The Effect of Potassium on the Fixation of Molecular Nitrogen by Root Nodules of *Vicia faba*

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**ABSTRACT**

The effect of potassium supply of *Vicia faba* on the fixation of molecular nitrogen by root nodules was studied by using $^{15}$N-labeled molecular nitrogen. Plants well supplied with potassium showed higher contents of $^{15}$N in the soluble amino fraction and in the protein fraction of various plant organs as compared with plants of a lower potassium status. This effect was evident particularly in the root nodules. Assimilation experiments, carried out with $^{15}$CO$_2$, revealed that the content of radioactivity in the sugars and amino acids of the root nodules was increased by the potassium supply of the host plants. In particular, the content of $^{15}$C amino acids in the root nodules was influenced beneficially by potassium, which means that potassium favored the provision of reduced nitrogen (NH$_3$). It is postulated that the better carbohydrate supply of nodules, by plants well supplied with potassium, results in a higher carbohydrate turnover in the nodules and thus the provision of ATP and reducing electrons required by the nitrogenase is enhanced.

Potassium in the form of K$_2$SO$_4$ was given in addition. The first experimental series comprised two, the second, three potassium levels: K$_o$, 0.5 meq/l; K$_o$, 1.5 meq/l; K$_o$, 4.5 meq/l. The K concentration of the nutrient solution was controlled every 2nd day. As soon as the K concentration in the K$_o$ treatment had fallen off to below 0.25 meq/l, the solution was replaced by fresh solution. As a result of the additions of NaOH, a constant pH 6 of the solution was kept throughout the experiment. During the 1st week of growth, the plants had been supplied also with NH$_4$NO$_3$ at a rate corresponding to 1 mM in the nutrient solution. The solution was aerated three times a day.

The plants of the first series were grown in this nutrient solution for a period of 4 weeks, those of the second series for 6 weeks. Then, they were exposed to $^{15}$N$_2$ to investigate their N$_2$-fixing potential. For this purpose a special box was constructed. The lid of the box (Fig. 1) had holes through which the plants could grow. The roots were kept in three compartments filled with nutrient solution of different K concentrations. A fourth compartment contained a bottle with labeled $N_2$ (1 liter with a 93% enrichment of $^{15}$N$_2$). Prior to the application of labeled $N_2$, the holes in the box were sealed by means of a plastic mass (Prestix) to prevent gas exchange with the outer atmosphere, and about 25% of the nutrient solution was removed to obtain a larger volume of air in the root medium, especially in the zone of major root nodule development. Then, the bottle with labeled $N_2$ was opened and $N_2$ was distributed evenly within the box by a pump. A more detailed description of the experimental setup has been published recently by Haghparast (7). The amount of $^{15}$N$_2$ in the root atmosphere of the first experiment was 5-atom % excess, and 4-atom % in the second. The plant roots were exposed to this atmosphere for a period of 12 hr. The plants were then harvested and divided into roots, nodules, stems, and leaves.

In another two series with two and three K levels, respectively, the content of $^{15}$C-labeled sugars and amino acids in the root nodules was determined. The plants used for these experiments were grown in the same way as described above. The plants were then placed into an airtight Plexiglas box containing labeled CO$_2$ (1 mCi of $^{15}$CO$_2$) and left there for 22 hr under artificial light with 20,000 lux. Details of this technique have been described by Haeder and Mengel (6).

The various plant organs were subjected to extraction three times with a solution of 75% ethanol. The extract was dried in a vacuum evaporator at 35 C, and the residue was dissolved in a water-chloroform mixture (1:1 v/v). The upper phase contained the soluble amino compounds. The ethanol-insoluble residue contained N mainly in the form of proteins. This fraction is designated in the following as a protein fraction. To determine the labeled N, both fractions were digested according to the Kjeldahl technique. The released labeled NH$_3$ combined

Today about 12,000 different species of higher plants are known living in symbiosis with N-fixing bacteria (17). Nearly 200 are legumes cultivated as crop plants in agriculture and horticulture. The N fixed by their root nodules does not affect only the N cycle of nature, but is also of economic importance. Therefore, greater attention should be paid to the question of whether the nutritional status of the host plant has a major influence on N$_2$ fixation by *Rhizobium*. Wu et al. (20) reported that abundant supplies of phosphate and potassium to soybeans had a beneficial effect on N$_2$ fixation by *Rhizobium japonicum*. Similar observations have been made by Gukova and Tjulina (5) with *Lupinus* and *Vicia*. It is not yet known whether K*+* exerts a direct influence in this respect or a more indirect one by improving the growth of the host plant. The object of the following experiment was to investigate this question more closely.

**MATERIALS AND METHODS**

The experiments were carried out with young plants of *Vicia faba* grown in solution culture. After being germinated on filter paper, the plants were placed in a special box (Fig. 1) and supplied with nutrient solution of the following composition: 3 mM MgSO$_4$, 1.5 mM NaH$_2$PO$_4$, 6 mM CaCl$_2$, 28 $\mu$M MnSO$_4$, 20 $\mu$M H$_2$BO$_3$, 3 $\mu$M CuSO$_4$, 3 $\mu$M ZnSO$_4$, 0.6 mM Na molybdate, 0.6 $\mu$M CoCl$_2$, and 20 $\mu$M Fe chelate.

535

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thus was of the liquid. Exchangers.

To determine the same amount, the dissolved sugars were used, except that anions and cations were eliminated from the dissolved residue by means of anion and cation exchangers. A detailed description of this separation technique has been given by Haedrich and Mangel (6). The radioactivity of the amino acids and the sugars was determined using a liquid scintillation counter, with aquasol as a scintillation liquid.

The treatments involved 10 plants each. For analysis of leaves, stems, roots, and nodules, five plants each were used, thus obtaining two samples from each treatment. To determine the 15N content, each sample was subjected to analysis three times. The data given below are means of 6 values, as a result of two “biological” and three analytical replications.

RESULTS

The plants grown in solution culture developed normally and showed no deficiency symptoms even in the low K treatment. Growth of root nodules in all plants was so vigorous that the number of nodules amounted to between 200 and 300 per plant at the time of harvest. Table I shows the effect of increasing both the K supply on the yield of plant organs and the K content of above ground plant parts (leaves and stems). In both experimental series plant growth was improved by K.

The number or size of the root nodules was also affected favorably by K application. In the first series with the younger plants, better K nutrition increased the number of root nodules per plant, while in the second series (6-week-old plants) it had a particularly beneficial effect on the nodule weight.

Table II shows the content of labeled N in the soluble amino acid fraction. It appears that with increasing K application the content of labeled N in all plant organs was raised considerably, with the exception of the leaves of the second experimental series. However, it has to be taken into account that the amount of labeled N in the stems of these plants showed a remarkable increase with improved K supply, so that this deviation cannot be considered as a general trend but rather as a question of N distribution in leaves and stems. The content of labeled N in the protein fraction is given in Table III. These data show that improved K supply increased the content of labeled N in the protein fraction. It appears that the root nodules of plants supplied with higher amounts of K fixed more N2 during the 12-hr exposure to labeled N than the plants low in K. This effect cannot be ascribed primarily to the larger size and number of nodules. When the K1 is compared with the K3 treatment of the second experimental series, it is seen that the number of nodules was only slightly increased and nodule size was about 20% larger, but N2 fixation went up by nearly 100%, for the amount of labeled N per plant in the K1 treatment was 116 μg of 15N whereas in the K3 treatment it rose to 226 μg of 15N. The conclusion that increased N turnover in the nodules as a result of improved K supply to the host plant had a greater effect on N2 fixation by Rhizobium than the number and size of the nodules is supported by the fact that the data in Tables II and III are expressed as contents (labeled N/g fresh weight) and not as quantities of labeled N per plant or plant organ.

with HCl to form NH4Cl which was subsequently oxidized according to the Rittenberg technique using NaOBr (16). 15N was determined by emission spectroscopy as has been described by Meier and Muhl (14) and Faust (4).

To extract the 14C-labeled sugars from the root nodules, the same technique as for the separation of the soluble amino compounds was used, except that anions and cations were eliminated from the dissolved residue by means of anion and cation exchangers. A detailed description of this separation technique has been given by Haedrich and Mangel (6). The radioactivity of the amino acids and the sugars was determined using a liquid scintillation counter, with aquasol as a scintillation liquid.

The treatments involved 10 plants each. For analysis of leaves, stems, roots, and nodules, five plants each were used, thus obtaining two samples from each treatment. To determine the 15N content, each sample was subjected to analysis three times. The data given below are means of 6 values, as a result of two “biological” and three analytical replications.

Table I. Effect of Increasing Potassium Supply on Yield and Root Nodules of Vicia faba

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Treatment</th>
<th>K Conc</th>
<th>Leaves</th>
<th>Stems</th>
<th>Roots</th>
<th>Nodules</th>
<th>Fresh Wt of Nodule</th>
<th>K in Dry Wt of Leaves and Stems</th>
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<tbody>
<tr>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>1</td>
<td>K1</td>
<td>0.5</td>
<td>8.74</td>
<td>7.36</td>
<td>12.9</td>
<td>298</td>
<td>3.5</td>
<td>3.2</td>
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<tr>
<td>2</td>
<td>K2</td>
<td>4.5</td>
<td>10.96</td>
<td>8.36</td>
<td>16.1</td>
<td>388</td>
<td>3.5</td>
<td>3.2</td>
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<tr>
<td>3</td>
<td>K3</td>
<td>0.5</td>
<td>15.6</td>
<td>13.4</td>
<td>17.0</td>
<td>233</td>
<td>6.5</td>
<td>1.0</td>
</tr>
<tr>
<td>4</td>
<td>K4</td>
<td>1.5</td>
<td>19.4</td>
<td>16.7</td>
<td>23.1</td>
<td>250</td>
<td>7.2</td>
<td>2.2</td>
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<tr>
<td>5</td>
<td>K5</td>
<td>4.5</td>
<td>21.9</td>
<td>17.3</td>
<td>25.0</td>
<td>251</td>
<td>8.0</td>
<td>3.6</td>
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Table II. Effect of Increasing Potassium Supply on the Content of Labeled N (15N2-supplied) in the Soluble Amino Fraction of Various Plant Organs of Vicia faba

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Treatment</th>
<th>K Conc</th>
<th>Leaves</th>
<th>Stems</th>
<th>Roots</th>
<th>Nodules</th>
<th>mg 15N g fresh wt</th>
</tr>
</thead>
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<tr>
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<td></td>
<td></td>
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<tr>
<td>1</td>
<td>K1</td>
<td>0.5</td>
<td>3.84</td>
<td>2.85</td>
<td>1.37</td>
<td>25.1</td>
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<tr>
<td>2</td>
<td>K2</td>
<td>4.5</td>
<td>4.03</td>
<td>3.54</td>
<td>1.70</td>
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<tr>
<td>3</td>
<td>K3</td>
<td>0.5</td>
<td>1.41</td>
<td>0.94</td>
<td>0.39</td>
<td>21.1</td>
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<tr>
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<td>1.35</td>
<td>1.49</td>
<td>0.30</td>
<td>34.9</td>
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Table III. Effect of Increasing Potassium Supply on the Content of Labeled N (15N2-supplied) in the Protein Fraction of Various Plant Organs of Vicia faba

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Treatment</th>
<th>K Conc</th>
<th>Leaves</th>
<th>Stems</th>
<th>Roots</th>
<th>Nodules</th>
<th>mg 15N g fresh wt</th>
</tr>
</thead>
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<td></td>
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<tr>
<td>1</td>
<td>K1</td>
<td>0.5</td>
<td>3.25</td>
<td>1.44</td>
<td>0.64</td>
<td>11.8</td>
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<tr>
<td>2</td>
<td>K2</td>
<td>4.5</td>
<td>4.34</td>
<td>3.52</td>
<td>0.66</td>
<td>14.6</td>
<td></td>
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<tr>
<td>3</td>
<td>K3</td>
<td>0.5</td>
<td>1.52</td>
<td>0.66</td>
<td>0.20</td>
<td>5.23</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>K4</td>
<td>1.5</td>
<td>2.23</td>
<td>0.52</td>
<td>0.29</td>
<td>5.86</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>K5</td>
<td>4.5</td>
<td>2.16</td>
<td>0.76</td>
<td>0.60</td>
<td>7.91</td>
<td></td>
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</table>
Thus, differences in number of root nodules and growth of the plants are eliminated to a high degree.

This statement is supported also by results of experiments with labeled C (Table IV). The content of 14C-labeled material increased with better K nutrition. K+ obviously promoted the transport of carbohydrates from the green plant parts into the root nodules. This had a marked effect on the content of labeled C in the amino acid fraction. In the second experiment, the C label of the sugar fraction of nodules from the K1 treatment was in the same order of magnitude as that of the amino acid fraction, whereas the label of the amino fraction in the K3 treatment was nearly twice as high as the label in the sugar fraction. This demonstrates clearly that K+ especially favored the formation of reduced N (NH3).

DISCUSSION

Legumes well supplied with inorganic nutrients are supposed to fix more N2 by their root nodules than plants that are deficient in one or another element. This may be a result of an indirect effect of plant nutrients, because plants well supplied with nutrients produce more leaf matter, resulting in an increased CO2 assimilation by the plant. The fact that photosynthesis favors N2 fixation by root nodules has been reported by various authors (13, 19). This possibly leads to an improved supply of carbohydrates to the roots so that number and size of the nodules will be increased. Such an effect is also exerted by K+, as can be seen from the data in Table I. This finding alone, however, cannot explain the increased N fixation of nodules from plants well supplied with K+, because the K effect on N2 fixation per plant was considerably higher than the K effect on nodule size or number. The beneficial action of improved K supply on the growth of the host plants does not furnish a satisfactory explanation for the higher contents of labeled N in the various plant parts, as in plants with larger amounts of leaf, stem, and root matter the fixed N is distributed among a larger volume of plant substance, and for this reason an increase in the content of labeled N in the different plant organs cannot be expected per se. Higher contents of labeled N in the various plant parts therefore indicate that also the N turnover of the root nodules was improved by K+. This statement is supported by the data given in Table IV showing that the amount of C-labeled sugars and in particular that of C-labeled amino acids per g fresh weight of root nodules increased with increasing K supply to the host plant. There might be a connection between this K effect and the provision of *Rhizobium bacteroides* with carbohydrates. According to recent publications by various authors, K+ promotes the translocation of carbohydrates in higher plants (1, 8, 18). This may also hold true of legumes. Hehl and Mengel (9) found higher contents of starch and sucrose in the roots of *Medicago sativa* and *Trifolium pratense* well supplied with K than in plants given lower K applications.

This is in agreement with the data shown in Table IV. The most important result of the experiment described was that K+ increased the label of the amino acids to a greater extent than that of the sugars. This means that K+ in some way had a beneficial effect on the formation of reduced N (NH3). If K+ exerted an effect only on the synthesis of keto acids as a result of a better supply of carbohydrates to the roots, there had not been such a great difference between the labels of sugars and amino acids with increasing K supply (Table IV). Rather an opposite effect was to be expected in this case, that means that a high supply of C skeleton had led to a relative shortage of reduced N.

Carbohydrates provided by the host plant to *Rhizobium bacteroides* affect the metabolism of the latter. In these processes the tricarboxylic acid cycle plays a decisive part (2). It supplies the nitrogenase with electrons and ATP, required for the reduction of N2 to NH3. According to investigations of Koch et al. (11) and Klucar and Evans (10) with extracts of soybean nodules, an ATP-generating system is indispensable for N2 fixation. It is assumed that ATP synthesis is particularly favored by K+. This assumption is in agreement with results obtained by Pfliiger and Mengel (15) who found that K+ improved the photophosphorylation of chloroplasts. The supply of electrons to the nitrogenase depends on the turnover of organic acids in the tricarboxylic acid cycle. If this turnover is enhanced by improved carbohydrate supply, there will also be better conditions for N2 reduction. It appears that improved carbohydrate supply to the root nodules as a result of the action of K+ may result in a better provision of the nitrogenase system with electrons and ATP. It is still an open question whether nitrogenase itself, as many other enzymes, is activated by K+ (3). In this respect an observation of Gukova and Tijulna (5) who reported that root nodules with a good capacity of N2 fixation are rich in K+ is of interest.

The beneficial effect of K+ on the incorporation of labeled amino acids into protein may be a result not only of the better N supply, but also of the favorable influence exerted by K+ on protein synthesis as has become manifest by recent experiments of Koch and Mengel (12).

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LITERATURE CITED