TRANSACTIONS, ILL. STATE ACAD. SCI.
Vol. 67, No. 4 (1974)

VARIATION OF THE CONTENT OF 2,4-DIHYDROXY-7-METHOXY-2H-1, 4-BENZOXAZIN-3(4H)-ONE GLUCOSIDE IN CORN (ZEA MAYS L.)

A. J. Scism*, J. N. BeMiller†, and A. L. Caskey†

School of Medicine*, and Department of Chemistry and Biochemistry†
Southern Illinois University at Carbondale,
Carbondale, Illinois 62901

Resistance of corn plants to stalk rot and European corn borer has been related to the quantity of 2,4-dihydroxy-7-methoxy-2H-1, 4-benzoxazin-3(4H)-one glucoside. Values calculated as μg of 6-methoxy-2-benzoxazolinone (MBOA) per gram of fresh corn tissue ranged from 14 to 394. Variations (from 18 to 32 μg MBOA per g fresh weight) observed in samples of the same genetic background grown at different time periods appear to be due to differences in the amount of heat and light received by the plant.

Some cyclic hydroxamic acids are antibiotics and all form very stable chelates with the ferric ion. They may be ubiquitous in aerobic microorganisms, and are produced in large quantities under iron-deficient grown conditions (Emery, 1971).

One group of cyclic hydroxamic acids, the 2,4-dihydroxy-2H-1,4-benzoxazin-3(4H)-one glucosides, are found in corn, wheat, rye, and barley. After removal of the D-glucose unit, the free aglycons are converted into 2(3)-benzoxazolinones which are antifungal compounds. For example, 2,4-dihydroxy-7-methoxy-2H-1,4-benzoxazin-3(4H)-one (DIMBOA) can be converted into 6-methoxy-2-benzoxazolinone (MBOA), and the concentration of both has been related to the resistance of corn plants to stalk rot (BeMiller, 1965) and to the European corn borer (Klun, 1966, 1969, 1970; Loomis, 1957; Smissman, 1957, 1957a) and the resistance of wheat plants to stem rust (Elnaghy, 1962).

Various methods have been used for measurement of these compounds (TABLE 1). It is difficult to compare reported amounts because of differences in tissue samples used. Some workers used fresh plants, others used dried plants; some used the entire plant, others used only portions of the plant; and various sizes and genetic backgrounds have been used. Final results have been variously reported as content of DIMBOA, DIMBOA glucoside, and MBOA.

In spite of the difficulties, an attempt was made to compare values reported for corn tissue (Scism, 1972). The various methods and reported values are given in TABLE 1. In this table, values from the various sources have been changed to μg of MBOA per gram of fresh corn tissue by appropriate conversions of the values reported. Dry sample weights were converted to a fresh weight basis by arbitrarily assuming fresh samples to be 90% water. It is obvious that the reported values differ greatly.

Age is a factor that influences the amount of DIMBOA glucoside found in corn plants, for 5 times as much DIMBOA per gram of fresh weight was found in 14-day old seedlings as in 2-month-old plants (Virtanen, 1963).

An infrared method for the determination of 2,4-dihydroxy-2_H_-1,4-benzoxazin-3(4_H_)-one glucosides in plant tissues has been developed in this laboratory (Scism, 1974). The method involves freezing and thawing of tissue samples to allow enzyme-catalyzed cleavage of the glucosides, fragmentation, extraction of the released aglycons, removal of chlorophyll, conversion of the 2,4-dihydroxy-2_H_-1,4-benzoxazin-3(4_H_)-ones to the more stable 2(3)-benzoxazolinones, removal of the 2(3)-benzoxazolinones by extraction, and quantitative ir measurement of the 2(3)-benzoxazolinones in methylene chloride solution.

In connection with this study, precision of the ir method was determined using a group of 10 replicates of a B37 X B14 population of corn seedlings grown for 14 days in August 1971 (Scism, 1974). When results of this study were compared with those from other populations grown in the greenhouse for other periods of time between August and December 1971, considerable variation in the μg of MBOA per g of fresh tissue was found (range 180-316; mean 246). In general, the later in the year the corn was planted, the longer the period required for the plants to reach a height of 45-60 cm and the lower the MBOA content.

The differences in MBOA content might be related to differences in temperature and light experienced by the plants during their growth period. Seedlings grown in the dark exhibited lower levels of MBOA than seedlings grown in the light, but exposure of the seedlings grown in the dark to light for a period prior to sample collection gave a fourfold increase in MBOA (Loomis, 1957).

A relationship between the time of planting and susceptibility to stalk rot in corn has been noted (Pappelis, 1966). Corn planted in cooler weather (1 April, 15 May) showed more stalk rot than corn planted later (15 June, 1 July). When the cold, wet conditions under which corn had been damaged by atrazine (to which it is normally tolerant) were simulated, it was found that root extracts from plants grown at lower temperatures (10°C to 15°C) had a decreased capacity to hydroxylate atrazine, compared to root extracts from plants grown at higher temperatures (25°C to 30°C), because of decreased amounts of benzoxazinones in roots of the plants grown at the lower temperatures (Thompson, 1970).

Thus, comparisons of the DIMBOA glucoside content found in different corn populations should be made from samples which have been grown under the same conditions, for it appears that samples of a single genetic background will have different contents of DIMBOA glucoside if they have different histories with respect to temperature and illumination. Seeds should be planted at the same time, in the same place; plants should experience the same growing conditions and be harvested at the same time, and the same portions of the plant should be taken for samples if valid comparisons and genetic selections are to be made.

Further research relating the amount of heat and light received by corn plants and the amount of available iron to their content of DIMBOA glucoside seems to be needed.

Table 1. -- Comparison of Selected Reported Values for DIMBOA Glucoside Content of Corn Tissue[a]

Substance reported	Corn type	Technique	Plant part	Quantity[b] as MBOA	Ref.
MBOA	W210D W204	isolation	tops	14-110	Loomis, 1957
DIMBOA		isolation	tops	150	Wahlroos, 1959
DIMBOA glucoside		isolation	tops	394[c]	Wahlroos, 1959
MBOA	R105-204 R105-206	fluorescence	whorl	180 50	Bowman, 1968
MBOA	C.I.31A Oh 45 WF9 B37 B14	isotopic dilution	whorl	214 100 16 20 32	Klun, 1966
DIMBOA	C.I.31A X B49 W22 X R101 B37 X B14	isotopic dilution	whorl	170 54 28	Klun, 1970
MBOA	B37 X B14	infrared	seedlings	32	Scism, 1974
MBOA		isolation	tops	15	Smissman, 1957
DIMBOA		isolation	etiolated shoots	390	Hamilton 1962

Table 1. (continued)

Substance reported	Corn type	Technique	Plant part	Quantity[b] as MBOA	Ref.
DIMBOA	Early Albert	isolation		70-350	Virtanen, 1963
DIMBOA	C.I.31A	isolation	etiolated tops	150	Tipton, 1967
MBOA	C.I.31A WFO	isotopic dilution	whorl	394 50	Klun, 1969

[a]When values were reported for different plant sizes and cultivars, the value for the plant size closest to that used by us was chosen, as were values for the cultivars which gave the extreme limits.

[b]μg per g of corn (fresh weight).

[c]Known to be quite impure.

LITERATURE CITED

BEMILLER, J. N., and A. J. PAPPELIS. 1965. 2,4-Dihydroxy-7-methoxy-1,4-benzoxazin-3-one glucoside in corn. I. Relation of water-soluble, 1-butanol-soluble glycoside fraction content of pith cores and stalk rot resistance. Phytopathology 55:1237-1240.

BOWMAN, M. C., M. BERZOA, and J. A. KLUN. 1969. Spectrophotofluorometric determination of 6-methoxy-2-benzoxazolinone, an indicator of resistance to European corn borer in Zea mays. J. Econ. Entomol. 61:120-123.

ELNAGHY, M. Z., and P. LINKO. 1962. The role of 4-0-glucosyl-2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one in resistance of wheat to stem rust. Physiol. Plant. 15:764-771.

EMERY, T. 1971. Hydroxamic acids of natural origin. Advan. Enzymol. 35:135-185.

HAMILTON, R., R. BANDURSKI, and W. REUSCH. 1962. Isolation and characterization of a cyclic hydroxamate from Zea mays. Cereal Chem. 39:107-113.

KLUN, J. A., and T. A. BRINDLEY. 1966. Role of 6-methoxybenzoxazolinone in inbred resistance of host plant (maize) to first-brood larvae of European corn borer. J. Econ. Entomol. 59:711-718.

KLUN, J. A., W. D. GUTHRIE, A. R. HALLAUER, and W. A. RUSSELL. 1970. Genetic nature of the concentration of 2,4-dihydroxy-7-methoxy-2H-1,4-benzoxazin-3(4H)-one and resistance to the European corn borer in a diallel set of eleven maize inbreds. Crop Sci. 10:87-90.

KLUN, J. A., and J. F. ROBINSON. 1969. Concentration of two 1,4-benzoxazinones in dent corn at various stages of development of the plant and its relation to resistance of the host plant to the European corn borer. J. Econ. Entomol. 62:214-220.

LOOMIS, R. S., S. D. BECK, and J. F. STAUFFER. 1957. The European corn borer, Pyrausta nubilalis (Hubn), and its principal host plant. V. A chemical study of host plant resistance. Plant Physiol. 32:379-385.

PAPPELIS, A. J., and L. V. BOONE. 1966. Effect of planting date on stalk rot susceptibility and cell death in corn. Phytopathology 56:829-831.

SCISM, A. J. 1972. Determination of 2,4-dihydroxy-7-methoxy-1,4(2H)-benzoxazin-3-one glucosides in corn (Zea mays L.). Ph.D. Dissertation. Southern Illinois University at Carbondale. 148 pp.

SCISM, A. J., J. N. BEMILLER, and A. L. CASKEY. 1974. Determination of 2,4-dihydroxy-1,4(2H)-benzoxazin-3-one glucosides in corn (Zea mays L.). Anal. Biochem. 58:1-13.

SMISSMAN, E. E., J. B. LAPIDUS, and S. D. BECK. 1957. Corn plant resistance factor. J. Org. Chem. 22:220.

SMISSMAN, E. E., J. B. LAPIDUS, and S. D. BECK. 1957a. Isolation and synthesis of an insect resistance factor from corn plants. J. Amer. Chem. Soc. 79:4697-4698.

THOMPSON, L., Jr., F. W. SLIFE, and H. S. BUTLER. 1970. Environmental influence on the tolerance of corn to atrazine. Weed Sci. 18:509-514.

TIPTON, C., J. KLUN, R. HUSTED, and M. PIERSON. 1967. Cyclic hydroxamic acids and related compounds from maize. Isolation and characterization. Biochemistry 6:2866-2870.

VIRTANEN, A. I., and O. WAHLROOS. 1963. Absence of 6-methoxybenzoxazolinone in uninjured corn tissue. J. Pharm. Sci. 52:713-714.

WAHLROOS, O., and A. I. VIRTANEN. 1959. The precursors of 6-methoxybenzoxazolinone in maize and wheat plants, their isolation, and some of their properties. Acta Chem. Scand. 13:1906-1908.