

Effect of Some Herbicides as Plant Growth Regulators on the Chemical Composition of *Zea maize* Grains

Raga'a O. Osman,^a F. A. Ahmed,^b F. A. Khalil^c & M. S. Ali^a

^a National Research Center, Doki, Cairo, Egypt

^b Biochemistry Department, Faculty of Agriculture, Cairo University, Cairo, Egypt

^c Faculty of Agriculture, Tanta University, Egypt

(Received 7 January 1987; revised version received 16 February 1987;
accepted 16 July 1987)

ABSTRACT

Field experiments were conducted to evaluate the effect of the foliar application of four potent herbicides, i.e. atrazine, linuron, promotryn, and pyrazone at three different concentrations, on the chemical constitution of Zea maize grains. Chemical analyses of the grains indicated the following effects of the herbicides. The dry weight was increased generally with all concentrations of herbicides used; however, some concentrations caused a moderate decrease. All treatments increased the protein content of the grains; the increase varied from 4.93% to 20.7% according to the type of treatment. Some of the amino acids increased (e.g. threonine, serine and glycine) and others decreased (i.e. isoleucine, tyrosine, phenylalanine and arginine); hydroxyproline appeared after most treatments and was not found in the untreated plants. The total carbohydrate and soluble carbohydrate were decreased with all treatments.

INTRODUCTION

Maize (*Zea maize* L.) is considered as one of the important feeding crops in Egypt. Any improvement in the nutritional value of the grains is highly beneficial. Many attempts have been made to achieve this goal by using plant growth regulators or sublethal doses of herbicides to improve either the quality or the quantity of maize grains. Herbicides cause various physiological and biochemical effects on the growth and development of

emerging seedlings as well as the established plants. The various physiological and biochemical processes affected by herbicides are grouped into four broad categories. These are respiration, photosynthesis, protein and nucleic acid metabolism and hydrolytic enzyme activities (Rao, 1981). Generally, the sublethal effects of herbicides are characterized by the growth and yield of crops, carbohydrate and nitrogen metabolism, moisture content, seed germination, etc. Studies have shown that protein in various crops is increased considerably when sublethal concentrations of S-triazines are applied. For instance, Eastino & Davis (1967), Ries *et al.* (1968), Vergara *et al.* (1970) and Majundar & Gautam (1968) established that application of sublethal concentrations of S-triazine increased the protein in plants. Wu *et al.* (1970) reported that the application of S-triazine gave an increase in total soluble amino acids. On the other hand, Bharat *et al.* (1972) found that foliar application of simazine, prometryn and ferbutryne increased the protein content of sweet corn. El Shafey & Nadia (1973) reported that treatment of maize with atrazine increased dry matter and total nitrogen content in the plants. Shetty *et al.* (1973) found that foliar application of simazine or atrazine on maize decreased starch and sugar contents in grains. The same results were obtained by Gomaa *et al.* (1978).

The aim of the present investigation is to study the effect of spraying maize plants with sublethal concentrations of some herbicides on the chemical constituents of *Zea maize* grain, especially the protein, amino acid and carbohydrate contents.

MATERIALS AND METHODS

Materials

Source of grains and herbicides.

- (1) Grains of corn (*Zea maize* L.) cultivar Giza 2 were obtained from the Corn Crops Research Division, Agricultural Research Centre at Giza.
- (2) The herbicides were obtained from the Weed Control Research Section, Agricultural Research Centre at Giza, ARE. The herbicides used are:
 - (A) Atrazine (Atrex) (2-chloro-4-ethyl amino-6-methyl mercapto-1,3,5-triazine).
 - (B) Linuron (Afolon, Lorex) (3-(3,4-dichlorophenyl)-1-methoxy-1-methyl urea).

- (C) Prometryn (Caparol) (2,4-bis(isopropyl amino)-6-methyl-thio-1,3,5-triazine).
- (D) Pyrazon (Pyramin) (5-amino-4-chloro-2-phenyl-3-pyridazinone). Atrazine, pyrazon, prometryn and linuron at 10^{-5} , 10^{-7} and 10^{-10} M concentrations were used.

Methods

Farm experiment: Cultivation

The farm experiment was carried out at the Agriculture station of the National Research Centre, El-Kanater El-Khairia, Kaluobia. The plan of the experiment was that of a randomised block design. The soil was first ploughed twice and well levelled, then divided into rows. The area was divided into 39 plots with 5 rows per plot. Each row was 5 m long by 60 cm. The experimental plot was protected by a 5 m wide strip encircling the whole area. Grains were sown at a rate of five grains per hole. Three weeks after sowing, plants were thinned to one plant per hole. Fertilizers were applied at the usual rates and proper time. The different field practices were followed in the usual manner for corn cultivation.

The plants were sprayed three times. The first spray was two weeks after sowing, the second was two weeks later and the third one month after the second. Corn grains at harvest were collected to analyse protein, amino acids and carbohydrate contents.

Chemical analyses

- (1) *Moisture content* was determined as outlined in AOAC (1970).
- (2) *Determination of protein* was by the method reported by the AOAC (1970).

Determination of total hydrolysable carbohydrates

A known weight (0.2–0.5 g) of defatted corn grains was placed in a test tube, then sulphuric acid (10 ml 1N) was added. The tube was sealed and placed overnight in an oven at 100°C. The solution was then filtered into a measuring flask (100 ml) and filled to the mark with distilled water. The total sugars were determined colorimetrically according to the method of Dubois *et al.* (1956).

Determination of total soluble carbohydrates

The total soluble carbohydrates expressed as glucose were determined colorimetrically according to the method of Dubois *et al.* (1956). The insoluble carbohydrates were determined by difference.

Determination of total amino acids

Hydrolysis was carried out according to the method of Block *et al.* (1958). A 118 cl Beckman Amino Acid Analyser equipped with a column (6 mm × 22 cm) packed with Resin, Type W₃ (Beckman) was used. Ninhydrin was flowed at a rate of 22 ml/h and buffers (pH 2.83, 0.2 N, Li⁺: pH 3.70, 0.2 N Li⁺ and pH 3.75, 0.1 N Li⁺) were flowed at a rate of 44 ml/h. The temperature of the column was 40–65°C.

Identification of amino acids

The method of Giddings & Keller (1965) was used. Peak identification was performed by comparing the area of each peak representing the unknown amino acid with the area of a known amino acid.

RESULTS AND DISCUSSION

Moisture, dry weight, protein and carbohydrate contents of corn grain treated with tested herbicides are presented in Table 1.

Moistures

The moisture content varied according to the type of treatment. A slight increase or a noticeable decrease were recorded among the different compounds, depending upon the concentration of the tested compounds.

Protein content

The results in Table 1 show an increase in the protein content after all treatments except for pyrazon at 10⁻¹⁰M.

The results in the present investigation are similar to those obtained by Ries & Kenworthy (1963) and Gomaa *et al.* (1973). Bharat *et al.* (1972) found that foliar application of simazine, promotryn and torbutryne increased the protein content of meat corn grain growing under field conditions, the increase ranging from 15.2 to 8.33%, compared with the control. Gautam & Bhardwaj (1977) investigated the effect of sublethal doses of simazine and found a high protein content in maize. Wu *et al.* (1970, 1971) cited that S-triazine influences protein production of plants, not only by the effect on nitrate reductase, but also by enhancing the activities of other enzymes, including those of starch degradation (β -amylase and starch phosphorylase) and amino acid formation (*trans*-aminase). Stimulation in the activities of glutamate dehydrogenase, pyruvate kinase, cytochrome oxidase and starch

TABLE 1
Effects of some Herbicides at 10^{-10} , 10^{-7} and 10^{-5} M Concentrations on Dry Weight, Protein and Carbohydrate Contents in *Zea* maize

Compounds	Concentration (M)	Dry weight (%)	Protein (%)	Total hydrolyzable carbohydrate (%)	Soluble carbohydrate (%)	Insoluble carbohydrate (%)
Control		87.37	9.94	69.3	5.66	63.7
Atrazine	10^{-10}	89.06	11.6	68.0	4.99	63.0
	10^{-7}	88.85	11.6	68.7	5.37	63.3
	10^{-5}	87.20	11.2	66.5	5.35	61.1
Pyrazon	10^{-10}	86.71	9.77	67.8	5.34	62.5
	10^{-7}	87.90	10.4	67.7	5.39	62.3
	10^{-5}	89.61	12.0	64.0	4.21	59.8
Prometryn	10^{-10}	88.04	11.0	68.9	4.68	64.2
	10^{-7}	87.57	11.3	64.2	5.05	59.1
	10^{-5}	87.40	11.4	65.4	5.09	60.4
Linuron	10^{-10}	87.58	11.9	68.1	5.53	62.9
	10^{-7}	87.38	10.9	66.4	4.91	61.5
	10^{-5}	87.03	10.5	65.2	5.30	59.9

phosphorylase is the consequence of an increased rate of protein synthesis. The applied herbicides may effect the pathway of nitrogen, and increase the amino acids which are precursors of storage protein.

Carbohydrate content

Table 1 shows that all treatments generally decreased total hydrolysable, soluble and insoluble carbohydrate. The maximum decrease in total hydrolysable carbohydrate was shown with prometryn— 10^{-7} M (8.54% decrease) followed by pyrazon— 10^{-5} M and linuron— 10^{-5} M.

The decrease in carbohydrate content of different plants by treatment with triazine compounds has been reported by many investigators. Bharat *et al.* (1972) reported that simazine, torbutryne and prometryn decreased the total carbohydrate. Gomaa *et al.* (1973) found that Atrazine and Linuron plus Atrazine decreased the carbohydrate content. Also Singh *et al.* (1970) reported that starch and total sugars were decreased after treatment with S-triazine in beans, peas and maize. The same results were obtained by Shetty *et al.* (1973) and Gomaa *et al.* (1978). It was observed that S-triazine increased the activity of starch phosphorylase, followed by enhanced rates of pyruvate kinase and cytochrome oxidase activities which supports the view that S-triazine stimulates the utilisation of carbohydrate for amino acid and protein synthesis. On the other hand, Chodova & Zomanek (1971) observed that a reduction in respiration was related to a decrease in respiration substrates. Many reports indicate that triazine reduces the amount of glucose, fructose and/or sucrose in plants.

It is clear that a decrease in carbohydrate content of maize treated with atrazine, pyrazon, prometryn and linuron is related to the effect on respiration enzymatic systems and stimulates the utilization of carbohydrate for amino acid and protein synthesis.

Total amino acids

Table 2 indicates that all the herbicides used at different concentrations increase the total amino acids and produce an amino acid pattern which varies according to the type of treatment and concentration. For instance, 10^{-10} M among all the treatments was the most pronounced concentration. Some amino acids increased while others decreased markedly according to the different treatments; others disappeared or appeared in comparison with the untreated plants. This might be due to the conversion of some amino acids to others or to the effects of the herbicides used on the biosynthesis of protein. Singh & Salunkhe (1970) and others have proposed that

TABLE 2
Individual and Total Amino Acids (g/100 g) of Corn Grains treated with Atrazine, Pyrazon, Prometryn and Linuron at 10^{-10} , 10^{-7} and 10^{-5} M

Amino acids	Compounds														
	Control			Atrazine			Pyrazon			Prometryn			Linuron		
	10^{-10} M	10^{-7} M	10^{-5} M	10^{-10} M	10^{-7} M	10^{-5} M	10^{-10} M	10^{-7} M	10^{-5} M	10^{-10} M	10^{-7} M	10^{-5} M	10^{-10} M	10^{-7} M	10^{-5} M
Aspartic	0.56	0.79	0.96	0.45	0.34	0.72	0.58	0.41	1.86	1.15	0.80	0.25	1.15	0.80	0.25
Threonine	0.29	0.34	0.05	0.24	0.49	0.21	0.35	0.39	0.86	0.81	0.67	0.30	0.81	0.67	0.30
Serine	0.46	0.00	0.60	0.44	0.86	0.41	0.83	0.66	0.61	0.81	1.41	0.35	0.81	1.41	0.35
Glutamic	2.66	2.68	3.05	1.73	2.70	1.38	0.91	2.05	3.01	3.37	1.38	1.66	3.01	1.38	1.66
Proline	0.79	0.69	0.78	1.36	0.90	0.87	0.52	0.87	0.74	1.22	0.08	1.08	0.74	0.08	1.08
Glycine	0.32	0.55	0.45	0.41	0.43	0.43	0.24	0.33	0.39	0.60	0.07	0.06	0.60	0.07	0.06
Alanine	0.83	0.74	1.11	0.59	0.96	1.75	0.78	0.75	1.04	1.47	0.09	0.77	1.47	0.09	0.77
Valine	0.33	0.27	0.42	0.49	0.40	1.06	0.28	0.21	0.33	0.59	0.31	4.45	0.59	0.31	4.45
Cystine	0.04	0.08	0.13	0.10	0.11	0.01	0.02	0.01	0.05	0.11	0.59	0.02	0.11	0.59	0.02
Methionine	0.05	0.08	0.05	0.04	0.23	0.01	2.45	0.003	0.02	0.14	0.00	0.01	0.14	0.00	0.01
Isoleucine	0.20	0.07	0.32	0.37	0.27	0.25	1.17	0.07	0.10	0.26	1.49	0.12	0.26	1.49	0.12
Leucine	0.47	0.47	0.64	1.00	0.46	0.96	0.19	0.53	0.43	0.44	0.34	0.81	0.44	0.34	0.81
Tyrosine	0.30	0.33	0.03	0.23	0.30	0.23	0.33	0.22	0.20	0.14	0.00	0.34	0.14	0.00	0.34
Phenylalanine	0.35	0.42	0.04	0.34	0.41	0.38	0.39	0.32	0.26	0.12	0.61	0.48	0.12	0.61	0.48
Lysine	0.18	0.16	0.02	0.17	0.12	0.43	0.08	0.26	0.09	0.33	0.38	0.28	0.33	0.38	0.28
Histidine	0.16	0.23	0.18	0.17	0.17	0.36	0.13	0.17	0.12	0.17	0.27	0.22	0.17	0.27	0.22
Arginine	0.41	0.41	0.05	0.04	0.34	0.67	0.21	0.36	0.26	0.30	0.58	0.63	0.30	0.58	0.63
Hydroxyproline	0.00	0.15	1.05	1.23	0.47	0.00	1.22	1.08	0.95	1.21	0.00	0.94	1.21	0.00	0.94
Total	8.40	8.46	9.93	9.40	9.96	10.13	10.68	8.67	11.39	10.32	9.07	9.77	10.32	9.07	9.77

subherbicidal levels of triazine create a metabolic condition in which carbohydrates are used for protein synthesis. The herbicides tested disturbed the biosynthesis of amino acids.

After the atrazine treatments the data in Table 2 show that glycine, cysteine and histidine were increased, while lysine and arginine were decreased. Hydroxyproline was detected only in the treated plants. Variations in the amounts of individual amino acids after treatments depended upon concentration. At 10^{-10} M, noticeable increases in the amounts of some amino acids such as aspartic acid, glycine, phenylalanine and methionine were recorded, ranging from 7.62% to 75.0% over the control; on the other hand, the amounts of proline, lysine and alanine were decreased. Serine disappeared and hydroxyproline was detected in the treated plants.

Generally, the treatment with pyrazon increased methionine, isoleucine, serine and phenylalanine. Hydroxyproline appeared after treatment with 10^{-10} and 10^{-5} M herbicide while it was undetectable in the control. The data obtained indicate that pyrazon at 10^{-10} M produced the highest increase in the amount of methionine (381% over the control) of all herbicide treatments in this investigation.

Table 2 illustrates that prometryn at 10^{-10} M increased some amino acids from 1.25% (histidine) to 44.2% (lysine) and decreased others from 9.94% (arginine) to 93.8% (methionine) as compared with the control. The other two concentrations showed a different trend either by increasing or decreasing the amounts of amino acids as reported in Table 2.

In connection with linuron treatments the data show an increase in threonine, phenylalanine, lysine, histidine and arginine. At 10^{-10} M, a varied increase in some amino acids, from 6.13% (histidine) to 176% (threonine) was recorded. On the other hand, leucine and glutamic acid were decreased.

Our results are in good agreement with those reported by Bharat *et al.* (1972), Gomaa *et al.* (1973) and Singh *et al.* (1970). Wu *et al.* (1970) reported that application of S-triazine gave an increase in total soluble amino acids. Salumkhe *et al.* (1971) mentioned that foliar application of sublethal amounts of S-triazine compounds affected the synthesis of amino acids and protein. Gomaa *et al.* (1973) found that S-triazine and atrazine plus linuron applied to maize increased total lysine, valine and threonine significantly. This increase may be related to the effect of these herbicides on the biosynthesis of amino acids. Gomaa *et al.* (1978) studied the effect of herbicides on the chemical composition of maize grains and concluded that the herbicides improved protein and essential amino acids. Rajov (1981) mentioned that atrazine applied to maize caused a significant increase in the amino acid content compared with the control.

REFERENCES

- AOAC (1970). *Official methods of analysis of the Association of Official Agriculture Chemists*. (11th, edn), Washington, DC.
- Bharat, S., Omp, V., Ming, T., Wu, M. T. & Salunkhe, D. K. (1972). Effect of foliar application of S-triazine on protein, amino acids, carbohydrates, and mineral composition of pea and sweet corn seeds, bush bean pods and spinach leaves. *J. Agric. Food. Chem.*, **20**, 1256–59.
- Block, R. J., Durrum, B. L. & Zweig, G. (1958). *Manual of paper chromatography and paper electrophoresis*. (2nd edn), Academic Press, Inc., Publishers, New York.
- Chodova, D. & Zemanek, J. (1971). Effect of triazines on carbohydrate constituents. *Biologia. Pl.*, **13**, 234–42.
- Dubois, M., Smith, F., Gilles, K. A., Hamilton, J. K. & Rebers, P. A. (1956). Colorimetric method for determination of sugar related substances. *Anal. Chem.*, **28**, 350.
- Eastino, E. F. & Davis, D. E. (1967). Effect of atrazine and hydroxyatrazine on nitrogen metabolism of selected species. *Weeds*, **15**, 306–9.
- El Shafey, Y. H. & Nadia, O. Monged (1973). A comparative study between atrazine and hoeing treatments in controlling corn weeds. *Egyptian Journal of Botany*, **16**, 257–63.
- Gautam, R. C. & Bhardwaj, R. B. (1977). Effect of herbicidal and sub-herbicidal doses of Simazine on maize crops. *Indian Journal of Weed Sci.*, **9**(1), 53–60.
- Giddings, R. & Keller. (1965). Identification of amino acids. *Advances in Chromatography.*, **1**, 229–53.
- Gomaa, E. A. A., Oraby, F. T. & Zidan, E. M. (1973). Effect of method of weed control and nitrogen fertilizer on weed density and growth and yield of maize. *Moshtohor Res. Bull*, **9**, 241–46.
- Gomaa, E. A. A., El-Kalla, S. E. & Khorshid, A. M. (1978). Effect of weed control methods on yield and chemical composition of corn (maize) grain. *Fur Ackerund pflanzenbau*, **146**(3), 197–203.
- Majunder, J. C. & Gautam, O. P. (1968). Weed control studies in hybrid maize. 2. Effect on maize and on the following wheat crop. *Z. Phekrank. Bfcank. Pfe Schutz.*, **75**, 543.
- Rao, V. S. (1981). *Principles of weed science (A Reference Cum-Textbook)*. Oxford and EBH Publishing Co., New Delhi, Bombay, Calcutta.
- Rajov, R. K. (1981). Herbicidal influence on the formation of plant constituents in field maize. *Pesticide*, **15**(7), 17–19.
- Ries, S. K. & Kenworthy, A. L. (1963). The apparent influence of simazine on nitrogen nutrition of peach and apple trees. *Weeds*, **11**, 270–3.
- Ries, S. K., Schweizer, J. & Chmiel, H. (1968). The increase in protein content and yield of simazine-treated crops in Michigan and Costa Rica. *Bioscience*, **18**, 205–8.
- Salunkhe, D. K., Wu, M. T. & Singh, B. (1971). Effect of foliar application of S-triazine on protein amino acids in leaves of beans, peas and corn under growth room and greenhouse conditions. *J. Amer. Soc. Hort. Sci.*, **96**, 489.
- Shetty, S. V. R., Brar, H. S. & Furtick, W. R. (1973). The regulatory effects of two S-triazine herbicides on nutritive composition of seeds and leaves of sweet corn (*Zea maize* L.). *India, J. of Weed Science*, **5**(1), 33–37.

- Singh, B. & Salunkhe, D. K. (1970). Some metabolic responses of bush bean plants to sub-herbicide concentration of S-triazine compounds. *Can. J. Bot.*, **48**, 2213-17.
- Singh, R. P., Moolani, M. K. & Sharma, D. (1970). Maize response to simazine dosage. *Labdev., B.* (1971), **9**(34), 229-30 (W.A. **22**, 296).
- Tweedy, J. A. & Ries, S. K. (1967). Effect of simazine on nitrate reductase activity in corn. *Plant physiol.*, **42**, 280-2.
- Vergara, B. S., Miller, M. & Avelino, E. (1970). Effect of simazine on protein content of rice grain. *Coryza Satival. Agron. J.*, **62**, 269-72.
- Wu, M. T., Singh, B. & Salunkhe, D. K. (1970). Influence of foliar application of simazine, igran, GS-14254 on S-aminolevulinic acid dehydratase of pea seedlings. *Phytochemistry*. Also in: *Herbicides: Physiology, Biochemistry, Ecology* (Aduis, C. F. L. J. (Ed.)), Academic Press, New York.
- Wu, M. T., Singh, B. & Salunkhe, D. K. (1971). Effect of foliar application of sublethal concentrations of several S-triazines on protein and amino acids in leaves of beans, peas and corn. *Plant Physiol.*, **48**, 517.