PHYSIOLOGICAL STUDIES ON THE WHEAT PLANT*

V. Diurnal Variations of Total Nitrogen and Amino-Acid Nitrogen in *Triticum vulgare*

BY SHRI RANJAN
AND
SANTOSH KUMAR BASU
(From the Department of Botany, Allahabad University)

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Introduction

It is not our intention to give, in this small paper, a full historical resumé of work done in connexion with the diurnal variations of total nitrogen and amino-acid nitrogen, nevertheless it will be necessary, in view of the points discussed in this paper, to give a brief account of the more recent and important work done on the subject. Chibnall, working on *Phaseolus vulgaris*, established a strong probability that there are diurnal variations in the nitrogen contents of the leaves—the total nitrogen increasing by day and diminishing by night. He argues, therefore, that the nitrogen gets transported during the night. But he also says that the decomposition of proteins takes place both in the day and at night, but in the day the process is masked due to a greater synthesis of the proteins.

Maskell and Mason also observed a diurnal variation of the total nitrogen in the cotton plant, so that the nitrogen increases by day and diminishes at night.

Regarding the work on the amino-acids Mc Kee thinks that probably ammonia is to be regarded as the starting point in amino-acid synthesis and Dhar and Mukerjee have found that amino-acids can be synthesized photochemically *in vitro*, using titanium dioxide as a catalyst. Blackman and Templeman working on grasses found that in full daylight when ammonium sulphate and calcium nitrate is added, the total nitrogen, largely in the form of protein, increases. At low light intensities, although there is a rise in total nitrogen due to manuring, elaboration of protein is reduced. Muenscher's work on chlorella shows that light is not a necessary factor in *in vivo* reactions. Nightingale has shown that amino-acids are formed in the dark in the roots of *Narcissus* and *Asparagus*. Russell working on the respiration

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* Four other papers of this series have been published in the 1940-41 *Proceedings of the National Academy of Sciences, India.*

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of Elodea densa finds that the amino-acid causes practically no increase in the rate of intake of oxygen.

The work on the diurnal variation of nitrogen, in the wheat plant, was undertaken in our laboratory to find out how far it was a photochemical process.

Material and Method

A. Material—

Samples of wheat plants (Pusa 52), growing in ordinary soil, were obtained from the botanical garden of the University. After removing the plants from the soil, they were immediately brought to the laboratory for analysis. For every experiment, samples were collected and analysed at 8 A.M., 12 noon and 4 P.M.

B. Sampling—

(i) LEAF.—The technique employed in sampling leaves closely follows that of Maskell and Mason (1924). Each leaf sample consisted of about ten to fifteen mature and healthy leaves of practically the same age for every experiment.

(ii) STEM.—The technique followed in sampling the stems was the same as that of the leaves. Sheathing leaf bases and very small portions of stems had to be employed as experiments were started when the plants were very young. From a number of plants, 25 to 30 equally thick pieces, each of about half an inch in length, were selected for every experiment.

C. Method of Analysis—

(i) LEAF.—Five grams of fresh and healthy leaves were quickly weighed, cut into small pieces and put into briskly boiling distilled water, sufficient to cover the material. The leaves were boiled for about five minutes. It was then finely crushed with some purified sand in a hand mortar and boiled again. It was then filtered and the residue was washed several times with hot distilled water. The filtrate was concentrated to a fixed volume and analysed for amino-acids. The residue was analysed for total nitrogen. The total nitrogen, therefore, represents the nitrogen of the non-soluble proteins only.

(ii) STEM.—The procedure, for stem analysis, was the same as for the leaf.

D. Estimations—

The amino-acid nitrogen was estimated by the Van Slyke’s method and the total nitrogen by Kjeldahl’s method using Nessler’s reagent. The colour
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Experimental Results

Section I—

The diurnal variation of total nitrogen and amino-acid nitrogen in stems and leaves during the ontogenic drift of the wheat plant.—The experiments were started with wheat seedlings grown in sawdust, which were from 10–14 days old. The amounts of total nitrogen and amino-acid nitrogen in the stems and leaves are given in Fig. 1. The total nitrogen rises from about 1.2 in the leaf in the morning to nearly 2 gms. per 100 gms. of leaves at noon and thereafter it falls to a little over 1.3 gms. In the stem also the total nitrogen rises from 0.6 to 0.8 at noon and then it falls off to 0.6 gms. The amino-acid nitrogen of the leaf keeps steady at 0.035 till noon and thereafter it falls off to 0.013. The amino-acid of the stem rises from 0.013 in the morning to 0.027 at noon and then slightly declines off to 0.022.

This sequence of rise from morning to noon and then a fall in the evening, in the total nitrogen, is seen in the wheat plant throughout its ontogenic drift, e.g., in plants 3 weeks, 4 weeks, 1½ months and 2 months old.
Total \( N_2 \) and amino-acid \( N_2 \) in leaves and stems of plants at different ages.

**FIG. 2**

(A) Age—3 weeks

(B) Age—4 weeks

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Fig. 4

(A) Total N\textsubscript{2} and amino-acid N\textsubscript{2} in gms. per cent of leaf and stem. Age—1\frac{1}{2} months.

Fig. 5

(A) Total N\textsubscript{2} and amino-acid N\textsubscript{2} in gms. per cent of leaf and stem. Age—12 months.
Figs. 2, 3, 4 and 5 show that even the magnitude of the rise and fall is nearly the same throughout the period the plant is growing. In the case of the amino-acid content also its rise and fall, during the ontogenic drift of the plant, is of the same type as in Fig. 1.

The above results, to a certain extent, support the views of Chibnall and Maskell and Mason who get an increase of total nitrogen in the day and a decrease at night.

Some experiments were also done on plants which were 3 months and over in age and in which flower buds had commenced to be formed. In these cases the swollen nodes near the bases of stems showed that the formation of these buds had taken place.

The total nitrogen and the amino-acid nitrogen of the stems and leaves of 3- and 4-months old plants are given in Figs. 6 and 7 respectively. From these figures it is apparent that the type of the increase of the total nitrogen is the same as that in the seedling stage except that the initial total nitrogen content and the subsequent rise at noon is slightly greater than the total nitrogen in the seedlings. The amino-acid nitrogen, however, in Figs. 6 and 7 shows lower values, inasmuch as in Fig. 5 when the plants were...
about 2 months old the amino-acid nitrogen of the leaf started at 0·031 gms. per cent. and increased to 0·036 at noon, but when the leaves were 3 months old it started at 0·026 only, and increased to 0·031. This amount one finds in Fig. 7 also where the plants were 4 months old. The slight increase in total nitrogen and a decrease of the amino-acid nitrogen in the leaves of plants 3 to 4 months old, over the young plants, is significant. In trying to explain this difference we venture to suggest that the protein metabolism in plants being obviously a reversible reaction, at a certain equilibrium position the hydrolytic and synthetic rates will become equal. But in plants the problem is far more complicated. Thus, among other things, the quantity of the amino-acid, in the leaf, will depend upon not only (1) the hydrolyses of the proteins to the amino-acids but also to (2) the synthesis of the amino-acids from the raw materials in light. As the production of the acid proceeds from these two sources, the equilibrium position, specific to the plant, is approached. With the approach of the equilibrium position, the condensation rate increases on the one hand, and on the other, the synthetic rate decreases. The decrease in the rate of the latter process is due to the increase of the products
of the reaction, in this case they being the amino-acids. If, however, the amino-acids are removed, say by translocation, then the formation of these acids, by the hydrolysis of the proteins on the one hand, and its synthesis on the other, will proceed at a rate depending upon its removal. In a young fast-developing plant, the amino-acids from the leaves move to the meristematic regions in the stem. Simultaneously with its removal, on the basis of mass action, increasing quantities of this acid will be produced. Conversely, in a mature plant such as plants 3–4 months old where, not only, vegetative growth has ceased but also the spikes have formed, the amino-acids do not travel to the stem. Their concentration in the leaves, therefore, causes increased condensation resulting in the increase of the proteins and a consequent lowering of the amino-acids (Fig. 7).

From the results another consistent feature emerges that from the seedling stage upwards, one always finds a steady increase, in both the stem and leaves, of total nitrogen and amino-acid nitrogen from morning to noon and thereafter a decrease.

These results, therefore, broadly speaking, support the views of Chibnall, Maskell and Mason who also get an increase of total nitrogen in the day, though they do not get a maximum rise at noon as we recorded.

These results confirm the view, that in light, both the amino-acid nitrogen and the total nitrogen increase, but they do not throw any light on the question as to whether this increase is due to the direct photochemical action or whether light only affects the synthesis in an indirect way by increasing the supply of some intermediate substances which may chemically unite with simple nitrogenous substances to increase the organic nitrogen compounds in the leaves. Light could also act in another indirect way and that is to increase the respiratory rate of a green leaf so that an excess of energy is supplied to bring about the endo-energetic reaction in the synthesis of proteins. To further clarify these points the following experiments were performed.

Section II—

Injection experiments on the wheat plant. Glucose injected. Strength 2% solution.—Wheat plants were removed in the morning at about 7 A.M. from the field and soon after were placed in beakers containing a little water. After about an hour 2% solution of glucose was injected into them. The plants were then kept covered, for the whole day, with a piece of black cloth.
Observations were recorded as usual at 8 A.M., 12 noon and 4 P.M. The amounts of total nitrogen and amino-acid nitrogen in the stems and leaves are given in Fig. 8.

The total nitrogen of the leaves rises from 1.35 to 1.55 at noon and thereafter falls off rapidly to 0.1 in the evening. The total nitrogen of the stem, however, continues to rise throughout the day.

The amino-acid nitrogen of the leaves arises from 0.03 to 0.043 at noon and then rapidly falls off to 0.008 by the evening. In the stem the amino-acid nitrogen is 0.014 in the morning, then it quickly rises to 0.036 at noon to fall off to 0.008 in the evening.

A control experiment was also carried out with plants similarly kept in dark without the glucose injections. Fig. 9 gives graphically the record of total nitrogen and amino-acid nitrogen of this set.
The total nitrogen of the leaf is 1.35 in the morning and by noon it falls rapidly to 0.33. The rapid fall then slows down and in the evening the value of the total nitrogen is 0.3. The total nitrogen of the stem throughout the day remains practically unchanged at 0.3.

The amino-acid nitrogen of the leaf falls from 0.01 in the morning to 0 by noon, and no amino-acid could be detected even in the evening. The amino-acid of the stem, however, rises from 0.004 in the morning to a little over 0.008 at noon. This amount then remains constant till evening.

Glucose in Shive's solution injected. Strength: 2% Glucose + Shive's solution with a slight excess Ca(NO$_3$)$_2$.—As in previous cases plants were brought from the botanical garden in the morning and were then injected with 2% glucose + Shive's solution, containing slightly higher percentage of nitrate. The plants were kept covered for the whole day with a piece of black cloth. The observations were recorded as usual at 8 A.M., 12 noon and 4 P.M.

Fig. 10 which gives graphically the total nitrogen and amino-acid nitrogen in the leaves and stems shows a rise and fall very similar to the rise and fall in Figs. 1-5 but the rise is slightly more accentuated here. Normally when the plants were growing in the open during their (Fig. 3) seedling stage the total nitrogen in the leaf and stem at 8 A.M. was 1.3 and 0.7 respectively while in this case it was 1.45 and 0.7 respectively. At 12 noon, the total nitrogen
increased to 2.4 and 0.95 in the leaves and stems of plants injected with the nutrient solution as opposed to 2.0 and 0.95 in the leaves and stems of plants growing in open sunshine (see Figs. 1-5). Thus the increase in this case at noon is appreciably higher. The fall towards evening is also much less, for the total nitrogen fell off to 1.8 and 0.8 in the leaves and stems while in Figs. 1-5 it fell off to such low values as 1.45 and 0.65.

But, one finds the biggest increase in the case of the amino-acid nitrogen.

Here the initial value was 0.040 and 0.020 in the leaves and stems respectively at 8 A.M., which rose rapidly to 0.060 and 0.037 respectively at noon. The corresponding initial amino-acid contents at the seedling stages (Figs. 1-5) was 0.030 and 0.013 respectively which rose at 12 noon to 0.035 and 0.024—these values are about half. One therefore arrives at the definite
conclusion that even in the dark but with the injection of glucose and Shive's solution containing excess of nitrates there is a greater increase of total nitrogen and amino-acid nitrogen than in the case of those plants that are growing in the open sunshine.

Discussion

**Total nitrogen and amino-acid nitrogen of the stem and leaf—**

At any moment the total nitrogen, *i.e.*, the total inorganic and organic nitrogen contents of a leaf will depend upon the following two variables:

(a) The rate of translocation from the stem into the leaf of nitrogenous compounds and

(b) the rate of translocation of nitrogenous substances from out of the leaf to the stem. If the rate (a) is more than (b) then there will be an accumulation of nitrogenous matter in the leaf and *vice versa*. It is, therefore, obvious, since movements of nitrogenous substances do take place in the stem and leaf, that there will be fluctuations in the amounts of this material at a particular moment in a given leaf.

Schulze and Schutz\(^{13}\) found for Acer a higher nitrogen content in the evening than in the morning. Chibnall, working on the scarlet runner bean, noted a fall in the total nitrogen at night. Miller\(^{7}\) also working on soybeans, cowpeas and garden beans, noted that in the majority of cases the total nitrogen increases in the evening and decreases is the morning.

The work done in this laboratory shows that at all stages of the development of the plants the leaves and stems show a low nitrogen content in the morning, a rapid rise to a maximum at noon and then a fall towards evening. These results are slightly different from the results of Chibnall, Miller and others who found a greater increase of total nitrogen towards evening.

The increase of protein nitrogen (shown in all the figures as total nitrogen), in the leaves is obvious if there is an increase of amino-acid nitrogen for, when a certain critical concentration of amino-acids has reached in the leaves, condensation to polypeptides and thence to higher proteins will necessarily take place and thus the total nitrogen, which in effect, is the total nitrogen of the proteins, will show an increase. Therefore, with the increase of the amino-acid nitrogen in the day a simultaneous increase of total nitrogen will also be seen in the leaves.

The rapid increase of the amino-acid nitrogen of the leaf in the day may be due to two factors *viz.*, (1) a greater accumulation of the inorganic nitrogenous material by the rapid translocation brought about by increased...
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transpiration in the day and (2) a rapid synthesis of organic nitrogen from inorganic sources, with the help of solar radiations acting directly or indirectly. The immediate source of energy for this endoenergetic reaction whether it is from the sun or from the chemical oxidations of the carbohydrates will be discussed subsequently. The amino-acids thus formed, get, on the one hand, partially condensed into the higher proteins in the leaves and on the other get translocated in the stem. This is obvious from Figs. 1–5 (B). The amino-acids of the stem as compared to those of the leaves rises till noon in all the cases much more rapidly. This must be due to the rapid translocation of this material from the leaf into the stem even during the time it is being formed in the leaf. The same Figs. 1–5 (B) show that the amino-acids of the leaf falls in the evening far more rapidly than in the stem. In fact in some cases like Fig. 1 (B) the curve for amino-acid of the stem cuts that of the leaf. This shows that the loss of this substance in the leaf is much greater than the stem. So that even though the production of amino-acid decreases in the afternoon, the translocation to the stem goes on at a fairly fast rate causing a depletion in the leaves of not only the amino-acids, but also of the proteins as is evident by the steep fall of the total nitrogen content of the leaf in the evening. On the basis of the balanced reaction a fall in the concentration of the amino-acids will naturally bring about the hydrolysis of the proteins.

From the accumulation of the amino-acids in the stem it is also evident that till noon the flow is greatest and thereafter this slightly slows down. Assuming now that the flow of any substance will depend upon (a) resistance and (b) a difference in the potential, then if the resistance is a constant factor, the flow will be guided primarily by the difference in the potential. Now, the time of the meristematic activities of the wheat cells is known to be at about noon. This is the time when new protoplasm is being laid and consequently a greater quantity of amino-acids are needed. Consequently the amino-acid nitrogen potential at the growing tip is nearly zero as it is being rapidly used up, or the difference of potential between the leaf and the growing tip is great. There is of course no change in the resistance for the channels, through which nitrogenous material flows, remains unchanged. Therefore till noon there is a greater flow of the amino-acids.

Energy requirements in the synthesis of proteins—

Experiments in section 2 throw some valuable light on the problem of the energy requirements in the synthesis of proteins. Figs. 8 and 9 are significant in this connexion. Fig. 8 (B) shows that when the excised leaves were injected with a 2% solution of glucose but kept in the dark, the
amino-acids increased considerably till noon, as also there was a slight increase of the total nitrogen in the leaf (Fig. 8 A). The leaves not injected with glucose but kept in the dark showed a rapid fall of total nitrogen (Fig. 9 A) by noon as also there was a rapid fall to zero of the amino-acids (Fig. 9 B). This in itself proves that the synthesis of amino-acids can take place in the total absence of solar radiations but with an excess of carbohydrate material. The fate of the glucose after injection and its rôle in the synthesis of proteins can be summarized as follows:—After the injection of glucose solution the intercellular spaces get filled with this solution. Ranjan\textsuperscript{10} working on Cherry-laurel leaves in Cambridge has, however, shown that even after injection enough oxygen enters the leaf to ensure perfect aerobic respiration. The solution after injection fairly rapidly enters every cell of the leaf abutting on the intercellular spaces. The glucose molecules, after their entrance, have now three alternative fates; \textit{viz.}, they get

1. Condensed into the polysaccharides.

2. Oxidised to $\text{CO}_2 + \text{H}_2\text{O}$.

3. Chemically united at some intermediate stage with other chemical substances present in the cell.

With the 1st we are not concerned. The 2nd and 3rd cases are important so far as it affects protein synthesis.

Taking the 3rd case we suggest that during some stage in the glycolysis of the carbohydrates the substance resulting therefrom unites with some derivative of the nitrates to form the amino-acids. Possibly there are at least two endoenergetic reactions involved in this requiring energy; \textit{viz.}, (1) the reduction of $\text{NO}_3$ to $\text{NO}_2$ during its change to some ammonium derivatives and (2) the synthesis of amino-acid compounds from the chemical union of the above with the glycolysed substance of the carbohydrate. The energy evidently is supplied by the oxidations of the carbohydrates. In uninjected cases, the leaves are also oxidising the carbohydrates in the dark, but evidently the energy is only sufficient to carry on the other metabolic activities of the plant cell and hence an excess is not available for the synthesis of amino-acids. But when glucose is injected, as shown by Ranjan,\textsuperscript{10} for Cherry-laurel leaves, the respiration rate temporarily increases to a great extent. Thus quite a lot of energy is released which is utilised in the synthesis of proteins. This increased energy is lacking in the leaves in dark, and hence there is no formation of amino-acids.

Again numerous injection experiments have shown that after injection the respiration rate only temporarily increases, the duration of the increase
depending upon the concentration of the solute so injected. Thus, after a
time, when the respiration rate climbs down to the normal the synthesis of
the amino-acids also decreases as is shown in Fig. 10 where towards the
evening both the total nitrogen and the amino-acid nitrogen decreased con-
siderably.

In the case of plants kept in dark but not injected one finds (Fig. 9 B)
that the amino-acid nitrogen of the stem increases at noon, though the
total nitrogen remained constant throughout. The total nitrogen and the
amino-acid nitrogen of the leaf, however, came down very low at noon. As
has been previously said the meristematic activity is maximum at noon in the
wheat plant. And thus proteins in the form of amino-acids travel from the
leaf into the stems at noon, causing an increase of these substances in the
stem at the cost of the leaves.

These experiments also show that after injection the energy of oxidation
of glucose is in excess to the requirements of energy during synthesis of pro-
teins. In other words, the energy is not the limiting factor, but the nitrate is
the limiting factor as Fig. 10 shows. When 2% glucose solution along with
Shive's solution with an excess of Ca(NO₃)₂ was injected, the amino-acid
content of the leaf started at 0·040 and reached 0·060 at noon whereas, in
only the glucose injected leaves, the amount was 0·008 in the morning rising
to 0·02 at noon. Thus the increase was nearly 3 times in the case of Shive's
solution with glucose. Here the percentage of glucose was the same as in
the experiment represented by Fig. 8, and so the energy of oxidation was
kept constant but only the nitrate was increased resulting in the increase of the
synthesis of amino-acids.

The synthesis of proteins in light—

In the light of the aforesaid discussion we venture to suggest that when
the wheat plants are normally growing, in light, the energy for the synthesis
of amino-acid is derived from the oxidation of the carbohydrates in light and
not by the energy of the solar radiations that are absorbed by the green leaf.

For if the solar radiations were responsible for the synthesis of proteins,
then at 4 P.M., as at noon, there should have been an increase in the total
nitrogen. But, as is evident from the various figures, it is not so. The
question then arises as to the cause of this fall, in the afternoon, in the total
nitrogen.

As has been said before, the translocation of the amino-acids cannot be
responsible for this fall for the simple reason that relatively little of this acid is
translocated in the afternoon. Yap¹⁴ has noted, however, that the rate of
photosynthesis of the sugarcane decreased from 10 A.M. to 4 P.M., the process being the most active from 8 A.M. to 10 A.M. This has also been noted for other plants by various other workers. When carbon assimilation is actively going on, the respirable sugar, on the one hand, increases which causes increased respiration and on the other, substances like propionic acid may be formed as shown in Fig. 11 which may chemically unite with NH₃ to form the amino-acids. If this be so, it will account for the increased formation of the amino-acids and the total nitrogen at noon and their relative decrease in the afternoon. Ranjan has also shown that the rate of respiration, in light, of a normal green leaf increases considerably over the rate of respiration in darkness. Thus the excess of energy so liberated gets transferred for the synthesis of the amino-acids. A tentative scheme of reactions is given in Fig. 11.

![Fig. 11](image-url)
We may then venture to suggest that the twin causes responsible for the increase of organic nitrogen in light are (1) increased respiration in light and (2) formation of intermediate substances, as a result of carbon assimilation, which eventually form the amino-acids. Thus light plays only an indirect part in the synthesis of proteins.

**Summary**

1. Samples of stems and leaves were separately obtained from the wheat plants at 8 A.M., 12 noon and 4 P.M. The total nitrogen and amino-acid nitrogen of the above samples were estimated. It was observed that the amounts of total nitrogen and amino-acid nitrogen in the leaves rise and reach a maximum value by noon, and thereafter decrease. In stems the total nitrogen varies directly with the variations in the total nitrogen of the leaves.

2. Excised leaves when kept away from light showed a rapid fall in the total nitrogen content from morning to noon, while the nitrogen in the stem remained constant throughout.

3. When the leaves were injected with 2% glucose solution and kept in dark the total nitrogen increased towards noon and thereafter it fell off. The nitrogen of the stem, however, showed throughout a slight rise.

4. The total nitrogen in the leaves when injected with 2% glucose + Shive’s solution and kept in dark, showed a marked increase towards noon. The increase, however, fell off towards evening.

5. In discussing the results the authors find that in the day the carbohydrates increase, as a result of photosynthesis, so also the total nitrogen. But how is it that there is such a relationship? The senior author has shown elsewhere that the respiration increases in light to a marked extent. The increased energy thus released by increased respiration in light brings about the synthesis of proteins. This is substantiated by experiments in plants kept in dark but injected with glucose + Shive’s solution. In this case both the total nitrogen and the amino-acid nitrogen rapidly increases. Here again, with the injection of glucose the respiration rate rises rapidly, and the increased energy given out in this exothermal reaction is enough to cause the synthesis of nitrogen from some derivative of the NO$_3$ of the Shive’s solution and the glycolysed product of the injected glucose. In the case of leaves in light, however, due to carbon assimilation, intermediate substances which ultimately unite with the derivatives of nitrates are formed and with the help of the energy of the respiration in light, organic nitrogen is built up. Light, therefore, plays only an indirect part in such a reaction, the direct being played by glucose. A tentative scheme of reactions is given in the text.
LITERATURE CITED