ABSTRACT
Norflurazon (NF) is a herbicide that is known to inhibit carotenoid biosynthesis, destroy chlorophylls, desintegrate chloroplasts, and ruin chloroplast ribosome. In this study, we clarify how light intensity modulates effects of NF on CO\textsubscript{2} fixation and activity of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) in some C\textsubscript{3} and C\textsubscript{4} plants. We investigate effects of NF on maize, barley, triticale, and radish under low light intensity (0.12 and 0.4 W/m\textsuperscript{2}), and on maize, wheat, bean, and radish under high light intensity (100 W/m\textsuperscript{2}). We show that the effects are modulated by light intensity and species peculiarities. Specifically, C\textsubscript{3} plants, with no mechanism for CO\textsubscript{2} recycling, lose carbon compounds easier compared to C\textsubscript{4} plants, resulting in earlier manifestation of photodestruction under NF treatment. Besides, we show that Rubisco activity increases as far as its content decreases.

Keywords: CO\textsubscript{2} fixation, Rubisco activity, light intensity, norflurazon, C\textsubscript{3} plants, C\textsubscript{4} plants, carotenoids, chlorophylls
NF (Bolychevtseva et al. 1988). In the present study, we aim at further clarifying how light intensity modulates effects of NF on CO₂ fixation and Rubisco activity in C₄ and C₃ plants.

**MATERIAL AND METHODS**

**Plant material and treatment**

In the first set of experiments, effects of NF were studied under low light intensity, either 0.12 or 0.4 W/m². Seedlings of maize, barley, triticale (cultivar AD-206), and radish were sprouted from the seeds in Petri dishes with wet filter paper within a luminostate, either with (treatment) or without (control) 10⁻⁴ M NF solution added. During the experiment, temperature was 23°C.

In the second set of experiments, effects of NF were studied under high light intensity, 100 W/m². Seedling of maize, wheat, bean, and radish were sprouted from the seeds in containers with soil (d=0.43 m), either with (treatment) or without (control) 75 ml of 10⁻⁴ M NF added per container on the 14th day. Then, the seedlings were cultivated for additional 12 days.

**CO₂ fixation**

CO₂ fixation rate was measured using radioisotope method, based on ¹⁴CO₂ absorption by leaves (Rusinova et al. 1992). 20–500 g of leaves was put into a chamber with ¹⁴CO₂ (V=150 ml), and kept there for 1 min, either in darkness, or under different light intensity (0.12, 0.4, and 4 W/m²). ¹⁴CO₂ content in the chamber was 0.9 %, equal to 19 Ci/mol. Then, the leaves were fixed by 80 % ethanol. After centrifuging the plant samples, radioactivity was measured using the SL-30 and SL-4000 liquid scintillation counters (France).

**Rubisco content and activity**

To extract Rubisco, 1 g of leaves were milled with 5 ml of 50 mM Tris-HCl buffer (pH=8.0) containing 10 mM of MgCl₂, 5 mM of dithiothreitol, and 1 mM of EDTA (T=2–4°C). The homogenate was filtered, and the filtrate was centrifuged for 40 min under 23 000 g. In the supernatant, two characteristics were measured: (i) total soluble proteins (TSP) contents — on sedimentation by 3 % trichloracetic acid solution (Lowry et al. 1951), and (ii) Rubisco carboxylase activity — based on velocity of NaH¹⁴CO₃ incorporation into acid-resistant products in the presence of ribulose-1,5-bisphosphate under 30°C, using radioisotope method (Ezhova et al. 1997).

The enzyme mixture contained: 10 mM of MgCl₂, 10 mM of dithiothreitol, and 50 mM of Na₃CO₃ in 0.05 M Tris-HCl buffer (pH=8.0); 0.5 mM of ribulose-1,5-bisphosphate. The enzyme mixture was incubated for 2 min. Protein content did not exceed 50–100 µg. The control enzyme mixture contained the same ingredients except ribulose-1,5-bisphosphate; the ingredients were incubated for 10 min under 30°C.

To stop the reaction, an equal volume of 2 N HCl solution was added to the enzyme mixture. Protein sediment was removed by centrifuging. To get rid of ¹⁴CO₂ excess, supernatant was kept for 30 min under 70°C. Then, 0.025 ml of the sample was put into scintillation liquid (100 ml of SL 108 and 30 ml of ethanol), and radioactivity was measured using the SL-30 liquid scintillation counter (France). Rubisco activity was expressed in µM of ¹⁴CO₂ fixed by 1 mg of protein per 1 min.

Rubisco was separated from the extract using electrophoresis; its content was measured according to Dean & Leech (1982). Disc electrophoresis was carried out in polyacrylamide gel with linear concentration gradient 5 to 15 % (pH=8.9) (1964). To build the calibration curve, 0 to 240 µg of Rubisco from bean leaves was used. The method precision is 20 %.

**Pigment content**

Pigments were extracted by 80% acetone; their content was measured according to Bolychevtseva et al. (1987).

**RESULTS AND DISCUSSION**

Effects of NF on CO₂ fixation, TSP and Rubisco content, and Rubisco activity in leaves of 7-day maize, barley, triticale, and radish seedlings under low light intensity (the first set of experiments) are reported in Table 1. Under 0.12 W/m², NF treatment intensified CO₂ fixation in all the plants, up to 2 times. TSP content increased in all the plants (1.4–2 times). Rubisco content increased in barley, triticale, and radish (1.2–1.9 times), while substantially decreased in maize (3.6 times). Rubisco activity per its own content considerably increased in maize (2.3 times). At the same time, Rubisco activity per TSP contents decreased in all the plants (1.2 times – in barley, radish, and triticale, 2.3 times – in maize), possibly as a result of NF-activated biosynthesis of other proteins. Under 0.4 W/m², NF treatment intensified CO₂ fixation in barley, triticale, and radish, while inhibited it in maize. TSP content decreased in maize, barley, and triticale, while rose in radish (1.3 times). Rubisco content drastically decreased (up to 30 times) in all the plants except radish. The treated maize and barley seedlings showed higher Rubisco activity per its own content (1.6–2 times) compared to control. At the same time, all the plants demonstrated lower Rubisco activity per TSP contents under the treatment.

Effects of NF on TSP and Rubisco content, and Rubisco activity in leaves of 26-day maize, wheat, bean, and radish seedlings under high light intensity (the second set of experiments) are reported in Table 2. There, the same consistent pattern holds, i.e. Rubisco activity per its own content increases (1.5–2 times) while the enzyme content in contrast decreases (1.4–2.7 times). At the same time, Rubisco activity per TSP content did not change significantly. The latter can be explained by indirect effects of NF on the enzyme: 10⁻⁴ M NF solution did not affect Rubisco activity *in vitro*, which is in line with literature data (Mayfield et al. 1986).

Effects of NF on pigment content in leaves of 7-day maize, barley, and radish seedlings under low light intensity (the first set of experiments) are reported in Table 3. NF treatment reduced pigment content, with morepronounced effect under 0.4 compared to 0.12 W/m². The highest NF-resistance under low light intensity (0.12 and 0.4 W/m²) was observed in sciophytic radish, which demonstrated less decrease in carotenoid and chlorophyll content compared to barley and maize. This can be explained by species peculiarities, e.g. by metabolic pathways (C₃ vs C₄). In radish,
NF treatment under low light intensity (0.12 and 0.4 W/m²) did not reduce Rubisco content and activity, while did raise CO₂ fixation and TSP content (Table 1). On the other hand, relatively high chlorophyll content together with carotenoid deficiency under NF treatment in radish created preconditions for fast chloroplast photodestruction in a shifting experiment: when radish seedling grown under low light intensity (0.12 W/m²) were exposed to high light intensity (100 W/m²), total necrosis of leaves developed within 1 day. This fast photodestruction may be associated with overloading of pigment systems by excitation and consequent structural changes in PSII components (Fedtke 1979). In contrast, heliophilous maize seedlings, although containing less carotenoids and chlorophylls both in control and under NF treatment, did survive in the same shifting experiment, with no significant changes in pigments and TSP content, as well as in Rubisco activity (Table 4).

Earlier, Bolychevtseva et al. (1988) showed that NF treatment does not cause photodestruction of 70S ribosome under relatively low light intensity, although carotenoid and chlorophyll content significantly decreases. Thus, it seemed reasonable to expect no changes in chloroplast proteins biosynthesis. However, NF treatment did result in both TSP and Rubisco content in barley, triticale, and radish under 0.12 W/m² (Table 1). The small Rubisco subunit is synthesized in cytoplasm, while the large one – in chloroplasts; one may confer from this that NF activates protein biosynthesis in both cell departments. Under high light intensity, TSP content and Rubisco activity in C₃ and C₄ plants under different light intensity

<table>
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<tr>
<th>Table 1. Effects of norflurazon on TSP and Rubisco content, Rubisco activity, and CO₂ fixation in 7-day maize, barley, triticale, and radish seedlings under low light intensity (the first set of experiments)</th>
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<td>Light intensity, W/m²</td>
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<th>Table 2. Effects of norflurazon on TSP and Rubisco content, and Rubisco activity in 26-day maize, wheat, bean, and radish seedlings under high light intensity (the second set of experiments)</th>
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<td>Light intensity, W/m²</td>
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<th>Table 3. Effects of norflurazon on pigments content in 7-day maize, barley, and radish seedlings under low light intensity (the first set of experiments)</th>
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<th>Table 4. Effects of norflurazon on pigments and TSP content, and Rubisco activity in maize seedlings under change in light intensity (shifting experiment)</th>
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<td>Light intensity, W/m²</td>
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tensity (100 W/m²) photodestruction is more pronounced, regardless of age and regime of treatment and growing, which reduces Rubisco content but simultaneously raises Rubisco activity per its own content (Tables 1, 2).

Comparing radish and maize cases indicate that chloroplast photodestruction under NF is sensitized by chlorophyll due to carotenoid deficiency, which is in line with Bolychevtseva et al. (1988). Possibly, maize and other C₄ plants show decrease in CO₂ usage due to its recycling by phosphoenolpyruvate carboxylase, which biosynthesis is not affected by NF (Bolychevtseva et al. 1987). C₃ plants do not have such mechanism and therefore lose carbon compounds easier compared to C₄ plants, resulting in earlier manifestation of photodestruction.

Thus, light intensity differentially modulates effects of NF on CO₂ fixation and Rubisco activity in C₃ and C₄ plants. In maize, a C₃ plant, Rubisco content decreases under 0.12 W/m², despite carotenoid and chlorophyll deficiency. At the same time, it appears more resistant under high light intensity, possibly due to the mechanism for CO₂ recycling. This conclusion is in line with the results of Bolychevtseva et al. (1987, 1988) showing that Rubisco keeps on functioning under severe PSII inhibition caused by NF. Rubisco activity increases under decrease in its content – due to either intermediates formed under photodestruction or activated biosynthesis of some chloroplast enzymes (e.g. Rubisco activase which raises Rubisco affinity to CO₂) (Streu sand & Portis 1987).

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LITERATURE CITED


