NITROGEN TRANSLOCATION IN HIGH- AND LOW-PROTEIN LINES OF WHEAT

by 6508

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B. S., Kansas State University, 1969

A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Agronomy

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1971

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# TABLE OF CONTENTS

- REVIEW OF LITERATURE................................................................. 1
- MATERIALS AND METHODS--------------------------------------------- 3
  - Experiment I-------------------------------------------------------- 3
  - Experiment II------------------------------------------------------- 4
- RESULTS--------------------------------------------------------------- 5
  - Experiment I-------------------------------------------------------- 5
  - Experiment II------------------------------------------------------- 7
- DISCUSSION------------------------------------------------------------- 12
- ACKNOWLEDGEMENTS----------------------------------------------------- 18
- REFERENCES------------------------------------------------------------ 19
- VITA----------------------------------------------------------------- 22
## LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Percent translocation of $^{14}$C-amino acid mixture from culm to grain of Atlas 66 and Triumph wheat injected at anthesis and sampled weekly to maturity. LSD (.05) between wheat lines is not significant; LSD (.05) between weeks is 0.68 for the alcohol soluble fraction, 0.85 for the water soluble fraction, and 7.73 for the NaOH soluble fraction.</td>
<td>6</td>
</tr>
<tr>
<td>2. Percent translocation of $^{14}$C-amino acid mixture from culm to grain of Atlas 66 and Triumph wheat injected weekly beginning at anthesis and sampled at maturity. LSD (.05) between wheat lines is not significant; LSD (.05) between weeks is 0.61 for the alcohol soluble fraction, 1.06 for the water soluble fraction, and 6.28 for the NaOH soluble fraction.</td>
<td>8</td>
</tr>
<tr>
<td>3. Nitrogen content (mg per plant) of flag, lower, and all leaf blades removed from four wheat lines at anthesis. LSD (.05) between high- and low-protein wheat lines is 65.4.</td>
<td>9</td>
</tr>
<tr>
<td>4. Nitrogen content (mg per plant) of culms at maturity after removing none, flag, lower, or all leaves of four wheat lines at anthesis. LSD (.05) between high- and low-protein wheat lines is 66.0.</td>
<td>10</td>
</tr>
<tr>
<td>5. Nitrogen content (mg per plant) of leaf blades remaining at maturity after removing none, flag, or lower leaves of four wheat lines at anthesis. LSD (.05) between high- and low-protein wheat lines is 64.8.</td>
<td>11</td>
</tr>
<tr>
<td>6. Nitrogen content (mg per plant) of grain at maturity after removing none, flag, lower or all leaves of four wheat lines at anthesis. LSD (.05) between high- and low-protein wheat lines is 64.8.</td>
<td>13</td>
</tr>
</tbody>
</table>
7. Nitrogen content (mg per plant) of whole plants of four wheat lines at anthesis and maturity. LSD (.05) between high- and low-protein wheat lines is not significant.
REVIEW OF LITERATURE

Nitrogen metabolism in grain crops has been investigated considerably to assist breeding high-protein varieties (3, 4, 5, 6, 7, 8, 9, 11, 12, 19, 20, 21). Crosses of South American wheats with soft red winter wheat varieties have increased yields and grain-protein concentration over standard varieties. Selections from the crosses, 'Atlas 50' and 'Atlas 66', (Middleton, Bode and Bales, 1954), have been crossed with hard red winter varieties to improve their grain protein concentration and yield (Johnson et al., 1963). Croy and Hageman (1970) reported that 'Ponca' wheat had a significantly higher level of nitrate reductase activity and more water-soluble protein in leaf tissue than 'Monon'. Ponca produced more grain protein per acre but the protein concentration in the grain of the two varieties was not significantly different. It was demonstrated that wheat varieties high in grain nitrogen concentration had both a high level of nitrate-reductase activity and a high efficiency of translocation of reduced nitrogen into the grain (4). Rao and Croy (17) found high-protein wheat seedlings and seeds had higher protease activity. That indicated high-protein wheats had higher efficiency of translocation of vegetative nitrogen to the grain by breakdown of the vegetative protein and subsequent translocation. Johnson, Mattern and Schmidt (8) reported high-protein wheat varieties consistently produced over two per cent more grain-protein concentration than low-protein varieties. A common wheat, 'Warrior', had a higher percentage of vegetative nitrogen and a lower percentage of grain nitrogen than high-protein lines. That was due to greater capacity to absorb soil nitrogen and lower efficiency to translocate vegetative nitrogen to the head. Absorption and translocation of nitrogen appeared to be separate and independent physiological processes. High-protein wheat lines showed an increase in percentage of grain nitrogen over low-protein
lines the last few weeks before maturation. The net result was an initial decrease in grain-nitrogen percentage with a later increase that continued until the grain matured. Environmental factors during a two-week preripe period that interfere with translocation of nitrogen from vegetative parts to the grain greatly affect the final level of protein in the grain. Haunold, Johnson and Schmidt (6) found the relationship between yield and protein content of two high- and two low-protein wheat lines varied inconsistently from year to year. If soil nitrogen was low, a negative correlation was noted. Atlas 66 had a threshold value of three per cent more grain protein than 'Wichita' if soil nitrogen was not limiting.

Seth, Hebert and Middleton (19) reported that high- and low-protein wheat lines did not differ in their vegetative-nitrogen content prior to heading. After heading, the high-protein lines increased more rapidly in per cent nitrogen in the spike than low-protein lines. This was due to more efficient translocation of nitrogen from vegetative parts to the grain or to more enzymes or more efficient enzymes involved in protein synthesis in the seed. Neales, Anderson and Wardlaw (15) found the increase in grain nitrogen after heading exceeded losses by the leaves and stem during grain development. This would indicate the balance of grain nitrogen came from the roots after anthesis. Leaf removal at anthesis reduced the uptake of nitrogen into the culm and nitrogen content of the grain at maturity. Removal of flag leaves induced a reduction in grain nitrogen at harvest in excess of that contained in the flag leaves at anthesis. Grain weight was reduced twenty per cent by removing the flag leaves and twelve per cent by removing the lower leaves at anthesis. Defoliation at anthesis reduced uptake of nitrogen by the culm forty-four per cent. The role of leaves in the migration of nitrogen to the grain was two-fold. First, while the leaves were actively transpiring and photosynthesizing, uptake of nitrogen by the culm was promoted.
Secondly, leaves directly supplied grain with nitrogen by mass transfer from the vegetative parts after heading.

Since translocation of vegetative nitrogen to the grain after anthesis appeared to be more efficient in high-protein wheat lines, several studies were designed to directly evaluate this factor in several wheat lines. The role of leaves also was studied for their effect on translocation, mobilization, and absorption of nitrogen in high- and low-protein wheat lines.

MATERIALS AND METHODS

Experiment I

Seed of 'Triumph' and Atlas 66 wheat was planted November 5, 1969, in 24-cm plastic pots containing 3 kg loam soil. The seedlings were germinated and placed outdoors for 6 weeks for vernalization. After vernalization, the plants were placed in the greenhouse at 20-25°C and after 2 weeks incandescent lights were placed 100 cm above the plants for a 16-hr day period.

At anthesis, March 4, 1970, 24 pots, 12 each of Triumph and Atlas 66, containing 3 plants per pot, were treated with 0.1 ml of 0.5 μc 14C-amino acid mixture (New England Nuclear) by the method of Brown and Neish (1). The pot was inverted on a ring stand and 3 heads were selected and labelled for injecting. A no. 26 syringe needle was inserted into the culm below the first node from the spike. At the base of the spike, 0.1 ml of the solution was injected from a syringe and no. 26 needle into the hollow part of the stem while air escaped through the upper needle. The upper needle was withdrawn and collodion was placed over the puncture and allowed to dry. The syringe and needle were then withdrawn and that puncture was sealed with collodion. After treating, the pots were randomized and sampled at weekly intervals. Atlas 66 and Triumph plants in another 30 pots were injected weekly beginning with anthesis and sampled at
maturity on April 24, 1970.

Sampling consisted of clipping the labelled heads below the spike and removing and drying the grain 2 days in a circulating air oven at 65°C. The dried grain was weighed and kernel numbers were counted. For analysis, a 1.0-g subsample of grain was ground to a fine powder with mortar and pestle and extracted 2 hrs with 90% (v/v) ethanol with constant stirring. The suspension was centrifuged at 2000 x g for 10 min and the supernatant was decanted and adjusted to 10-ml volume. The pellet was taken up in 10 ml of water during a 2-hr extraction period. The extract was centrifuged and the resulting supernatant was decanted and adjusted to 10-ml volume. The remaining pellet was solubilized with 25 ml of 5 M NaOH and diluted to 50 ml with distilled water.

Radioactivity in each fraction was analyzed by plating 0.5 ml, 0.2 ml, and 0.2 ml of the ethanol soluble, water soluble, and NaOH soluble fractions, respectively, on Whatman no. 3 filter paper discs 2.3 cm in diameter. After drying, the discs were placed in low-potassium glass vials containing 5 ml scintillation mixture (0.4% (w/v) 2,5-diphenyloxazole (PPO) and 0.01% (w/v) p-bis-2(2-(4-methyl-5 phenyloxazolyl)-benzene (dimethyl POPP) in absolute toluene) and counted in a liquid-scintillation system.

Experiment II

Seed of Triumph and 'Kaw' (low protein) and Atlas 66 and 'Atlas 50' (high-protein) wheat were planted in 3.75 x 3.75-cm peat pots containing 200 g loam soil February 28, 1970. The seed was germinated in an environmental chamber at 25-15°C day-night temperature for 1 week. The temperature was then lowered to 7°C for 6 weeks. The vernalized seedlings were transplanted to the Kansas State University agronomy farm in a randomized-block design with 4 replications. These plants were subjected to leaf-removal treatments at anthesis.
At anthesis, four treatments were imposed randomly on individual plants of each wheat line. Treatments were a control with no leaves removed, plants with all leaf blades removed, plants with all leaf blades except the flag leaf blades removed (lower leaves), and plants with only the flag leaf blades removed. Whole plants and vegetation removed from the plants at anthesis were dried to constant weight at 70 °C. Total dry weights were determined and the material was ground to 20-mesh size. A 1-g subsample was analyzed for Kjeldahl-nitrogen content (16) using boric acid in the receiving flask (18).

At maturity, the plants were harvested and separated by hand into three fractions: grain, leaf blades, and stems plus leaf sheaths (culms). The fractions were dried to a constant weight at 70 °C, weighed, ground and analyzed for Kjeldahl-nitrogen content.

RESULTS

Experiment I

Total translocation of amino acids from culms to grain did not differ significantly between Atlas 66 and Triumph wheat at any sampling date when plants were injected at anthesis and sampled weekly (Fig. 1). Atlas 66 grain had significantly more $^{14}$C-labelled amino acid in the ethanol-soluble fraction at the last week’s sampling than at the previous sampling dates, and had significantly more water-soluble labelled amino acid during the last two sampling dates than during the previous three weeks. Triumph grain similarly contained significantly more water-soluble label during the last two weeks than during the first weeks. Significantly more $^{14}$C label was found in NaOH-soluble protein in Atlas 66 during the fourth week sampling and in Triumph during the third and fourth samplings than during the first and last samplings.

Translocation from plants injected at weekly intervals after anthesis and
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Fig. 1. Percent translocation of $^{14}C$-amino-acid mixture from culm to grain of Atlas 66 and Triumph wheat injected at anthesis and sampled weekly to maturity. LSD (.05) between wheat lines is not significant; LSD (.05) between weeks is 0.68 for the alcohol-soluble fraction, 0.85 for the water soluble fraction, and 7.73 for the NaOH-soluble fraction.
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Fig. 1.
sampled at maturity is shown in Fig. 2. The amount of $^{14}C$ label in the ethanol- and NaOH-soluble fractions of the grain did not differ significantly between Atlas 66 and Triumph. Triumph contained significantly more label in the water-soluble fraction than Atlas 66 at the second- and fourth-week injections, however. Mature Atlas 66 grain had significantly more ethanol-soluble label from the first injection after anthesis than from subsequent weekly injections. Atlas 66 grain also contained significantly more label in the NaOH-soluble fraction from the second and third weekly injections, while Triumph contained significantly more from the second, third and fourth weekly injections.

Experiment II

Amounts of vegetative nitrogen removed from the low- and high-protein wheat lines at anthesis are shown in Fig. 3. When all leaves were removed, significantly more vegetative nitrogen was removed from Atlas 66 than from Kaw or Triumph and from Atlas 50 than from Kaw. There was no significant difference between Triumph and Atlas 50. When only the lower leaves were removed, significantly more nitrogen was removed from Atlas 66 and Atlas 50 than from Kaw and Triumph. The amount of nitrogen removed in the flag leaves did not differ significantly among the four lines.

Amounts of nitrogen in the culms at maturity for the various leaf-removal treatments are shown in Fig. 4. Culms of Atlas 50 and Atlas 66 contained significantly more nitrogen than Kaw or Triumph when none, all, or lower leaves were removed. When flag leaves were removed, culms of Kaw and Triumph contained more nitrogen than Atlas 50 or Atlas 66.

Amounts of nitrogen in leaves remaining at maturity are shown in Fig. 5. Flag-leaf nitrogen content of the four wheat lines did not differ significantly when the lower leaves were removed at anthesis. Kaw had significantly more
Fig. 2. Percent translocation of $^{14}$C-amino acid mixture from culm to grain of Atlas 66 and Triumph wheat injected weekly beginning at anthesis and sampled at maturity. LSD (.05) between wheat lines is not significant; LSD (.05) between weeks is 0.61 for the alcohol-soluble fraction, 1.06 for the water-soluble fraction, and 6.28 for the NaOH-soluble fraction.
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Fig. 5. Nitrogen content (mg per plant) of leaf blades remaining at maturity after removing none, flag, or lower leaves of four wheat lines at anthesis. LSD (.05) between high- and low-protein wheat lines is 64.8.
nitrogen in lower leaves than either Atlas 50 or Atlas 66 and Triumph had significantly more than Atlas 66 when flag leaves were removed at anthesis. In control plants, which had no leaves removed, Kaw had significantly more leaf nitrogen at maturity than Triumph and Atlas 66.

The effect of different leaf removals at anthesis on grain-nitrogen content at maturity is shown in Fig. 6. Atlas 50 and Atlas 66 control plants contained significantly more grain nitrogen than Kaw or Triumph. Removing flag leaves at anthesis significantly reduced the grain-nitrogen content of Atlas 50 and Atlas 66, but not of Kaw and Triumph, when compared to control plants. Similarly, removing the lower leaves at anthesis significantly decreased the grain nitrogen content of Triumph, Atlas 50, and Atlas 66 but not of Kaw. When all leaves were removed at anthesis, the grain-nitrogen content was decreased in the high-protein lines and in Triumph.

Whole-plant nitrogen content of the four wheat lines at anthesis and at maturity is shown in Fig. 7. Although there were significant differences between low-protein lines at anthesis and high-protein lines at maturity, whole-plant nitrogen did not differ among lines within growth stages or between growth stages within lines.

DISCUSSION

Translocation of labelled amino acids from culms to grain was essentially the same in Atlas 66 and Triumph wheat grown in the greenhouse. The rate was low immediately after anthesis, increased to a peak several weeks after anthesis, and declined at maturity. That pattern was reported also by Carr and Wardlaw (2). The decrease in $^{14}$C label in grain of plants injected at anthesis and sampled periodically was likely due to respiration, although retranslocation, leaching, and shattering might have been involved. The decrease in $^{14}$C label in grain of
Fig. 6. Nitrogen content (mg per plant) of grain at maturity after removing none, flag, lower, or all leaves of four wheat lines at anthesis. LSD (.05) between high- and low-protein wheat lines is 64.8.
Fig. 7. Nitrogen content (mg per plant) of whole plants of four wheat lines at anthesis and maturity. LSD (.05) between high- and low-protein wheat lines is not significant.
plants injected periodically and sampled at maturity, however, indicates translocation was more efficient midway during grain development than earlier or later. The lack of difference in translocation from culm to head between varieties indicated, in any case, that other factors were responsible for differences in grain-protein content.

Studies with high- and low-protein spring wheat lines showed the high-protein wheat lines contained more stem and leaf nitrogen at anthesis (11). Similar studies showed that vegetative parts of high- and low-protein wheat lines contained the same amounts of nitrogen (19). The lines in this study contained equal nitrogen in the flag leaves but more was in the lower leaves of the high-protein lines than in corresponding leaves of the low-protein lines at anthesis. Our results indicated high-protein lines had more total nitrogen available for incorporation into the grain.

The greater amount of nitrogen in the grain of control plants of the high-protein lines than the low-protein lines at maturity was noted by other investigators (8, 11). That could result from the greater amount of nitrogen in the vegetative parts of the high-protein lines at anthesis if the amount of nitrogen ultimately translocated to the grain depended on the level of nitrogen in the plant (7). The decrease in grain nitrogen of the high-protein lines when flag leaves were removed at anthesis indicated the importance of flag leaves in mobilization and translocation of vegetative nitrogen to the developing grain. Reports that removing flag leaves of wheat plants decreased grain-nitrogen content in excess of that contained in the flag leaves (15) were verified in the high-protein lines from this study. Removing the lower leaves at anthesis affected grain-nitrogen content more in the low-protein lines than in the high-protein lines. That indicated the remaining flag leaves were less actively involved in absorption of soil nitrogen and translocation of vegetative nitrogen.
to the head in the low-protein lines. The opposite was noted in the high-
protein lines and may be due partly to greater uptake of soil nitrogen by the
plants (15), and subsequent reduction by upper plant parts (5). High-protein
wheat lines probably also have more nitrate-reductase activity over a longer
period of time (3, 14). The high-protein lines incorporated more nitrogen into
the grain when all leaves were removed than when only the flag-leaf blades were
removed. It would be expected this treatment would cause less incorporation of
nitrogen in the grain. Because the culm-nitrogen content was also higher when
all leaves were removed, it can be concluded only that the head is probably as
active in nitrogen assimilation as it is in photosynthesis (2, 10).

Studies with spring wheat showed that high- and low-protein lines had the
same nitrogen content in leaves at maturity (11). In this study, a difference
in leaf-nitrogen content at maturity was noted (Fig. 5) in control plants.
When flag-leaf blades were removed at anthesis, nitrogen content was decreased
greatly in lower leaves of the high-protein lines, but not the low-protein lines,
as compared with control plants. That indicated the marked effect of the flag
leaves of the high-protein lines on plant-vegetative nitrogen as well as grain
nitrogen at maturity.

Comparisons of nitrogen content of leaves at anthesis (Fig. 3) and cor-
responding leaves at maturity (Fig. 5) suggested that flag-leaf nitrogen content
decreased in the low-protein lines and increased slightly in the high-protein
lines. Nitrogen content in the lower leaves increased in the low-protein
lines and decreased in the high-protein lines. That indicated the lower leaves
were less efficient in mobilizing and translocating nitrogen in the low-protein
lines than in the high-protein lines. However, the ranking between lines for
most and least nitrogen present in all leaves reversed between anthesis and
maturity. This again indicated Kaw accumulated nitrogen in the leaves while
Atlas 66 utilized more nitrogen for grain development.

Nitrogen content of culms varied similarly to that of grain among the varieties, lines and treatments. The higher content of nitrogen in control plants of high-protein lines than in low-protein lines also occurs in spring wheat (11). This is probably due to the same factors that govern grain-protein content. Removing flag leaves at anthesis decreased culm nitrogen in the high-protein lines, indicating the flag leaves were necessary for uptake of soil nitrogen or mobilizing lower-leaf nitrogen. The same effect was noted when lower leaves were removed from the low-protein lines at anthesis. Removing all leaves at anthesis should decrease the culm-nitrogen content at maturity below the level caused by the other treatments. It appears that the high-protein lines accumulated nitrogen in the culms without any leaves. That might be due to assimilation by remaining leaf sheaths, since they remain viable and active even longer than corresponding leaf blades (5).
ACKNOWLEDGEMENTS

The author wishes to express his sincere appreciation to Dr. Gary M. Paulsen, major professor, for his assistance in planning and conducting the experiments and in preparing the thesis.

Appreciation is extended to members of the committee for their suggestions concerning the thesis.

Appreciation is also extended to fellow graduate students in this laboratory for their assistance.

Thanks are also due to the author's wife, Nancy, for her help and encouragement.
REFERENCES


VITA

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Translocation of $^{14}$C-labelled amino acids from culms to grain of high- and low-protein wheat lines during grain development was low initially after anthesis, increased at mid-maturity, and decreased slightly at full maturity. Efficiency of translocation did not differ between high- and low-protein lines.

Total nitrogen content was similar in flag leaves and greater in lower leaves removed at anthesis from high-protein wheat lines than from low-protein wheat lines. Grain-nitrogen content at maturity was greater in the high-protein wheat lines when no leaves were removed at anthesis. Removing flag-leaf blades at anthesis had little effect on grain-nitrogen content of the low-protein wheat lines, but greatly decreased nitrogen content in the high-protein wheat lines. Removing the lower leaves or all leaves at anthesis decreased grain-nitrogen content markedly in all lines.

Nitrogen content at maturity was greater in culms of high-protein lines than of low-protein lines when none, all, or lower leaves only were removed at anthesis. When flag leaves only were removed, culms of low-protein lines contained more nitrogen. Nitrogen content in leaves at maturity did not differ consistently among high- and low-protein lines when no leaves were removed at anthesis. Nitrogen content of mature lower leaves remaining after flag leaves were removed at anthesis was greater in the low-protein lines. When lower leaves were removed at anthesis, the remaining flag leaves contained similar amounts of nitrogen at maturity in all wheat lines. Whole-plant nitrogen content of all four lines of wheat were similar at anthesis and maturity.