BRIEF PAPERS

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 Murneck (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 Stehsl (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. 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.

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 nm 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 blender. The

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
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% Na₂CO₃. 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.

**Determination of Various Quantities of Indole-3-acetic Acid Added to Corn Kernels to Establish the Validity of the Colorimetric Assay**

<table>
<thead>
<tr>
<th>Mg IAA added</th>
<th>Mg per 500 gm measured</th>
<th>Mg per 500 gm minus blank</th>
<th>Percent error</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>4.0</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>12.7</td>
<td>16.5</td>
<td>12.5</td>
<td>- 2</td>
</tr>
<tr>
<td>12.8</td>
<td>24.0</td>
<td>20.0</td>
<td>+ 2</td>
</tr>
<tr>
<td>18.6</td>
<td>22.5</td>
<td>18.5</td>
<td>- 1</td>
</tr>
</tbody>
</table>

**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, ethylnidole-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**