphogly -cerate kinase (16 $U \cdot L^{-1}$). One unit of Rubisco was expressed as the amount of enzyme that caused changes in absorbance at 340 nm for 60 s.

Photosynthesis parameters: The net photosynthetic rate (Pn), transpiration rate (Tr), stomatal conductance (Gs) and intercellular CO_2 concentration (Ci) were measured using a portable photosynthesis system (Li-6400 XT, USA) as described by Zhang *et al*. (48).

Measurement of hydrogen peroxide (H_2O_2) and the release rate of superoxide anion ($O_2^{\cdot-}$)

H_2O_2: The H_2O_2 contents were determined as per Patterson (18), with some modifications. Cucumber leaves (0.5 g) were homogenized in 3 mL of 50 mM phosphate buffer (pH 6.8) and centrifuged at 6,000 g for 25 min. One mL supernatant was mixed with 1 mL of 0.1 % titanium sulphate diluted by 20 % H_2SO_4 and incubated for 10 min at room temperature. Then the mixture was centrifuged at 6,000 g for 15 min. The H_2O_2 contents were determined using H_2O_2 as a standard and expressed on fresh weight basis as $mol \cdot g^{-1}$ based on absorbance at 410 nm.

$O_2^{\cdot-}$ generation: The $O_2^{\cdot-}$ generation was determined as per Wang *et al.* (42) with slight modifications. Cucumber leaves (0.5 g) were homogenized with 2 mL of 50 mM phosphate buffer (pH 7.8) and centrifuged at 10,000 g for 20 min. The supernatant was transferred into a new tube, which was filled with phosphate buffer (pH 7.8). The mixture of 0.5 mL enzyme extract, 0.5 mL 50 mM phosphate buffer (pH 7.8) and 1.5 mL 1 mM hydroxylammonium chloride was incubated at 25°C for 1 h, then mixed with a solution containing 2 mL of 17 mM *p*-aminobenzene sulfonic acid and 2 mL of 7 mM α-naphthylamine at 25 °C for 20 min. The $O_2^{\cdot-}$ generation was measured at 530 nm and its contents were quantified from a linear calibration curve of $NaNO_2$ and expressed as mg fresh weight $mol \cdot min^{-1}$.

Activity of antioxidant enzymes

Superoxide dismutase (SOD) and peroxidase (POD) activities were determined as per Giannopolitis and Moerschbacher (14,27) with some modifications. Cucumber leaves (0.5 g) were homogenized in precool 0.2 M sodium borate buffer (pH 8.8) containing 5 mM mercaptoethanol and 1 mM EDTA. The extract was vibrated for 3 min, incubated for 2 h and then centrifuged (15,000 g) at 4 °C for 10 min. The supernatant was prepared to determine the SOD and POD activities. One unit of SOD activity was described as the amount of enzyme that causes 50 % inhibition of nitro blue tetrazolium (NBT) at 560 nm. Additionally, one unit of POD activity is defined as the amount of enzyme that causes an increase in absorbance of 0.01 at 470 nm per minute.

Polyamine oxidase activity

The enzymatic activity of polyamine oxidase (PAO) was determined as per Zhao (49). The frozen cucumber leaves (0.5 g) were homogenized in 1 mL of 0.1 M precool citrate buffer (pH 6.0) and added to 0.5 M NaCl and 0.01 mM pyridoxal phosphate (PLP). To collect supernatant, the homogenate was centrifuged at 12,000 g for 30 min. The reaction included 1 mL supernatant and 4 mL 0.1 M citrate buffer (pH 6.0) and was incubated at 30 °C. After 2 min, the 1 mL spermine (10 mM) was added into the reaction and then incubated at 30 °C again for 2 min. The mixture was added in solution containing 1 mL vitriol (3.6 M), 1 mL KI (8 %), 0.15 mL ammonium molybdate (10 %) and 0.5 mL starch solution (1 %), before monitoring the absorbance at 550 nm. One unit of PAO activity was described as an increase of 0.001 in absorbance per minute.

Polyamines Content

The extraction and analysis of free polyamines (PAs) were done as per Flores *et al.* (12) with some modification. 0.5 g frozen cucumber leaves were powdered in liquid nitrogen and homogenized with 4 mL precool perchloric acid (5%, v/v). The homogenates were incubated in ice for 60 min and centrifuged (15,000 g) at 4 °C for 30 min. For benzoylation of PAs, 500 μL supernatant were combined with benzoyl chloride (7 μL) and NaOH (1 mL 2 M), vortexed 20 s and kept at 37 °C for 30 min. Then 2 mL saturated NaCl was added into the mixture to stop reaction. The benzoyl-PAs was extracted in 2 mL diethyl ether. After centrifugation at 1,500 g for 5 min, 1 mL ether phase was collected, evaporated and solubilized again in 200 μL methanol (HPLC grade). The benzoyl-samples were stored at -80 °C to prepare for HPLC analysis. The free polyamine standards of Put, Spd and Spm (Shanghai Yuanye Bio-Technology Co., Ltd), were subjected to the same procedure as the samples.

The samples containing the benzoyl-PAs and the benzoyl-PAs standards were injected in HPLC system (Breeze2） coupled to an Waters 2998 UV detector. Ten μL volume was injected into a reverse phase column (Hypersil BDS C18 5 μm, 4.0 mm×150 mm) at temperature of 25 °C. Samples were eluted with a mobile phase of methanol: water (64:34, v/v) at a flow rate of 0.7 $mL \cdot min^{-1}$. PAs were quantified at 230 nm.

Quantitative real-time (qRT- PCR) analysis

Total RNA was isolated from the leaves of cucumber seedlings using Trizol-Reagent according to instructions provided by the manufacturer. Contaminated DNA and protein were removed with chloroform, isopropanol and ethyl alcohol (75%). RNA was reverse transcribed to cDNA using the M-MLV Reverse Transcriptase kit (BioTeke Inc). Twenty μL reaction mixture was prepared with cDNA using 2×Plus SYBR real-time PCR mixture kit (BioTeke Inc). Amplification was done using qTower3G Real-Time PCR Stystem (Analytik Jena AG, Germany). Gene expression analysis was done using $2^{-\Delta\Delta Ct}$ method and relative mRNA expression levels were normalized to actin. The primer sets used were as under:

Actin-F: 5’-CAGGAATCCACGAAACTACT-3’

Actin-R: 5’-AGACCCTCCAATCCAAACAC-3’

SOD-F: 5’-CCTAAACTCTCGTGAATGA
SOD-R: 5’-CAGCAGACAAGTATGGATA
POD-F: 5’-TTGTAATAATGGCGGCTT
POD-R: 5’-GTGTCATAGAAGGTGGAG
PAO-F: 5’-GGAATGAGGGTTCGTCTA
PAO-R: 5’-CAAAGCAGGGTCCAAGTC
RbcL-F: 5’-AGCCTGTTGCTGGAGAAG
RbcL-R: 5’-AGGGCGACCATACTTGTT
RbcS-F: 5’-GCCTCAAATCTTCCGCTGGT
RbcS-R: 5’-AATCCGCTTCCGATGTCGAAT
RCA-F: 5’-CGGGTGCTGGTCGTCTT
RCA-R: 5’-GCTCCTCTGGTAATTGCGTCT
TGase-F: 5’-TTACTGTCCGAACTTGAAG
TGase-R: 5’-TTGTCTCCACTCTGTCTT

Statistical analysis

The experiments Data were subjected to analysis of variance (ANOVA) and differences among means were determined by Student’s *t* test (P <0.05). The statistical analysis was performed with SAS 9.2 software.

RESULTS AND DISCUSSION

Cucumber Yield, leaves photosynthesis and Rubisco activity

Yield: Cucumber showed a similar yield under monocropping and wheat intercropping (Fig. 1a). This situation suggests that wheat growing with cucumber did not have a negative impact on factors that are crucial for cucumber production such as soil chemical properties, soil microflora and nutrient availability (3).

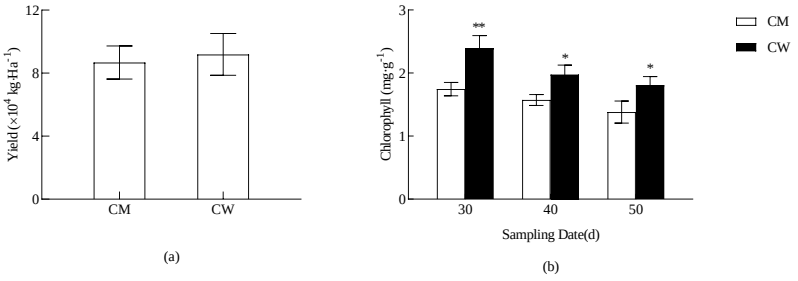

Figure 1. (a) Cucumber fruits yield and (b) chlorophyll contents of cucumber leaves in cucumber + wheat intercropping (CW) and cucumber monocropping (CM). Bar values are means represented together with their standard errors. One asterisk indicates a significant difference (P<0.05) and two asterisks indicate an extremely significant difference (P<0.01) between treatments based on Student’s *t* test

Photosynthesis parameters: Leaves of cucumber under monocropping and intercropping showed a gradual decrease in the content of chlorophyll over time (Fig. 1b), although the cucumber leaves collected in CM suffered a fall higher than those from CW ($P < 0.05$). The values of the photosynthetic parameters Pn, Gs and Tr also declined along time in the cucumber leaves subjected to both cropping systems (Fig. 2a, b, d), with a value of Pn in CW higher than in CM ($P < 0.05$). In the case of Ci, it reached the lowest levels on monocropping and intercropping at day 40 with an increase at day 50 that was stronger for CM (Fig. 2c). A reduction in photosynthetic efficiency is one of the initial signs of leaf senescence (43). Based on it, the chlorophyll content and Pn values indicate that wheat intercropping delayed the senescence of the cucumber leaves.

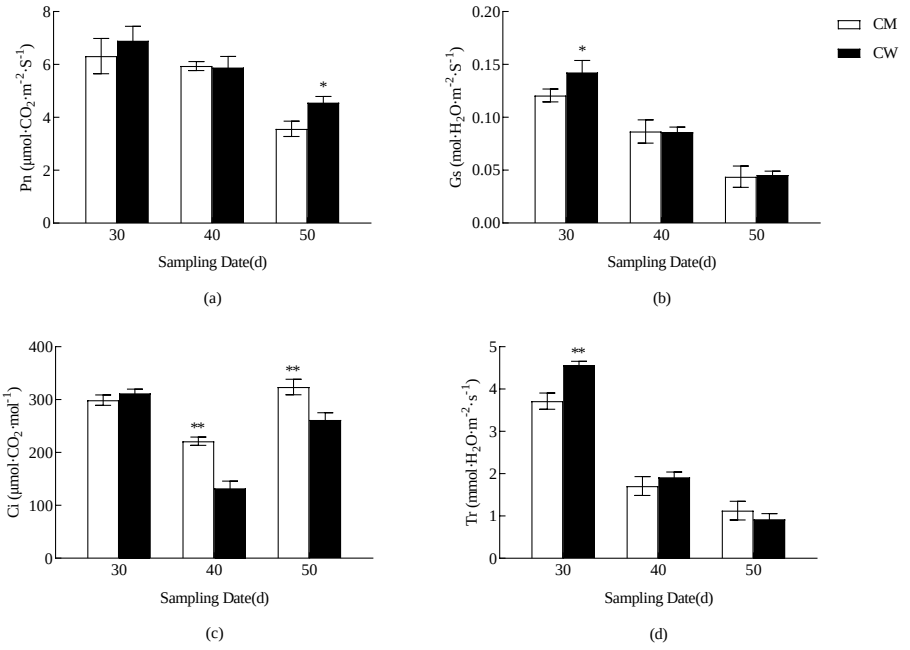

Figure 2. (a) Photosynthetic rates (Pn), (b) stomatal conductance (Cs), (c) CO_2 concentration (Ci) and (d) transpiration rates (Tr) of cucumber leaves in cucumber + wheat intercropping (CW) and cucumber monocropping (CM). Bar values are means represented together with their standard errors. One asterisk indicates a significant difference ($P<0.05$) and two asterisks indicate an extremely significant difference ($P<0.01$) between treatments based on Student's *t* test.

Rubisco Activity : The same can be concluded for the Rubisco activity which is critical in fixing CO_2 during photosynthesis and was in CW almost three and two folds higher than in CM at day 40 and 50, respectively (Fig. 3a) ($P < 0.01$) (38). The molecular basis for the changes observed in the Rubisco activity were also investigated through the expression of the *RbcL*, *RbcS* and *RCA* genes (Fig. 3b, c, d). The *RbcL* and *RbcS* codify a large subunit

and a small subunit, respectively, which are active sites that affect the catalysis of the Rubisco (9). The cucumber leaves under CW showed an expression of *RbcL* and *RbcS* lower than that observed in CM during the days 30 and 40 (Fig. 3b, c) (P <0.01). A contrary trend was seen in the level of the *RCA* expression, which was the lowest at day 30 and increased up to the next sampling dates in both treatments except when it turned down at day 50 in the sample collected from CM (Fig. 3d). These data suggest that the CW triggered the *RCA* expression in cucumber leaves across the time till reach the highest expression value at day 50 (Fig. 3d). RCA plays a crucial role in removing inhibitors from the active sites of Rubisco (29). Hence, our results suggest that the Rubisco activity was increased under CW by an enhanced expression of *RCA* with low expression of *RbcS* and *RbcL*. The increased in the Rubisco activity should be responsible of the higher level of Pn recorded for CW in the cucumber leaves (4,38).

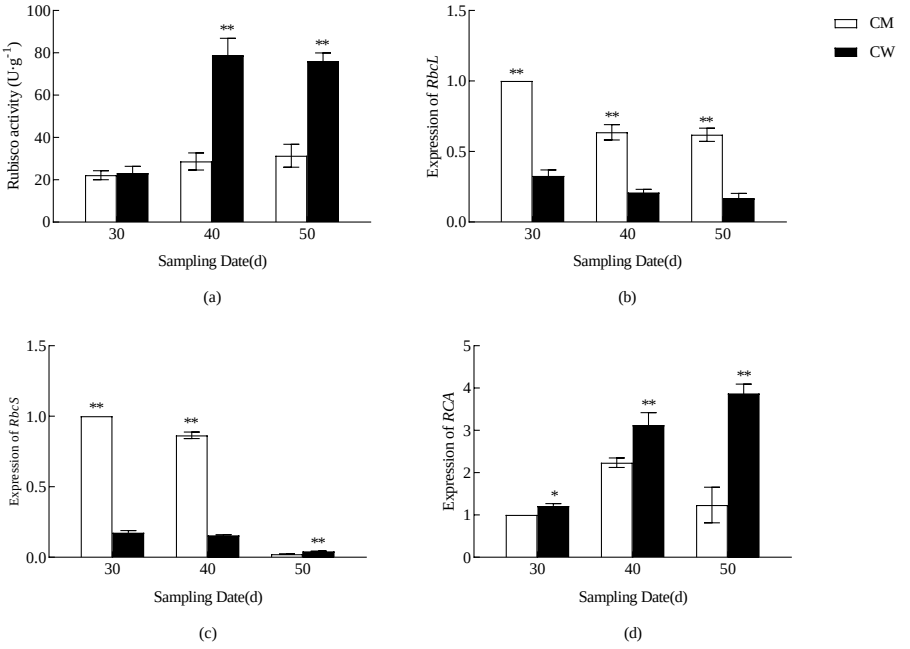

Figure 3. (a) Rubisco activity and expression of (b) *RbcL*, (c) *RbcS* and (d) *RCA* of cucumber leaves in cucumber + wheat intercropping (CW) and cucumber monocropping (CM). Bar values are means represented together with their standard errors. One asterisk indicates a significant difference (P<0.05) and two asterisks indicate an extremely significant difference (P<0.01) between treatments based on Student's t test.

Cucumber leaves ROS content, antioxidant enzymes and gene expression

ROS contents: Our results showed that the levels of H_2O_2 and the release rate of $O_2^{\cdot-}$ recorded for the cucumber leaves increased over time in both cropping systems (Fig. 4).

However, the increase was more obvious in CM leaves as compared to CW (P <0.05). The highest values were observed at day 50 for both cropping systems when the leaf levels of H_2O_2 and $O_2^{\cdot-}$ increased 1.4 and 1.6 folds, respectively, in CM as compared with CW (P<0.05).

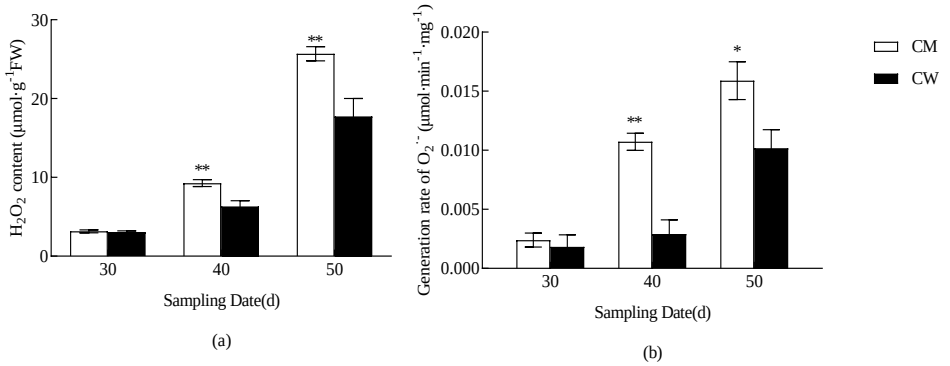

Figure 4. (a) Levels of H_2O_2 and (b) rate of $O_2^{\cdot-}$ generation of cucumber leaves in cucumber + wheat intercropping (CW) and cucumber monocropping (CM). Bar values are means represented together with their standard errors. One asterisk indicates a significant difference (P<0.05) and two asterisks indicate an extremely significant difference (P<0.01) between treatments based on Student's t test.

Antioxidant enzymes and gene expression: In the case of SOD activity, it increased till day 40 in both CW and CM, with significant differences between the treatments and then suddenly decreased in the last sampling date (Fig. 5a) (P <0.01). A similar trend was observed for SOD in the cucumber leaves, although the decrease in its expression at day 50 was not clearly related to the SOD activity observed in the same sampling date (Fig. 5c). Concerning results of leaf POD activity, it increased with the progress of time under both cropping systems till day 50 (Fig. 5b). However, POD activity in CW was higher than in CM at days 40 and 50, which was associated to a higher POD expression (Fig. 5d). Altogether, these results indicated that the leaf antioxidant system was stimulated under CW. This situation might avoid the ROS burst usually associated to the senescence of the cucumber leaves (8,39).

Cucumber leaves Polyamines contents, PAO and expression of *PAO, TGase*

Polyamines contents: The contents of Put and Spd significantly decreased in CW as compared to CM (P <0.01). The (Spd+Spm)/Put ratio of free leaf PAs decreased under CM but not under CW (Table 1). Exogenous polyamines applied to plants delay senescence by protecting the photosynthetic apparatus from the environmental stress (36). This finding allows us to hypothesize that free PAs scavenge oxygen free radicals which in turn delay the cucumber leaf senescence. The contents of endogenous polyamines often increase in the plants over the time (33).

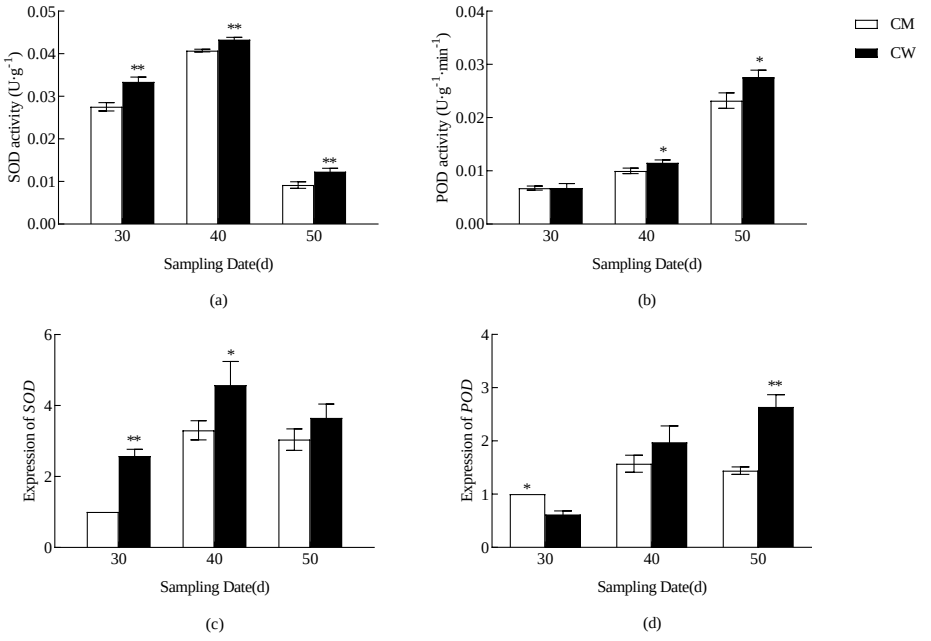

Figure 5. Activities of (a) the superoxide dismutase (SOD) and (b) peroxidase (POD) and expression of (c) SOD and (d) POD of cucumber leaves in cucumber + wheat intercropping (CW) and cucumber monocropping (CM). Bar values are means represented together with their standard errors. One asterisk indicates a significant difference (P<0.05) and two asterisks indicate an extremely significant difference (P<0.01) between treatments based on Student's t test.

Table 1. Free polyamines contents of cucumber leaves in cucumber + wheat intercropping (CW) and cucumber monocropping (CM).

Sampling dates	Treatments	Putrescine (×10^{-4}nmol/g)	Spermidine (×10^{-4}nmol/g)	Spermine (×10^{-4}nmol/g)	Spermine+Spermidine /Putrescine
30 d	Cucumber	6.03±0.39	21.96±0.37	18.98±0.34	6.79
	Cucumber +Wheat	8.63±0.42**	26.22±0.54**	20.67±1.02	5.43
40 d	Cucumber	6.88±0.57**	19.00±0.32**	27.16±0.39	6.71
	Cucumber +Wheat	4.07±0.28	14.83±0.68	28.16±0.74	10.56
50 d	Cucumber	10.59±0.47**	18.05±0.44**	47.90±1.34	6.23
	Cucumber +Wheat	3.48±0.19	12.57±0.38	46.94±1.02	17.08

Values are expressed as mean ± standard error. Two asterisks indicate an extremely significant difference between treatments based on Student's t test (P<0.01).

PAO expression and *TGase*: The conversion rate of Put to Spd/Spm, PAO activity and *TGase* expression made the difference between the two monocropping systems investigated in our work (Fig. 6). The ratio (Spd+Spm)/Put was inversely correlated with the senescence. Covalent linkage of PAs with proteins are catalyzed by transglutaminases

(*TGase*), a family of enzymes converting free Put in bound Put (2). In this study, CW increased PAO activity and *TGase* expression during initial sampling dates (P <0.01) but decreased the expression of *PAO* at the end as compared to CM (P <0.01) (Fig. 6). PAO is closely related to Spm, Spd and its derivatives (1). It is not only catalyzing the catabolism of Spm and Spd, in which H_2O_2 produced by PAO plays an important role in maintaining ROS balance, but also participates in the relevant conversion of PAs (24). The increased of PAO activity resulted in a significant decrease in the leaf contents of Spd in the CW treatment as compared to the CM treatment (P<0.01). In CW, it was enhanced both the conversion of Put and the degradation of Spd, but the difference of ratio (Spd+Spm)/Put indicated that the enhancement in Put conversion influenced more the senescense delaying than the Spd degradation (Table 1). In addition to the direct effects such as alteration of PAs, programmed cell death (PCD) and free radical scavenging (28), PAs regulate the sugar and nitrogen metabolism of plants by nitrate reductase so delaying senescence indirectly (15,16). Li *et al.* (23) showed that intercropping with wheat enhanced nitrogen metabolism of cucumber leaves and delayed senescence. Hence, it can be assumed that cucumber senescence was delayed in CW due to a change in the sugar and nitrogen metabolism derived from the action of PAs in the plant metabolism.

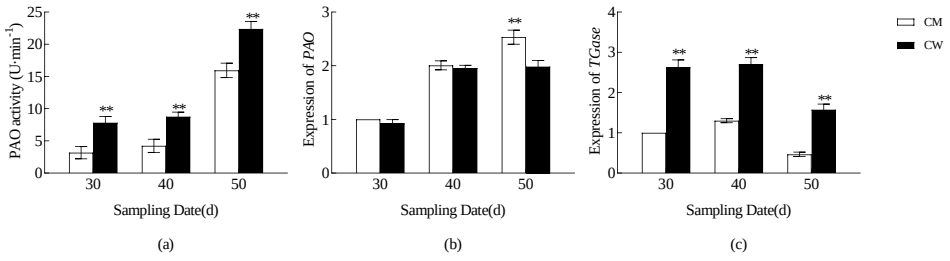

Figure 6. (a) PAO activity and the expression of (b) *PAO* and (c) *TGase* of cucumber leaves in cucumber + wheat intercropping (CW) and cucumber monocropping (CM). Bar values are means represented together with their standard errors. One asterisk indicates a significant difference (P<0.05) and two asterisks indicate an extremely significant difference (P<0.01) between treatments based on Student's *t* test.

The mechanism underlying the delayed leaf senescence observed in the cucumber plants grown with wheat needs further research. However, wheat might exert its effect on cucumber by its root exudates which can directly or indirectly change the soil microbial community structure (19,22,34,45,52,53) and increased rhizosphere bacterial diversity (20). These changes might be directly involved in the delay of leaf senescence (21).

CONCLUSIONS

Wheat intercropping increased the chlorophyll content, the Rubisco activity and the expression of *RCA* in cucumber leaves as compared to monocropped cucumber. The accumulation of ROS was reduced by an enhanced activity and expression of SOD and POD. Conversion of free polyamines was increased by PAO activity and *TGase* expression in cucumber + wheat intercropping, which promoted the ratio of (Spd+Spm)/Put. These factors were involved in the delayed senescence of cucumber leaves.

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