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Full Length Article

Effects of Chloramphenicol on Pigmentation, Proline Accumulation and Chlorophyll Fluorescence of Maize (*Zea mays*) Seedlings

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ABSTRACT

Effects of Chloramphenicol (CAP) on the maize (*Zea mays*) seedlings were investigated using some physiological indexes like the pigmentation, proline accumulation and the fast rise chlorophyll a fluorescence. This study shows that chlorophyll synthesis of maize seedling is stimulated by CAP at 50 and 100 mg/L but inhibited by CAP at 250 mg/L. Proline content was slightly altered under stress of 50 and 100 mg/L CAP and increased markedly under stress of 250 mg/L CAP, suggesting that the cell membrane structure is only affected in the presence of high level of CAP. The maximum quantum yield for primary photochemistry (Fv/Fm) and performance index on absorption basis (PI_{ABS}) showed the same change pattern as the chlorophyll content in response to CAP treatment, indicating that photosystemII (PSII) function was enhanced by CAP at 50 and 100 mg/L but significantly inhibited at 250 mg/L. However, the decreasing of carotenoid content, enery flux and electron flux through PSII reaction centers (RCs) with increasing CAP concentration shows that CAP at all experimental concentrations exerts adverse effect on pigment synthesis other than chlorophyll photosynthetic physiology that can not be indicated by Fv/Fm or PI_{ABS}.

Key Words: Chloramphenicol; Chlorophyll fluorescence; Pigments; PhotosystemII

INTRODUCTION

The issue of antibiotics in the environment has raised a growing concern in recent years. Veterinary and human uses are the most important sources of antibiotics in environment (Halling-Sorensen *et al*., 1998 & 2000; Kummerer, 2001). Many antibiotics used are poorly absorbed by organism and most of them are excreted into the environment (Alcock *et al*., 1999). On one hand, a number of antibiotics such as chloramphenicol (CAP), amoxicillin, erythromycin and tetracycline were resistant to biodegradation (Richardson & Bowron, 1985). On the other hand, although many antibiotics have relatively short environmental half-lives, they assume the qualities of highly persistent pollutants because they are continually introduced into the environment (Daughton & Ternes, 1999). A few of recent studies show that some antibiotics such as chlortetracycline and tetracycline are acutely toxic to algae and higher plants (Holten-Lutzhoft *et al*., 1999). Therefore, ecological risk of antibiotics in the environment should not be underestimated.

CAP is one of the most commonly used antibiotics in veterinary practice in that it inhibits a broad spectrum of aerobic and anaerobic microorganisms and thus is extensively used (Huang *et al*., 2006). CAP is known to being toxic to human bone marrow and can cause aplastic anemia (Anadon *et al*., 1994). Therefore, consumption of CAP is restricted by most countries (Sarmah *et al*., 2006). However, CAP has been still widely but illegally used and frequently detected in the surface water, wastewater and soil (Peng *et al*., 2006; Nikolaou *et al*., 2007). CAP has a potential for persistence resulting in bioaccumulation in the environment due to its low biodegradability (Tong *et al*., 2009), which may probably exert effects on terrestrial and aquatic ecosystems. Limited studies show that CAP at mg/L dosages is highly toxic to microalgae (Campa-cordova *et al*., 2006; Lai *et al*., 2009). Nevertheless, effects of CAP on pigment synthesis and photosynthesis of higher plants are largely unknown.

When illuminated with high intensity of actinic light, dark-adapted oxygenic photosynthetic organisms show a polyphasic rise with the basic steps from the 'origin'(O)

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through two 'phase'(J & I) to 'peak' fluorescence level (P) (Strasser *et al*., 2000). The fast rise Chl a fluorescence (O-J-I-P) transient provides important information on the photochemical activity of photosystemII (PSII) (Krause & Weis, 1991). Strasser and Strasser (1995) established the so called JIP-test, a procedure that quantitatively calculates several phenomenological and biophysical parameters on the basis of the O-J-I-P curve. The fast rise Chl a fluorescence and the JIP-test has been proved to be useful and non-invasive tool for investigation of PSII function under various environmental stresses (Strasser & Strasser, 1995; Eullaffroy *et al*., 2007). The aim of this study was to investigate the effects on CAP on the pigment synthesis, proline accumulation and photosynthesis of maize (*Zea mays*) seedlings by biochemical methods and chlorophyll a fluorescence test.

MATERIALS AND METHODS

Plant materials: The maize seeds were collected from XinJiang agriculture research institute and sterilized in 10% hydrogen peroxide for 30 min. After soaked for 48 h in distilled water, maize seeds were sowed in the matrix (vermiculite: perlite=1:2) at 25^0C. Three days after germination, seedlings were watered moderately with half-strength Hoagland solution. After 7 days of seedlings growth, seedlings were gently transferred to triangular flasks (200 mL) containing full-strength Hoagland solution. The nutrient solution was renewed twice a week. The seedlings were cultured at $25/20^0$C (day/night) with a 14 h/10 h light/dark period of illumination (280 μmol/m^2.s).

Plant treatment: CAP (purity of 95%) was obtained from Amresco, USA. The stock CAP solution of 0.1 mol/L was prepared with deionized water.

After 12 days of seedlings growth, the third leaf of each maize seedling was full expanded. The seedlings with equal size were selected as test samples. 0.1 mol/L CAP solution was added into the samples to make the final CAP concentrations to 50, 100, 250 mg/L, respectively. The sample without addition of CAP was used as the control. The volume of solution in each container was kept as the same by addition of full-strength Hoagland solution. Chlorophyll fluorescence of the third leaf of each maize seedling was measured every morning at 11:00 to 14:00. Plant pigments and proline contents were measured after 12-day CAP treatment.

Determinations of chlorophyll: The third leaf was used for determination of pigment content. 0.1 g of fresh leaf was extracted with 80% acetone (v/v) at 4℃ for 5 min and centrifuged at 1500×g. The absorbance (A_λ) at 663, 645 and 440 nm of the supernatant was determination using a spectrophotometer (UV-2000, Unico, Shanghai, China). Total chlorophyll and carotenoid contents were calculated according to Lihtenthaler (1987).

Determination of free proline: Fresh fraction (0.05 g) of the third leaf of the seedling was ground and extracted with 80% ethanol. Some permutite and active carbon was added into the extraction in order to wipe off the disturbance of aminophenol. Proline was determined by the method of Bates *et al*. (1973).

Measurement of fast rise chlorophyll fluorescence transient: The samples were dark-adapted for 5 min before measurement of chlorophyll fluorescence. The chlorophyll fluorescence transient was recorded up to 1s on a logarithmic time scale, with a data acquisition of every 10 μs for the first 2 ms and every 1 ms thereafter, by a handheld fluorometer (Fluopen-100, Brno, Czech). Each measured O-J-I-P induction curve was analyzed according to the JIP-test (Strasser & Strasser, 1995).

JIP-test analysis: The following data were directly obtained from the fast rise kinetic curves: Fo, the initial fluorescence, was measured at 50 μs, at this time all reaction centers (RCs) are open; F_J and F_I are the fluorescence intensity at J step (at 2 ms) and I step (at 30 ms); Fm, the maximal fluorescence, was the peak fluorescence at P step when all RCs were closed after illumination; $F_{300\mu s}$ was the fluorescence at 300 μs. Selected JIP-test parameters quantifying PSII behavior were calculated from the above original data as the formulae in Table I (Strasser *et al*., 2000).

Statistical analysis: The data in this work are the average of fourteen replicates per treatment and the results were presented as mean±SE. All the analysis were done using SPSS (16.0). In all cases, a significance level of $p<0.05$ was used.

RESULTS

Effects of CAP on proline accumulation and pigment content: The contents of proline and pigments in leaf of maize seedling were clearly affected by CAP treatment (Fig. 1). The proline content was slightly reduced in the presence of 50 and 100 mg/L CAP but increased by 29.45% in the presence of 250 mg/L CAP (Fig. 1A). The total Chl content increased significantly after treatment with 50 and 100 mg/L CAP (Fig. 1B). This suggests that exposure to moderate level of CAP can enhance chlorophyll synthesis. However, as the CAP increased to 250 mg/L chlorophyll synthesis was inhibited, with the chlorophyll content decreasing to 88.9% of the control. In the case of carotenoid, its content decreased drastically with increasing CAP concentration (Fig. 1C).

Effects of CAP on polyphasic fast fluorescence induction: The polyphasic fast fluorescence induction curves of maize seedlings untreated and treated with various concentration CAP were measured (Fig. 2A). There was no significant change in original fluorescence (Fo), while the fluorescence yield at phases J, I and P increased under stress of 50 and 100 mg/L CAP. However, treatment with 250 mg/L CAP reduced the fluorescence at P to below that of the control. This result indicated that treatment with moderate concentration CAP would enhance overall fluorescence

Table I: Summary of the JIP-test formulae using data extracted from the O-J-I-P chlorophyll a fluorescence transient

Formulae and terms	Illustrations
$V_J=(F_{2ms}-Fo)/(Fm-Fo)$	Relative variable fluorescence intensity at the J step
$V_I=(F_{30ms}-Fo)/(Fm-Fo)$	Relative variable fluorescence intensity at the I step
$Mo=4(F_{300\mu s}-Fo)/(Fm-Fo)$	Approximated initial slope of the fluorescence transient
Quantum efficiencies or flux ratios	
$\varphi_{po}=TRo/ABS=[1-(Fo/Fm)]=Fv/Fm$	Maximum quantum yield for primary photochemistry(at t=0)
$\varphi_{Eo}=ETo/ABS=[1-(Fo/Fm)].\psi_0$	Quantum yield for electron transport(at t=0)
$\psi_0=ETo/TRo=1-V_J$	Probability that a trapped exciton moves an electron into the electron transport chain beyond Q_A(at t=0)
Specific fluxes or specific activities	
$ABS/RC=Mo.(1/V_J).(1/\varphi_{po})$	Absorption flux per RC
$TRo/RC= Mo.(1/V_J)$	Trapped energy flux per RC(at t=0)
$ETo/RC= Mo.(1/V_J).\psi_0$	Electron transport flux per RC(at t=0)
DIo/RC=(ABS/RC)-(TRo/RC)	Dissipated energy flux per RC(at t=0)
Performance indexes	
$PI_{ABS}=(RC/ABS).[\varphi_{po}/(1-\varphi_{po})].[\psi_0/(1-\psi_0)]$	Performance index on absorption basis

yield of maize seedling while high concentration CAP would reduce fluorescence yield at P. This was in accordance with the response of chlorophyll content to CAP treatment.

In order to further investigate the effect of CAP on the polyphasic fast fluorescence, we examined the relative variable fluorescence at any given time *t* (Fig. 2B), which is defined as V*t*=(F*t*-Fo)/(Fm-Fo). Both the relative variable fluorescence at J (V_J) and at I (V_I) increased markedly with increasing CAP concentration (Fig. 2C & 2D).

Effects of CAP on energy flux through PSII RC: In order to obtain more information on the effects of CAP on photosystem II performance of maize seedlings, the polyphasic fast fluorescence induction curves were subjected to JIP-test analysis. The apparent size (ABS/RC) increased significantly with increasing CAP concentration (Fig. 3A). The trapping per active RC (TRo/RC) increased markedly under stress of 50 and 100 mg/L CAP and then changed little with further increasing CAP concentration (Fig. 3B). The dissipated energy flux per RC (DIo/RC), calculated as ABS/RC-TRo/RC, decreased slightly under moderate stress of CAP (at 50 & 100 mg/L) but increased significantly after treatment with high level of CAP (Fig. 3D). Electron transport per active reaction centre (ETo/RC) showed an opposite trend to DIo/RC, that is, ETo/RC increased slightly under moderate stress of CAP but decreased significantly at high level of CAP (Fig. 3C).

Effects of CAP on electron transport and photosynthetic performance: It was found that electron transport yield (φEo) changed little under stress of CAP at 100 mg/L or below but decreased pronouncedly after exposure to higher level(250 mg/L) of CAP (Fig. 4A). However, the electron transport beyond Q_A (ψo) showed a clear decreasing trend with increasing CAP (Fig. 4B). Treatment with moderate concentration of CAP (50 & 100 mg/L) resulted in an increase in the maximum quantum yield for primary photochemistry (Fv/Fm) and higher level (250 mg/L) of CAP reduced Fv/Fm (Fig. 4C). The PSII performance index (PI_{ABS}) on the basis of absorption showed the same change pattern as the Fv/Fm (Fig. 4D).

DISCUSSION

This study demonstrated that CAP at concentrations from 50 to 250 mg/L affected proline accumulation, pigment synthesis PSII activity of maize seedlings.

Proline can act as a source for cellular membrane osmotic adjustment (Mansour, 1998) and accumulates in many plants under a variety of environmental stressors such as heavy metal and salinity (Backor *et al*., 2004; Jampeetong & Brix, 2009). In the present study, proline content changed slightly in response to moderate concentration CAP but increased markedly under the stress of high level of CAP. This might indicate that the cell membrane structure is not modified after exposed to moderate level of CAP but is significantly affected at high level of CAP.

Chlorophyll synthesis CAP is slightly stimulated when the maize seedling is treated with CAP at moderate concentrations (50 & 100 mg/L) and is inhibited by CAP at high concentration (250 mg/L). Interestingly, it has to be noted that carotenoids respond to CAP with a different pattern from the chlorophyll. Its synthesis is inhibited by CAP at all the experimental concentrations and the inhibitory effect increased with increasing CAP concentration. Kviderova and Henley (2005) showed that streptomycin and amoxicillin did not significantly affect pigment content of *Picochlorum oklahomensis* and *Dunaliella* sp. in low light but reduced chlorophyll content in high light. Pomati *et al*. (2004) demonstrated that erythromycin at dosage of 1 mg/L significantly affected the growth of both *Synechocystis* and *Lemna* and tetracycline at intermediate dosages had inhibitory effects on *Synechocystis*.

Fig. 1: Proline (A), Chlorophyll (B) and Carotenoid (C) content of maize leaf untreated and treated with various concentration of CAP. Values represent means±SE (n=14). Bars represent the standard error from the mean

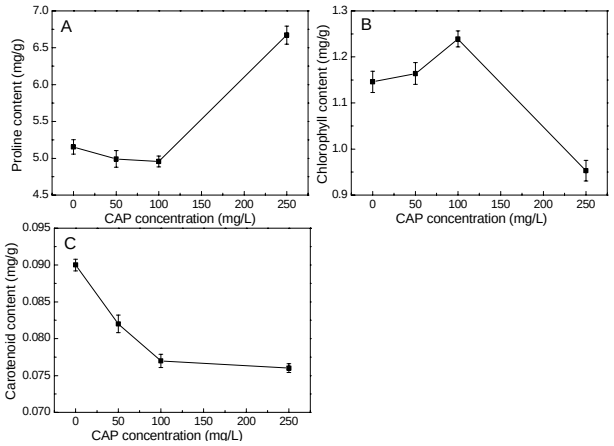
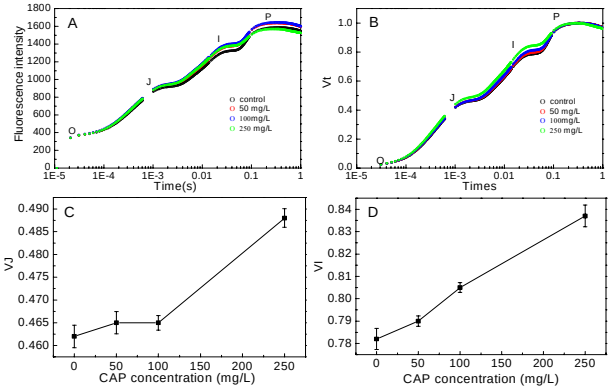


Fig. 2: The exemplified fast rise transient (O-J-I-P) curves for maize seedlings untreated and treated with CAP at various concentration for 12 days (A). All the curves were normalized as the relative variable fluorescence (V*t*), calculated as (Ft-Fo)/(Fm-Fo), from 0 to 1 (B). The variable fluorescence yield at J step (V_J) (C) and the variable fluorescence yield at I step (V_I) of maize seedlings untreated and treated with CAP at various concentrations for 12 days (D). Each data point (in C & D) represents the mean value of 14 independent measurements. Bars represent the standard error from the mean



Chlorophyll fluorescence showed the same change pattern as the chlorophyll under stress of CAP. An exposure to CAP with moderate concentrations increased the chlorophyll fluorescence yield at J, I and P steps as well as the maximum quantum yield for primary phtotchemistry (Fv/Fm) and PSII performance index (PI_{ABS}). Higher level of CAP caused decreases in the fluorescence intensity at P step together with the Fv/Fm and PI_{ABS}. These results indicated that the reduction of Fv/Fm and PI_{ABS} by CAP

Fig. 3: ABS/RC (A), TR_0/RC (B), ET_0/RC (C), DI_0/RC (D) for maize seedlings untreated and treated with CAP at various concentrations for 12 days. Each data point represents the mean value of fourteen independent measurements. Bars represent the standard error from the mean

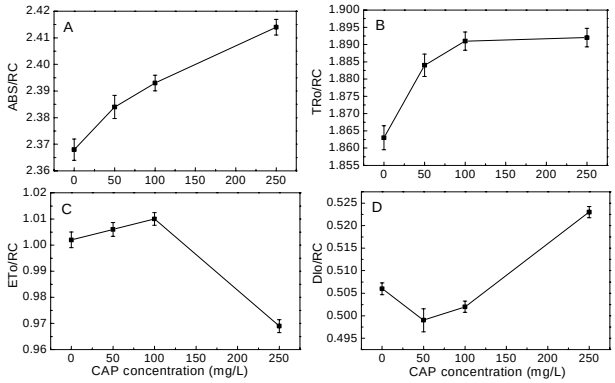
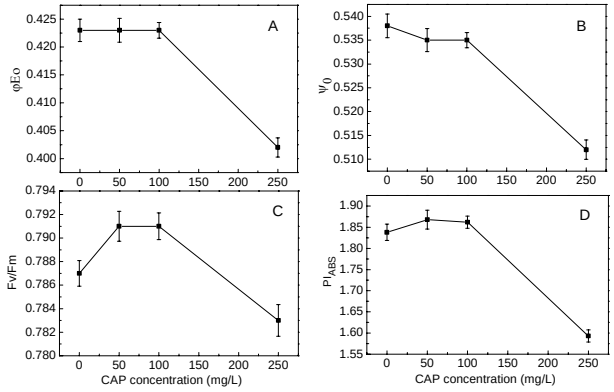


Fig. 4: φEo (A), ψo (B), Fv/Fm (C), PI_{ABS} (D) for maize seedlings untreated and treated with CAP at various concentrations for 12 days. Each data point represents the mean value of 14 independent measurements. Bars represent the standard error from the mean



may be ascribed to the disturbance of chlorophyll synthesis under stress of high level of CAP.

The drop of Fm in the presence of high level of CAP might be interpreted as an increase of the number of the closed PSII RCs that did not participate in electron transport. Toth *et al*. (2005) attributed the quenching of Fm to the presence of oxidized PQ molecules. The increase in V_J might also indicate a rise in the proportion of closed PSII RCs and thus in the proportion of reduced Q_A at J step. The increase in V_I can be attributed to the accumulation of reduced Q_A and plastoquinone, which cannot transfer electrons to the dark reactions. This was in well accordance with the result that electron transport beyond Q_A (ψo) decreased with increasing CAP concentration. That carotenoid showed the same decreasing trend as ψo and the chlorophyll content increased in response to moderate concentration CAP indicate that the inhibition of electron

transport be at least partially resulted from the disturbance of carotenoid synthesis under CAP stress. Our previous studies showed that exposure to amoxicillin or levofloxacin hydrochloride resulted in a substantial increase of the proportion of inactivated PSII (PSIIx) centers in *Synechocystis* sp. and thus decreased the capacity of electron-transport (Pan *et al*., 2008 & 2009).

The increase of ABS/RC with increasing CAP concentration suggests that maize seedling is not able to regulate the light-harvesting capacity in order to adapt to CAP stress (Adams & Demmig-Adams, 2004). The increase of DIo/RC means that PSII reaction centers are transformed into dissipative sinks for excitation energy under stress of CAP (Tevini *et al*., 1998). Increases of both TRo/RC and DIo/RC shows the inactivation of RCs with a less than proportional loss of absorbing antenna pigments and part of the energy absorbed by the antenna of inactivated RCs is dissipated by non-photosynthetic events (Nussbaum *et al*., 2001). Similarly, exposure to amoxicillin or levofloxacin hydrochloride at mg/L dosages could decrease the density of the active photosynthetic reaction centers and increased the dissipated energy flux per reaction centers in *Synechocystis* sp. (Pan *et al*., 2008 & 2009).

In conclusion, chlorophyll synthesis and improvement of PSII performance of maize seedlings is promoted by moderate level of CAP and clearly inhibited by high level of CAP. If only these results were considered, it seems that CAP contamination would not have adverse effects on the plants since CAP concentrations at the level of hundreds of mg/L rarely happened in the real environment. However, the increasing inhibitory effects of CAP at all experimental concentrations on carotenoid synthesis, energy flux and electron transport through PSII RCs show that CAP contamination may pose a threat to the health of plants. The mechanisms involved in these adverse effects of CAP on plant physiology need further study.

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