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SHORT COMMUNICATION

INFLUENCE OF FOLIAR APPLICATION OF SIMAZINE, IGRAN, AND GS-14254 ON DELTA-AMINOLEVULINIC ACID DEHYDRATASE OF PEA SEEDLINGS

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Abstract—Foliar application of simazine (2-chloro-4,6-bis-ethylamino-*s*-triazine), igran (2-methylmercapto-4-ethylamino-6-isobutyl-amino-*s*-triazine), and GS-14254 (2-methoxy-4-isopropylamino-6-butylamino-*s*-triazine) on 3-week-old pea seedlings caused an increase in the activity of delta-aminolevulinic acid dehydratase, an enzyme involved in the biosynthesis of porphyrins, heme compounds, and chlorophyll.

INTRODUCTION

s-Triazines increase protein accumulation in several plant species,[1-6] which could be adequately explained by the enhanced rate of nitrate reductase,[7-9] transaminase, amylase, starch phosphorylase, adenosine triphosphatase,[9] and respiration.[8] When it was demonstrated that the sub-herbicidal levels of *s*-triazines did not decrease the dry weight and yield[10] or photosynthesis in plants, it was also anticipated that these compounds, at their sublethal levels, would lack the ability to decrease the chlorophyll contents.[10] In fact, our findings showed that foliar application of 2 mg/l. of simazine, igran, and GS-14254 did not change the chlorophyll content of pea seedlings. In the present communication, the effects of foliar application of 2 mg/l. of simazine, igran, and GS-14254 on the activity of delta-aminolevulinic acid dehydratase in leaves of pea plants are reported.

RESULTS AND DISCUSSION

Foliar applications of simazine, igran, and GS-14254 caused significant increases in delta-aminolevulinic acid dehydratase activity of pea leaves on all the three dates of harvest when compared with those of control plants (Table 1). The maximum stimulation of the enzymatic activity in this study was caused by GS-14254. These three *s*-triazines are different in the side chains of the molecules; however, they showed very similar effect when applied to plants. While Paromenskya and Lyalin[10] demonstrated that simazine decreased the chlorophyll content in green algae, our results indicated no measurable difference in the chlorophyll contents of treated and control plants (Table 2).

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Table 1. Influence of simazine, igran, and GS-14254 on the activity of delta-aminolevulinic acid dehydratase of pea leaves*

Days after treatments	Specific activity (μmoles PBG/mg protein/hr)			
	Control	Simazine	Igran	GS-14254
5	397	699†	681†	735†
10	405	673†	661†	720†
15	409	665†	674†	692†

* Analysis of variance and comparison of means by Tukey's ω-procedure.
† Significantly different at 0·01 level from control.

The formation of porphyrins, heme compounds, and chlorophyll requires delta-aminolevulinic acid dehydratase. It appears, then, that an increase in the activity of delta-aminolevulinic acid dehydratase would result in an increased rate of synthesis of porphyrins, and subsequently, heme compounds and chlorophyll. Since our study showed that foliar application of simazine, igran, and GS-14254 had no influence on the content of chlorophyll of treated pea plants, it is, therefore, possible that an increase in the enzymatic activity may result in more synthesis of heme compounds as cytochromes. This, in turn, might affect the electron transport system and give explanation that simazine increased the respiration rate of rye plants.[8]

Table 2. Influence of simazine, igran, and GS-14254 on the total chlorophyll of pea leaves*

Days after treatments	Total chlorophyll (mg/g fresh leaves)			
	Control	Simazine	Igran	GS-14254
5	4 77	4·85†	4 57†	4·59†
10	4·49	4·63†	4·60†	4·54†
15	4·64	4·72†	4·74†	4·78†

* Analysis of variance and comparison of means by Tukey's ω-procedure.
† Not significantly different at 0·05 level from control.

EXPERIMENTAL

Pea seeds (*Pisum sativum* L., cv Perfected Freezer, Joseph Harris Co., Rochester, New York) were sown in perlite in the greenhouse. The plants were watered with Hoagland solution twice a week. When the seedlings were 3 weeks old, 2 mg/l. solutions of simazine, igran, and GS-14254 were uniformly sprayed on the leaves. Triton B-1956 (Rohm and Haas, Philadelphia, Pennsylvania) was used as a surfactant. Control plants were sprayed with H_2O solution of the surfactant only The plants were harvested 5, 10, and 15 days after treatments

Delta-aminolevulinic acid dehydratase study: Enzyme extraction was made by an adaptation of the method described by Shetty and Miller.[11] A 2-g sample of leaves was homogenized for 2 min in a pre-chilled Ten Broeck homogenizer with 10 ml 0·05 M K-phosphate buffer, pH 7 4, containing 0·01 M cysteine. The homogenate was strained through four layers of cheesecloth and centrifuged for 15 min at 20,000 *g* The supernatent was used for enzyme assay. Enzyme activity was measured according to the method des-

[11] A. S. Shetty and G. W. Miller, *Biochem. J.* **114**, 331 (1969).

scribed by Mauzerall and Granick.[12] A standard reaction mixture contained 10 mM cysteine, 10 mM delta-aminolevulinic acid, and 10 mM Mg, in addition to the enzyme extract, and 50 mM K-phosphate buffer at pH 7·4 in a final volume of 1 ml. Incubation was at 37° for 30 min. The reaction was stopped by adding 1 ml 0·1 $HgCl_2$ in 10% TCA. The reaction mixture was centrifuged at 12,000 *g* for 10 min. The supernatant, 1·5 ml, was mixed with equal volume of Ehrlich's reagent (*p*-dimethylaminobenzaldehyde solution in glacial HOAc and $HClO_4$). The absorptivity at 555 nm was measured. The amount of porphobilinogen (PBG) formed was calculated by using $\epsilon = 6{\cdot}2 \times 10^4$. Protein was measured by the method of Lowry *et al.*[13] and chlorophyll by the method of Goodwin.[14]

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