Promotion of Sorghum Callus Growth by the s-Triazine Herbicides

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ABSTRACT

Growth-promoting action of simazine and other s-triazine herbicides was detected by the use of sorghum (Sorghum bicolor [L.] Moench) callus tissue and the chlorophyll retention test. Soil application of simazine [2-chloro-4,6-bis(ethylamino)-s-triazine] at sublethal levels nearly doubled the growth-promoting action of sorghum root exudates. Treated plants yielded up to 26% more total protein than untreated plants. This indicated that the level of callus growth-promoting action in the root exudate of the plant has a positive effect on its final total protein yield and confirms a positive effect of simazine on total protein content in certain instances. The results may provide a new understanding of the mode of action of s-triazines applied at sublethal levels in increasing protein content and certain enzymic activities of treated plants. It is speculated that the growth-promoting action of these herbicides is hormonal in nature and most likely kinetin-like.

Several studies over a wide spectrum of economic crops showed that the application of the s-triazine herbicides (mainly simazine) to plants at sublethal levels may cause an increase in total protein content as well as alteration of certain biological activities such as nitrate reductase (17, 18, 21, 26). Since all known cytokinin active compounds are found to be nucleotide derivatives (4, 5, 10, 11), and the s-triazine ring is pyrimidine-like in structure with the number 5 carbon atom replaced by a nitrogen atom, it is possible that the action of the s-triazines in increasing protein content is due to alteration in hormonal control similar to that caused by kinetin.

No chemical method has been developed to detect cytokinins easily. There are many biological assay systems to detect them, of which the callus tissue system may be the most reliable (13). Sorghum callus tissue developed in our laboratories from the hybrid RS 671 grain sorghum (Sorghum bicolor [L.] Moench) was dependent on an external source of cytokinin, which made it an excellent material to use in bioassays to detect the suspected growth-promoting action of the s-triazines. This paper reports that the effect of sublethal levels of simazone and other s-triazines

MATERIALS AND METHODS

Growth and Treatment of Plants. Seeds of RS 671 hybrid grain sorghum (Sorghum bicolor [L.] Moench) were planted in 2-liter containers filled with soil and placed in growth chambers set on 14-hr days at 27 C and a light intensity of 240 umole/m²/sec. After germination the plant population was adjusted to two plants per container. The plants were harvested and the root exudates were collected 10 weeks after planting dates. Four containers (eight plants) were assigned for each treatment.

s-Triazine Treatments. The s-triazines used in this investigation were: (a) Simazine, 2-chloro-4,6-bis(ethylamino)-s-triazine; (b) Atrazine, 2-chloro-4-ethylamino-6-isopropylamino-s-triazine; (c) Propazine, 2-chloro-4,6-bis(isopropylamino)-s-triazine; (d) Prometryne, 2-methoxy-4,6-bis(isopropylamino)-s-triazine; (e) Prometryne, 2-methylmercapto-4,6-bis(isopropylamino)-s-triazine; (f) Ametryne, 2-methylmercapto-4-isopropylamino-6-ethylamino-s-triazine.

Soil applications of simazine were made to the containers 2.5 cm below the surface by removing the top 2.5 cm of soil, spraying the calculated dose suspended in sufficient amount of water, and then replacing the soil. The dose was calculated as a fraction of the recommended field application rate corresponding to the area of the container surface. The soil treatments of simazine were added at concentrations 0.25, 0.5, 0.75 and 1.00 times the recommended amounts for application as a herbicide in the field (1.12 kg/ha). The application took place immediately prior to planting. A foliar application was administered 4 weeks after planting at concentrations 0.25, 0.5, 0.75 and 1.00 times the simazine saturated solution of 5 µg/ml (25). The plants were sprayed once until completely soaked with the solution.

Root Exudate Sampling. The aerial portions of the plants were removed 3.5 cm above the soil surface and a piece of parafilm rolled around the stubs which extended above the cut surfaces. The exudates were collected periodically by means of a syringe until 5 to 10 ml was accumulated from each exuding plant. The exudates were filtered through Whatman No. 1 filter paper and added to the callus growth medium to test for their growth-promoting action.

Testing for Growth Promotion. Callus was induced from hybrid RS 671 on Murashige and Skoog medium (15) supplemented with 2 to 5 µg/ml of 2,4-D and 1% (v/v) sorghum seed extract (16). The seed extract was prepared by extracting 400 g of germinated sorghum seeds in 1 liter of 95% ethyl alcohol in a Waring Blender. After filtration through cheesecloth the ethyl alcohol was removed by evaporation. The volume of the resulting concentrated solution was made up to 1 liter by addition of deionized H₂O and then filtered through Whatman No. 1 filter paper. The resulting filtrate was stored frozen and used as needed. The

1 Published as Paper No. 3939, Journal Series, Nebraska Agricultural Experiment Station. Research reported was conducted under Project No. 12-69, and was partially supported by grants from the Rockefeller Foundation and United States Agency for International Development.
growth media were prepared as usual except that the cytokinin source was replaced by the material to be tested. The concentrations used for the materials tested were as follows: zeatin and s-triazines 0.5 μg/ml (w/v), nucleotide bases 1 and 10 μg/ml (w/v), sorghum seed extract 1% (v/v) and root exudates 10% (v/v). The media were distributed into small vials at 7 ml/vial. After the media were autoclaved and cooled, a 50- to 100-mg portion of callus was transferred to each vial. Vials were incubated at room temperature in the dark for 4 to 5 weeks. The callus was removed, blotted on filter paper to remove excess moisture, and weighed to the nearest milligram. The mean value of at least eight replicates of each treatment was used for treatments' comparison. Both fresh and dry weights were recorded. The regression of dry weight on fresh weight was found to be 0.926, thus only fresh weight values were reported.

Chlorophyll Retention Test. Three-centimeter sections of 7-day old Avena sativa seedling leaf tips were used as the tissue source. Ten sections were placed in Petri dishes containing two layers of filter paper saturated with the desired concentration of s-triazines to be tested. The s-triazines were the same as those tested with callus tissue. Solutions were tested at saturated and 5 μg/ml concentrations for all s-triazines compared to 8 μg/ml kinetin solutions. This concentration of kinetin was the optimum for retention of Chl under the conditions of the test used in this experiment. Eight replicates of each treatment were placed in the dark for 72 hr at room temperature. Chlorophyll was then extracted by boiling the leaf sections in 12 ml of 80% (v/v) ethanol for 10 min. The absorbance was determined at 652 nm on solutions made to 15 ml final volume.

**RESULTS AND DISCUSSION**

The general concept of the biological functions of auxins and cytokinins is that the former controls cell expansion while the
latter controls cell division. This classification is not consistent
but differs with different plants investigated. Haber and Luijpool
(6) found that the mechanism by which kinetin stimulated lettuce
seed germination was related to the initiation of cellular expansion
and not to cell division. Yamaki (27) on his work with mung bean reported that auxins not only control cell expansion,
but also regulate cell division and enzyme synthesis by binding
to a special type of soluble RNA. To ensure that the increase
of callus weight over the control was not due to natural auxins
present in the root exudate or an auxin-like action of the com-
ounds tested, a concentration test was conducted using 2,4-D
on media with no cytokinin. The results in Table I substantiate
that increasing the auxin concentration above 2.5 µg ml did not
cause increased callus growth. In fact, average callus weight
decreased significantly with the increase of auxin concentration
over 5 µg ml in the medium.

Growth Promotion of Sorghum Callus by the s-Triazine Herbi-
cides. Simazine was used with sorghum plants to study the influence
of a sublethal herbicidal application on total protein content
and the cytokinin activity in root exudate of the treated
plants. The two highest doses of the soil application (0.75 and
1.00 times the recommended amount for field application as
herbicide) were lethal to most plants. Therefore, these two treat-
ments were excluded from the experiment. Protein content,
determined as per cent of dry matter, was significantly higher in
the soil-treated plants, while their fresh weight production per
pot was lower than that of the untreated plants (Table II). The
protein values, when corrected to a constant weight, still showed
an increase for the 0.25 and 0.5 treatments over the control. The
increase in the protein content caused by the 0.5 treatment was
much higher than that caused by the 0.25 treatment. Probably,
the 0.5 treatment would be considered the optimum level of
application for simazine to produce maximum protein increase
in the treated plants under conditions of this experiment.

Growth-promoting action of the root exudates of treated and
untreated plants, as measured by fresh callus weight, is shown
in Figure 1. These results confirmed the presence of callus growth
promoters in the root exudate of sorghum plants. Soil applica-
tions of simazine to sorghum plants, at sublethal levels, pro-
duced a significant increase in the growth-promoting action of
the root exudate of the treated over the untreated plants. Foliar
applications had no influence on the growth-promoting action
of the root exudates.

This parallelism between total protein content of the plant and
the growth-promoting action in root exudate might be a cause
and effect relationship. In other words, the more growth pro-
moters transported upward, the more protein synthesized by
the plant cells. This kind of relationship also might apply to the root
application of simazine as long as the optimum level of applica-
tion was maintained. These findings would support the idea sug-
gested by Maranville et al. (12) that the action of simazine for
increasing protein content of the treated plants might be due to
kinetin-like action. The low solubility, volatility, and photo-
degradation of simazine or the suspected slow translocation of
growth-promoting substances through the leaves may be respon-
sible for the lack of response from the foliar applications. Sima-
azine and atrazine have been reported to move readily into roots
and then to stems and leaves of corn and sorghum (14, 19). The
uptake was proportional to the simazine concentration in the
soil (19). Roeth and Lavy (20) found that atrazine was metabo-
lized in sorghum primarily to 2-chloro-4-amino-6-(isopropyl-
amino)-s-triazine. In other words, the s-triazine ring remains

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**Fig. 3. Growth-promoting action of DNA bases, six s-triazines, seed extract and zeatin as measured by the increase in sorghum callus fresh weight.**
Table III. Effect of s-Triazine Compounds on Chl Retention in Avena sativa Leaf Tips as Compared to Kinetin

<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentration in Solution (mg/l)</th>
<th>Op Absorbance at 652 nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kinetin</td>
<td>5</td>
<td>0.601a</td>
</tr>
<tr>
<td>Ametryne</td>
<td>5</td>
<td>0.356b</td>
</tr>
<tr>
<td>Prometone</td>
<td>5</td>
<td>0.373c</td>
</tr>
<tr>
<td>Atrazine</td>
<td>5</td>
<td>0.199gh</td>
</tr>
<tr>
<td>Propazine</td>
<td>5</td>
<td>0.308e</td>
</tr>
<tr>
<td>Prometryne</td>
<td>5</td>
<td>0.263e</td>
</tr>
<tr>
<td>Simazine</td>
<td>5</td>
<td>0.277de</td>
</tr>
<tr>
<td>Control</td>
<td>5</td>
<td>0.272e</td>
</tr>
</tbody>
</table>

1 Means with a different letter are different at the 5% level according to Duncan’s New Multiple Range Test.

2 The higher concentration for each compound indicates the saturated solution.

Fig. 4. Growth-promoting action of different concentrations of ametryne (A), compared with the same concentrations of zeatin (Z) in the nutrient medium as measured by the increase in sorghum callus fresh weight.

This study has shown that s-triazine compounds, at very low concentrations, possess activity other than auxin-like which enhances sorghum callus growth, in the absence of other cytokinin sources in the medium. Regardless of their chemical structure, cytokinins are known to be substances capable of causing cell proliferation (1, 7). Cytokinins were also reported to cause an increase in protein synthesis in the plant tissue (2, 8, 9, 22–24). The high correlation between fresh and dry weights of the callus suggests that the increase in fresh weight is most likely due to cytokinesis. The low concentration of s-triazines used in the medium (2.5 μM) supports the idea that their action is hormonal. It is speculated that the action of the s-triazine herbicides in increasing the total protein content of the treated sorghum plants and enhancing sorghum callus growth is a hormonal action and most likely cytokinin-like.

LITERATURE CITED


