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Fluoranthene, a polycyclic aromatic hydrocarbon, inhibits light as well as dark reactions of photosynthesis in wheat (*Triticum aestivum*)

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ABSTRACT

The toxic effect of fluoranthene (FLT) on seed germination, growth of seedling and photosynthesis processes of wheat (*Triticum aestivum*) was investigated. Wheat seeds were exposed to 5 μM and 25 μM FLT concentrations for 25 days and it was observed that FLT had inhibiting effect on rate of seed germination. The germination rate of wheat seeds decreased by 11% at 25 μM FLT concentration. Root/shoot growth and biomass production declined significantly even at low concentrations of FLT. Chlorophyll *a* fluorescence and gas exchange parameters were measured after 25 days to evaluate the effects of FLT on Photosystem II (PSII) activity and CO_2 assimilation rate. The process of CO_2 assimilation decreased more effectively by FLT as compared to the yield of PSII. A negative correlation was found between plant net photosynthesis, stomatal conductance, carboxylation capacity and biomass production with FLT. It is concluded that inhibiting effects of FLT on photosynthesis are contributed more by inhibition in the process of CO_2 fixation rather than inhibition of photochemical events.

1. Introduction

Photosynthetic organisms, especially higher plants, are dominating components of the ecosystem and experience several environmental stress conditions. With increasing environmental pollution by a large number of hazardous chemicals with various structures and toxicity levels, the study of abiotic stress response in plants has become ever more important in agriculture, forest management and ecosystem restoration strategies. Polycyclic aromatic hydrocarbons (PAHs) are one of the recalcitrant group of pollutants known to be highly persistent in the environment (Cooke and Dennis, 1983; Neff, 1979). Over 90% of PAHs in the environment reside in surface soil (Wild and Jones, 1995). Furthermore, plants grown in PAH-contaminated soils absorb PAHs and pose a problem in terms of crop yield. It is thus important to understand exactly how PAHs act on plant photosynthetic mechanism and influence the overall plant growth and yield.

Ecotoxicity of hydrocarbons is highly variable, depending on their type, concentration, exposure time, state, environmental conditions and the sensitivity of affected species. Previous studies have demonstrated that PAHs toxicity can inhibit plant growth and development by influencing several biochemical and physiological processes, qualitatively as well as quantitatively (Jajoo et al., 2014; Li et al., 2008; Marwood et al., 2003). PAHs can enter the plant via stomata as well as via the root system and may inhibit plant growth (Kuhn et al., 2004; Ren et al., 1996). PAHs can lead to a range of effects – from impact on primary metabolic processes, such as decrease in photosynthetic and respiration activity and changes in enzyme activities, photosynthetic pigments content (Alkio et al., 2005; Huang et al., 1996, 1997) or injury to membranes by lipid oxidation (Branquinho et al., 1997; Chiang et al., 1996) to changes in plant growth and development and involved regulatory mechanisms. PAHs are lipophilic in nature and therefore they can alter membrane permeability and activity of several enzymes (Liu et al., 2009). PAHs have been shown to accumulate preferentially in thylakoids (Duxbury et al., 1997) affecting the ultrastructure of the thylakoid membrane and thus regulating structural and functional properties of the photosynthetic machinery adversely (Kreslavski et al., 2014). With increasing concentration of PAHs in the soil or other environment, a decrease in the content of photosynthetic pigments, change in plant hormones and inhibition of net photosynthesis rate have been reported (Ahammed et al., 2012; Kummerova et al., 2010). Photosynthesis is regarded as a very sensitive indicator of plant stress. Estimating photosynthetic rate was found to be useful in assessing the potential toxic effects of xenobiotic contaminants (including PAHs) on plants (Huang et al., 1997). It has been confirmed by several

Abbreviations: Chl, chlorophyll; Ci, internal CO_2 concentration; FLT, fluoranthene; F_M, F_O, F_V, maximal, minimal and variable fluorescence; Gs, stomatal conductance; LER, leaf elongation rates; PAH, polycyclic aromatic hydrocarbon; Pn, net photosynthesis; Pn/Ci, carboxylation capacity; PSII, photosystem II; qL, fraction of open PSII reaction centers; Y(II), quantum yield of PSII; Y(NPQ), yield of regulated energy dissipation; Y(NO), yield of non-regulated energy dissipation; Tr, transpiration

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previous researches that PAHs have an adverse impact on plant photosynthesis, particularly on electron transport and PSII activity (Kummerova et al., 2006; Tomar and Jajoo, 2013a, 2013b). However little attention has been paid on the effects of PAHs on dark reactions (carbon fixation) in higher plants.

In the present study, effects of fluoranthene (FLT) on wheat (*Triticum aestivum*) plant have been studied. Wheat is a staple food crop of economic importance and commonly cultivated all over the world. Four ring PAH, FLT is one of the most abundant PAHs, and it belongs to the environmental pollutants recommendation for investigation by the US environmental protection agency (Dabestani and Ivanov, 1999) because of its toxicity and ubiquitous presence in environment. However phytotoxicity of FLT in wheat, especially on the physiological growth, is still poorly understood. Therefore it is important to examine the effect of FLT on growth and photosynthetic activity of crop plants. The main aim of the present work is to evaluate the effect of FLT on various photosynthetic parameters in wheat plants. The specific objectives are (i) to study the responses of wheat seedling growth with FLT treatment, (ii) to elucidate the effect of FLT toxicity on PSII photochemistry and (iii) to investigate effect of FLT on carbon fixation parameters. The measurement indices were germination rate, length of shoot and root of seedling, fresh and dry weights, leaf elongation rate, chlorophyll fluorescence and gas exchange parameters. Gas exchange parameters and Chl *a* fluorescence may be used as biomarkers of organic pollutants in the environment and may become a suitable tool for the study of toxic effects of other environmental contaminants on higher plants.

2. Materials and methods

2.1. Seed germination

Lok-1 cultivar of wheat (*T. aestivum*) was used in this study. Seeds were placed into petri dishes (12 cm diameter, 50 seeds per dish) on double disks of Whatmann filter paper with 15 ml without FLT (control) and with FLT concentrations. The seeds were kept in dark for germination at 23 ± 2 °C. The germination rate was expressed as the percentage of germinating seeds to the total number of seeds. The root and shoot lengths were determined after five days of seedling germination.

2.2. Soil cultivation

For soil cultivation wheat seeds were allowed to germinate in 18 in. (length) black poly bags containing soil with 1/10th strength of Knop's solution. Ten seeds were sown in each bag. Plants were replenished every day with nutrient solution without FLT (control) and with FLT (50 ml). After 25 days of cultivation, various measurements were performed.

2.3. Fluoranthene preparation

Fluoranthene (FLT; Himedia Chemical Pvt. Ltd., India) was dissolved in acetone to make stock solution of 50 mM. This FLT stock solution was delivered to 1/10th strength of Knop's solution to final FLT concentrations of 5 μM and 25 μM. It was found that the concentration of dissolvent (acetone) did not affect seed germination and other physiological parameters (Kummerova et al., 1996; Tomar and Jajoo, 2013b). However control seeds were always exposed to the same concentration of acetone.

2.4. Pigments and growth analysis

The content of photosynthetic pigments (chlorophyll *a* and *b*, carotenoids in mg g^{-1} fresh leaves) was determined spectrophotometrically (Visiscan 167, Systronic). For the extraction of pigments, 100 mg of leaves (third leaf) tissues in 5 ml of 80% acetone was used. The calculation was made according to Arnon (1949).

Leaf elongation rate was measured in random leaves ($n=4$ per plant, five sets of each treatment) at 25 days of cultivation by placing a marker at the base of the youngest accessible leaf. The distance between the marker and the base was measured after 24 h (Mateos-Naranjo et al., 2010). Fresh and dry weights of shoot were determined in five replicates (three plants of each set randomly selected) of control and FLT-treated plants. For dry weight estimation individual plants were oven-dried at 80 °C (Vanova et al., 2009) for 4 h.

2.5. Chl a fluorescence measurements

Chl *a* fluorescence measurements were performed using a Pulse amplitude-modulated (Dual PAM-100, Walz, Effeltrich, Germany) system in intact leaves (third leaf) of wheat plants. Plants were dark adapted for 30 min at 23 ± 2 °C before measurements. A weak modulated light (12 μE) was given to get minimal fluorescence (F_O), followed by actinic light (53 μE), and saturating pulse (6000 μE) to obtain maximum fluorescence (F_M). Leaves were exposed with saturating pulse (one pulse per min) for 10 min in order to obtain steady state fluorescence. Recording and calculation were performed with the Dual PAM system. Quantum Yield of PSII Y(II), yield of regulated energy dissipation Y(NPQ), yield of non-regulated energy dissipation Y(NO) and fraction of open PSII reaction centers (qL) were calculated following the equations given by Kramer et al. (2004).

$$\mathrm{YII} = (F_M' - F)/F_M'$$

$$Y(\mathrm{NPQ}) = 1 - Y(\mathrm{II}) - 1/(\mathrm{NPQ} + 1 + \mathrm{qL}(F_M/F_O - 1))$$

$$Y(\mathrm{NO}) = 1/(\mathrm{NPQ} + 1 + \mathrm{qL}(F_M/F_O - 1))$$

$$\mathrm{qL} = (F_M' - F)/(F_M' - F_O')F_O'/F$$

2.6. Gas exchange measurements

Single-leaf gas exchange rates were measured with a portable photosynthesis system (IRGA-LI-6400, LI-COR, Lincoln, NE, USA) which has chamber area of 6 cm^2. The measurements were made on portions of leaves (third leaf) exposed directly to the sunlight. Leaves were maintained at right angles to incident solar radiation. Photosynthetic parameters like net photosynthesis (Pn) (μmol CO_2 m^{-2} s^{-1}), internal CO_2 concentration (Ci) (μmol CO_2 mol^{-1}), stomatal conductance (Gs) (mol H_2O m^{-2} s^{-1}), transpiration rate (Tr) (mmol H_2O m^{-2} s^{-1}) and carboxylation capacity (Pn/Ci) (mmol m^{-2} s^{-1}) of the leaves were measured. Five measurements were made per plot. Measurements were made between 11:00 AM and 12:00 PM (Li et al., 2013; Kataria and Guruprasad, 2014) under natural sunlight, ambient temperature and CO_2 concentration. On clear days at noon photosynthetic photon flux density (PPFD) was 1300–1600 μmol m^{-2} s^{-1}, air flow (500 μmol s^{-1}), and CO_2 concentration (350–380 ppm).

2.7. Statistical analysis

Data was analyzed by using Graphpad Prism 5.01 software, Inc., La Jolla, CA, USA. Results were analyzed using one way analysis of variance (ANOVA) followed by Newman–Keuls Multiple Comparison test. Significance was determined at $p < 0.05$ and the results are expressed as mean values and standard deviation (SD) and all the assays were carried out in replicates (three to five sets of each analysis).

3. Results and discussion

3.1. Effect of FLT on seed germination, pigment content and growth

We explored the impact of PAH e.g. fluoranthene (FLT) on overall growth of wheat plants. The critical stages of development are early stages of ontogenesis, the germination and root elongation (Baud-Grasset et al., 1993). FLT inhibited all stages of plant growth starting from seed germination to biomass production. As compared to control plants, FLT treated plants showed inhibition in germination of seeds and late development of seedling. A slight decrease in rate of seed germination was observed at 5 μM (5%) while significant decrease in germination rate was observed at 25 μM FLT (11%, $p < 0.05$) treated seeds as compared to the control seeds (Table 1). The uptake of water is one of the main conditions of germination and transition of the seed from latent into the active stage, but the uptake of water by seeds was not found to be affected by FLT (data not shown). The change in germination rate of seeds may be because of change in the endogenous level of hormones (cytokinin and ethylene and ABA) in the presence of FLT (Kummerova et al., 2010, 2012). Beside seed germination, root and shoot elongation is regarded as a convenient indicator of the environmental toxicity. FLT induced a dramatic decrease in root and shoot length of wheat seedling after five days of germination. A 20% decrease in root length and 18% decrease in shoot length