STUDIES IN PLANT METABOLISM. IV. COMPARATIVE EFFECTS OF 2,4-DICHLOROPHENOXACYCETIC ACID AND OTHER PLANT GROWTH REGULATORS ON PHOSPHORUS METABOLISM IN BEAN PLANTS

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Accumulated evidence has indicated that the physiological effect of 2,4-dichlorophenoxyacetic acid (2,4-D) in plants may function through its effect on processes of respiration (1, 2, 3, 4, 5) as well as on photosynthesis (6). Since the precise mechanism by which plant cells respond to 2,4-D has yet to be elucidated, and the participation of phosphorus in biological oxidation-reduction reactions had been found in both higher animals and microorganisms, an investigation was initiated to study the comparative effects of 2,4-D and other plant growth regulators on phosphorus metabolism in order to clarify, in part, the herbicidal effect of 2,4-D.

The immediate objectives of the work reported here are as follows: the comparative effects of 2,4-D and other plant growth regulators on (1) the phosphorus uptake, (2) the movement and the distribution of phosphorus, and (3) the incorporation of radioactive phosphorus into some intermediate compounds in bean plants.

MATERIALS AND METHODS

GROWTH REGULATOR SOLUTIONS: Immediately prior to use, 95 % ethanol solutions, each containing 0.1 % plant growth regulators (2,4-D acid, indoleacetic acid, indolebutyric acid or a-naphthaleneacetic acid) and 0.5 % Tween-20, were prepared and were used throughout this experiment.

TREATMENT OF PLANTS: Bean plants (Phaseolus vulgaris, var. Black Valentine) were grown in sand culture under greenhouse conditions unless otherwise noted. The nutrient solution of Biddulph (7) was employed. In several trials, the plants were randomized and divided into four groups. Three groups were treated with 10 μg, 50 μg or 100 μg of 2,4-D per plant on the midrib of one of the primary leaves which was almost fully expanded but whose terminal bud was still small. The fourth served as controls. The same nutrient solution containing 10 to 50 μg P\textsubscript{32} per liter was then applied daily to the medium in which the plants were growing. All plants were harvested after 7 days, and the roots were carefully washed under running water to remove the unabsorbed radioactive P. All samples were composited, weighed and homogenized in a Waring Blender with 20 times their weight of water. An aliquot of each homogenate was taken out with a pipette and dried in a 1 inch diameter stainless steel cup under an infra-red lamp. The radioactivities of all samples were measured directly with a thin mica window G-M counter (1.7 mg/cm\textsuperscript{2}). They represent a relative fraction of the total P absorbed and accumulated during the experimental period. Other aliquots were centrifuged with a Servall angle centrifuge to remove the residue and equivalent amounts of the supernatants were dried in the same manner and the radioactivities of these samples which represent the water soluble P were again measured. A typical set of data is shown in Table I.

In the study of the effect of 2,4-D and other plant growth regulators on P metabolism, 60 bean

<table>
<thead>
<tr>
<th>Table I</th>
<th>EFFECT OF VARIOUS AMOUNTS OF 2,4-D TREATMENT ON PHOSPHORUS UPTAKE IN BEAN PLANTS (EACH GROUP CONSISTS OF 5 PLANTS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,4-D TREATMENT, μg</td>
<td>0</td>
</tr>
<tr>
<td>Total P\textsubscript{32} activity per 25 mg fresh sample, cpm</td>
<td></td>
</tr>
<tr>
<td>Leaf</td>
<td>490</td>
</tr>
<tr>
<td>Stem</td>
<td>330</td>
</tr>
<tr>
<td>Root</td>
<td>9800</td>
</tr>
<tr>
<td>Water soluble P\textsubscript{32} activity per 25 mg fresh sample, cpm</td>
<td></td>
</tr>
<tr>
<td>Leaf</td>
<td>348</td>
</tr>
<tr>
<td>Stem</td>
<td>295</td>
</tr>
<tr>
<td>Root</td>
<td>8050</td>
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</table>

plants were grown in the same manner in 2 flats and divided into 6 randomized groups consisting of 10 plants each. The first and fourth groups received no treatment. The second group received 50 μg 2,4-D treatment to each plant (the chemical was applied on one of the primary leaves) and the 3rd, 5th and 6th groups received 250 μg of indoleacetic acid, 250 μg indolebutyric acid and 250 μg a-naphthaleneacetic acid per each plant respectively. A nutrient solution containing 10 μc per 1 of P\textsubscript{32} was then given daily to the plants after 24 hours of treatment. Any difference in absorption and distribution of P\textsubscript{32} in the bean plants and in the incorporation of P\textsubscript{32} into the P intermediate compounds if found between growth regulator treated and control bean plants would be the result obtained from the physiological effects which occurred after the treatment. All plants were
Fig. 1. Radioautograph of control bean plant showing the distribution of P\textsuperscript{32} after a 7-day absorption period.
harvested after 7 days. Two plants from each group were used for making radioautographs to show the distribution of $^{32}\text{P}$ (9). The remaining 8 plants were divided into leaf and stem portions, the root being discarded. The samples were composited, weighed, and homogenized with 20 times their weight of water. The homogenates were decanted into flasks and steamed immediately for 10 minutes to inactivate any enzymes present. An aliquot was centrifuged to remove the residue, and the supernatant was preserved under a layer of toluene and kept in a refrigerator. These samples were subjected later to chemical analyses for inorganic P, organic P, and measurement of radioactivity. Chromatographic separation, identification and determination of the P intermediate compounds of these samples were also performed.

**Measurement of Radioactivity:** A Tracerlab 64 scaler equipped with a thin mica window G-M counter, or a windowless gas flow counter was used depending upon the degree of activity of the sample. Equal amounts of supernatant solutions from growth regulator treated or from control bean plants were used in every case in order to minimize the error from the self-absorption. No correction for self-absorption was made in any of the measurements. In many instances, an Autoscaler equipped with an automatic sample changer was used for measuring the radioactivity of paper chromatograms. All samples were measured to within 5% probable error.

**Radioautography:** The radioautographs were prepared by exposing x-ray film to the plants containing $^{32}\text{P}$, for 22 to 48 hours, then developed with a Kodak D-72 developer.

**Chromatographic Separation:** One-dimensional chromatograms of the supernatant fluids were prepared in the usual way. The supernatant liquid was added dropwise with a glass dropper in a small spot to paper strips, about 6 cm from one end of the strip, until the desired amount had been applied. Whatman #1 paper strips ($1'' \times 22''$) were used. The strips were developed with a mixture of isopropyl ether and 90% formic acid in a volume proportion of 9:6, respectively. After development the chromatographic strips were thoroughly dried in air and cut into 1-cm sections, starting from the spot and consecutively numbered. Each section was placed in a stainless steel, cupped planchet and the radioactivity was measured. The relative radioactivity of the spots was then computed as described previously (9).

In other strips, after enough radioactivity from the supernatant liquids had been applied on the strips, known amounts of phosphoglyceric acid, glucose-1-phosphate and hexosediphosphate (about 50 $\mu$g each) were also applied to the spot. After development, the chromatograms were sprayed with a perchlorate-molybdate reagent. The technique of Hanes and Isherwood (10) was followed in producing the blue color of the P chromatograms. After recording the positions of P spots, the strips were again cut into 1-cm sections and the radioactivity of each section was measured in the same manner. The locations of blue P spots and the radioactive spots were compared. Several radioactive spots were identified by this process as inorganic phosphate, phosphoglyceric acid, glucose-1-phosphate and hexosediphosphate.

**Determination of Total and Inorganic Phosphorus:** The method of Fiske and Subbarow (11) was followed. The color of the solution was measured with a Coleman electric photometer and was compared with standards produced from known amounts of P.

**Results and Discussion**

**The Effect of Various Amounts of 2,4-D on the Uptake and Distribution of $^{32}\text{P}$ in Bean Plants:** Data regarding the $^{32}\text{P}$ activities per 25 mg fresh samples of leaf, stem and root from the 2,4-D treated and the control bean plants are represented in Table I. In all cases, the $^{32}\text{P}$ activities of leaves from 2,4-D treated plants were much less when compared to the leaves from the control plants, while the activities in either the stem or root from these two groups of plants showed no significant differences. When 2,4-D treatments were increased from 10 $\mu$g to 100 $\mu$g per plant, the accumulation of $^{32}\text{P}$ activity in the leaf from different treatments decreased accordingly. This observation indicated that 2,4-D treatment affected the upward movement and the supply of P to the leaves. Consequently, the insufficient supply of P in the leaf may directly or indirectly affect both the important photosynthetic and respiratory processes of the plant.

**The Effect of 2,4-D and Other Plant Growth Regulators on the Distribution of $^{32}\text{P}$ in Bean Plants:** As shown in figures 1 to 4, the distribution of $^{32}\text{P}$ in growth regulator treated plants differed from that found in the control plants. The radioautograph of the IBA treated plants was quite similar to that of the IAA treated one; therefore it was not included. In general, the $^{32}\text{P}$ was concentrated in the petiole of the treated leaf, the midrib of the treated leaf, and in the first internode. The concentration of $^{32}\text{P}$ was extended to part of the hypocotyl in a-naphthaleneacetic acid treated plants. The accumulation and the distribution of $^{32}\text{P}$ in the bean plants are probably determined by the plant growth regulator. The distribution pattern of $^{32}\text{P}$ in 2,4-D treated plants is very similar to the accumulation of

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**Fig. 2.** Radioautograph of 2,4-D treated bean plant showing the distribution of $^{32}\text{P}$ after a 7-day absorption period. (50 $\mu$g of 2,4-D were applied on the left primary leaf.)

**Fig. 3.** Radioautograph of indoleacetic acid treated bean plant showing the distribution of $^{32}\text{P}$ after a 7-day absorption period. (250 $\mu$g of IAA were applied on the left primary leaf.)

**Fig. 4.** Radioautograph of a-naphthaleneacetic acid treated bean plant showing the distribution of $^{32}\text{P}$ after a 7-day absorption period. (250 $\mu$g of NAA were applied on the right primary leaf.)
pattern of 2,4-D (12). This observation may suggest that the distribution of P$^{32}$ is associated with the distribution of the growth regulators. In 1948, Brunstetter et al. (13) demonstrated that when bean plants were treated with 3-indoleacetic acid, the P content in the treated stem increased about 5 times over that of the control after 148 hours. Our result showed that the accumulation of P$^{32}$ in IAA treated stems was the same as compared to the control stems. However, the total P content in the treated stems was increased about 60% over that of the controls and presumably this increase was due to the transport of P from the leaf. Whether or not the physiological effects of 2,4-D and of other growth regulators on bean plants, in regard to the distribution of radio-active P, are the same has yet to be studied. However, the growth regulator treatment will certainly modify the distribution and accumulation pattern of phosphorus in bean plants.

**The Effect of 2,4-D and Other Growth Regulators on the Incorporation of P$^{32}$ into Some Phosphorus Intermediates:** It is obvious that both the total water soluble P$^{32}$ activity and the specific activity decreased in the leaves of all bean plants treated with growth regulators. The effect was more pronounced in 2,4-D treated plants. The total P$^{32}$ activity was found to be greatly increased in 2,4-D treated stems over that of the control, slightly decreased in the IBA treated stem, and without significant changes in IAA- and NAA-treated stems. The percentage of inorganic P was found to be higher in the leaf and lower in the stem of 2,4-D treated plants as compared to the control. Also, the incorporation of P$^{32}$ into glucose-1-phosphate and hexosediphosphate was less in the leaf and greater in the stem of 2,4-D treated plants. A great increase in hexosediphosphate was observed only in the 2,4-D treated stem while no significant, or slight, change was found with the other plant growth regulator treatments. The results demonstrated clearly that all of the growth regulators affected the incorporation of P$^{32}$ into one or more P intermediates. 2,4-D has the greatest effect over the other growth regulators on the incorporation of P$^{32}$ into P intermediates.

**Summary**

The effects of 2,4-D and of other growth regulators on the P metabolism in bean plants was investigated. It was found that 2,4-D treatments greatly reduced the upward movement of radioactive P to the leaves; the degree of reduction was proportioned to the amount of 2,4-D supplied.

Treatment with 2,4-D, IAA, IBA, or NAA modified the distribution and accumulation pattern of radioactive P in bean plants.

The incorporation of P$^{32}$ into some P intermediate compounds in the water-soluble fraction was affected by plant growth regulator treatment. The greatest effect was observed in 2,4-D treated plants.

**Literature Cited**