dye 2,3,5-triphenyltetrazolium chloride was only weakly reduced in the copper-deficient plants. Maximum reduction of TTC was obtained in plants grown on a naturally calcareous soil or an organic soil to which copper was added.

Comparative ascorbic acid oxidase activity, catalase activity and the concentration of copper and iron in corn and wheat showed that the reduction of 2,3,5-triphenyltetrazolium chloride in these plants could best be correlated with copper than with iron nutrition.

Literature Cited

1. Allen, P. J. and Goddard, D. R. A respiratory study of powdery mildew of wheat. Amer. Jour. Bot. 25: 613–621. 1938.
2. Brown, A. H. and Goddard, D. R. Cytochrome oxidase in wheat embryos. Amer. Jour. Bot. 28: 319–324. 1941.
3. Brown, J. C. and Hendricks, S. B. Enzymatic activities of copper and iron deficiencies in plants. Plant Physiol. 27: 651–666. 1952.
4. Brown, J. C. The effect of a dominant iron or copper requiring metabolic system in the plant on the development of lime-induced chlorosis. Submitted for publication in Plant Physiol.
5. Dufrenoy, J. and Pratt, R. Histophysiological localization of the site of reducing activity in stalks of sugar cane. Amer. Jour. Bot. 35: 211–229. 1948.
6. Goddard, D. R. Metabolism in relation to growth. Growth: Supplement to Vol. XII. 17–45. 1948.
7. Hewitt, E. J. and Agarivala, S. C. Reduction of triphenyltetrazolium chloride by plant tissues and its relation to molybdenum status. Nature 169: 545–546. 1952.
8. James, W. O. The terminal oxidases in the respiration of the embryos and young roots of barley. Proc. Roy. Soc. London B 141: 289–299. 1953.
9. Lakon, Georg. Topographischer Nachiveis der Keimfahgkeit der Getreidefruchte durch Tetrazoliumsalze. Ber. Deutsch. Bot. Ges. 60: 299–305. 1942.
10. Porter, H. H., Durall, M. and Romm, H. J. The use of 2,3,5-triphenyltetrazolium chloride as a measure of seed germinability. Plant Physiol. 22: 149–159. 1947.

THE EXTRACTION AND COLORIMETRIC ESTIMATION OF INDOLE-3-ACETIC ACID AND ITS ESTERS IN DEVELOPING CORN KERNELS [1, 2, 3]

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The chemical identification of auxin, indole-3-acetic acid (IAA), isolated from the ethanol extracts of immature corn kernels by Haagen-Smit, Dandliker, Wittwer and Murneek (3) initiated considerable interest in both the quantitative and qualitative aspects of the auxin economy of corn kernels during their ontogeny. Avery, Berger, and Shalucha (1), Wittwer (8) and Stehsel (6) have studied these changes in auxin content during the development of the corn kernel. Recently Redemann, Wittwer and Sell (5) have reported the isolation of ethylindole-3-acetate from corn kernels and found that this substance was approximately 100 times more effective than IAA as a tomato fruit-setting agent.

To supplement biological methods of auxin assay, a colorimetric method was first described by Mitchell and Brunstetter (4). This procedure was later improved by Tang and Bonner (7), and by Gordon and Weber (2). Essentially, the method takes advantage of the red coloration formed through the mild oxidation of IAA.[4] Although sensitive, it is not always suitable for the determination of the auxin concentration in plant materials because of the presence of other pigments and the frequent formation of relatively stable colloidal suspensions. Since difficulties are encountered with extracts of plant materials, it seemed advisable to describe further refinements of the colorimetric methods developed in this laboratory and especially those procedures adapted for the quantitative determination of IAA and its esters in corn kernels during the various stages of development.

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[2] Journal Article No. 1465 from the Michigan Agricultural Experiment Station, East Lansing, Michigan.

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[4] This oxidation product will be described in another publication.

Experimental

Colorimetric Measurements: The procedure is modified from that of Gordon and Weber (2), in that 0.05 M ferric chloride in 10% (by volume) perchloric acid was used to develop the red oxidation product, maximum intensity of which was attained in 45 minutes when held at 30°C. The 10% perchloric acid was used in preference to 35% to facilitate extraction in the final separation. The red color was quantitatively extracted with isobutyl alcohol, and the isobutyl alcohol layer centrifuged to remove the suspended water droplets. Since the red color is unstable in this medium, the time after extraction to reading was standardized at five minutes; the absorbancy was determined at 530 mμ on a Beckman D.U. spectrophotometer. Under these conditions, Beer's law was obeyed over a concentration range of 0 to 0.8 mg/25 ml isobutyl alcohol and an absorbancy index of 2.7 ml (cm mg IAA)$^{-1}$.

Application of the Method to Plant Material: To ascertain the accuracy of the method, weighed amounts of IAA were added to 500 gm samples of immature corn kernels at the milk stage and macerated with ethyl acetate in a Waring blendor. The

resultant pulp was centrifuged, the solvent decanted, and the centrifugate washed 3 successive times with 25 ml portions of ethyl acetate. The combined ethyl acetate fractions were extracted with 25 ml of 2% Na_2CO_3. Two 10 ml aliquots of the aqueous layer were added to each of two 25 ml volumetric flasks. To one of the flasks 5 ml of 0.05 ferric chloride in 10% perchloric acid solution were added and both flasks made to volume with 10% perchloric acid. After permitting the reagent to react for 45 minutes, both solutions were extracted with 25 ml of isobutyl alcohol. The absorbancy of the color developed in the flask containing the iron solution was compared with the control having no iron solution. A similar experiment as a blank was made using corn kernels to which no IAA had been added. The readings obtained from the latter procedure were subtracted from those obtained by the addition of weighed quantities of IAA to the corn extracts. The results of these determinations are tabulated in table I. With this procedure esters of IAA do not interfere. However, if one refluxes the esters in 3% alcoholic KOH solution, the esters are completely hydrolyzed at the end of one hour and the same method may be used to determine the ester content in the corn kernels.

TABLE I

DETERMINATION OF VARIOUS QUANTITIES OF INDOLE-3-ACETIC ACID ADDED TO CORN KERNELS TO ESTABLISH THE VALIDITY OF THE COLORIMETRIC ASSAY

MG IAA ADDED	MG PER 500 GM MEASURED	MG PER 500 GM MINUS BLANK	PERCENT ERROR
0	4.0	...	..
12.7	16.5	12.5	-2
19.7	24.0	20.0	+2
12.8	16.2	12.2	-5
18.6	22.5	18.5	-1

DETERMINATION OF INDOLE-3-ACETIC ACID AND ITS ESTERS IN CORN KERNELS: Eight samples of corn kernels were collected at various stages of development from pre-fertilization through maturity. Immediately after harvest the kernels were cut away from the cobs, placed in sealed pliofilm bags, and stored at -25°C. For analysis, one kg (fresh weight) samples were macerated with ethyl acetate in a Waring blendor, and extracted as outlined above to estimate the quantity of IAA. Esters of IAA were determined first by removing the ethyl acetate in vacuo and then refluxing with 3% alcoholic KOH for 1.5 hours to hydrolyze the esters of IAA. The alkaline solution was neutralized to litmus with 10% HCl and the alcohol removed under reduced pressure. The residue was dissolved in ethyl acetate and the esters, as the equivalent of the IAA formed, were determined colorimetrically by the procedures previously described. The results of these determinations are tabulated in table II.

Dry weights were obtained by taking representative 20 gm fresh weight samples at each stage of maturity, drying at 70°C and 5 mm pressure, reweighing, and converting the fresh weights to the equivalent dry weights by these factors.

TABLE II

THE COMPARATIVE CONCENTRATIONS OF INDOLE-3-ACETIC ACID AND ITS ESTERS IN CORN KERNELS AT VARIOUS DEVELOPMENTAL STAGES

STAGE OF DEVELOPMENT	μG IAA PER KG	μG IAA PER 100 MG	μG IAA ESTERS PER KG	μG IAA ESTERS PER 100 MG
	(*fresh weight*)	(*dry weight*)	(*fresh weight*)	(*dry weight*)
Pre-fertilization ..	525	0.891	None	None
Fertilization	525	0.891	None	None
Blister	750	0.990	None	None
Young milk	1275	0.768	None	None
Intermediate milk	2175	0.828	1500	0.571
Late milk	1050	0.281	800	0.214
Dough	870	0.219	560	0.141
Mature	None	None	None	None

SUMMARY

A colorimetric method for the separate determinations of IAA and its esters as they occur in developing corn kernels is presented. While some IAA was present at all stages except in the mature kernels, it reached a peak in fresh corn during the intermediate milk stage. The tomato fruit-setting factor, ethylindole-3-acetate was found only in kernels which had matured to—or beyond—the intermediate milk stage. The highest concentration of the ester occurred at the same stage of development which characterized the peak in concentration of IAA.

LITERATURE CITED

1. AVERY, G. S., BERGER, J. and SHALUCHA, B. Auxin content of maize kernels during ontogeny from plants of varying heterotic vigor. Amer. Jour. Bot. 29: 765–772. 1942.
2. GORDON, S. A. and WEBER, R. P. Colorimetric estimation of indoleacetic acid. Plant Physiol. 26: 192–195. 1951.
3. HAAGEN-SMIT, A. J., DANDLIKER, W. B., WITTWER, S. H. and MURNEEK, A. E. Isolation of 3-indoleacetic acid from immature corn kernels. Amer. Jour. Bot. 33: 118–120. 1946.
4. MITCHELL, J. W. and BRUNSTETTER, B. C. Colorimetric methods for the estimation of 3-indoleacetic acid. Bot. Gaz. 100: 802–816. 1939.
5. REDEMANN, C. T., WITTWER, S. H. and SELL, H. M. The fruit-setting factor from the ethanol extracts of immature corn kernels. Arch. Biochem. and Biophys. 32: 80–84. 1951.
6. STEHSEL, M. Thesis, Univ. of California, Berkeley. 1950.
7. TANG, Y. W. and BONNER, J. Enzymatic inactivation of indoleacetic acid. I. Some characteristics of the enzyme contained in pea seedlings. Arch. Biochem. 13: 11–25. 1947.
8. WITTWER, S. H. Growth hormone production during sexual reproduction of higher plants. Univ. Missouri Research Bull. 371. 1943.