

Nitrogen mobilisation during germination and growth of soybean (*Glycine max.*)

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The growth in plants has various phases which starts with the activation of the embryo and ends with the maturation of seed. It is well known that there is a translocation of nutrients from the pods of the *Leguminosae* to the developing seeds. The available evidence indicates that the nitrogenous nutrients do not pass directly from the leaves to the seed, through the pods, but are at least in part at first stored in the pods in the form of protein, which is then used to supply the developing seeds. Many workers have studied the transfer of the pod proteins to the seeds, but although it is certain that the proteins must be broken down before translocation, the form in which the nitrogen passes to the seeds is still rather uncertain.

In continuation to our previous work on phosphorus and nucleic acid metabolism of soybean during growth and germination (ARORA & GANDHI 1969) it seemed desirable to study the changes in different anatomical parts, during growth and germination of soybean.

MATERIAL AND METHODS. — The germination of soybean seeds of variety Pb. No. 1 was carried out in washed and sterilised sand. Sand was filled in trays and levelled properly. The sand for sowing was sterilised because the seeds of soybean are susceptible to fungus growth. Seeds for germination were kept at the depth of one inch in sand. The trays were kept in the screen house, under normal atmospheric conditions. The samples of seedlings were taken, each after 2, 4, 6 and 8 days of germination. Seedlings were washed with distilled water to make them free from adhering sand particles and were then separated into cotyledons, radicles, plumule and leaves wherever possible.

For ripening study, open flowers were tagged and samples for the present study were drawn periodically only from pods set on tagged flowers. Tagging of flowers was done during the peak flowering period. Samples of pods and leaves were collected after 17, 27, 37 and 47 days after tagging of flowers; the pods were separated into seed covers and seeds.

The samples of seeds, seed covers, leaves and seedlings were dried at low temperature (70°C) and grinded in a grinder and stored in glass stoppered tubes for further analysis.

The following estimations were carried out in duplicate and recovery was tested in each case and results were calculated on that basis.

The total nitrogen was estimated by micro-kjeldahl's method as described by MACKENZIE and WALLANCE (1954). Total soluble nitrogen was estimated by the method of VICKERY *et al.* (1935). Ammoniacal & amide, nitrogen by PUCHER *et al.* (1935). Nitrate nitrogen by NICHOLAS and NASON (1957). Protein nitrogen was calculated as the difference between the total nitrogen and total soluble nitrogen.

For the estimation of amino nitrogen the following procedure was adopted.

Estimation of amino nitrogen - Ninhydrin reagent (freshly prepared). — It was prepared by dissolving 100 mg ninhyddrin in 37.5 ml of acetone and 12.5 of sodium citrate buffer (pH 5.5) and to it added 20 mg stannous chloride. It was stored in dark bottle.

Procedure. — 2 ml of plant extract was taken in the pyrex test tube with 1 ml of 2.5 N sodium hydroxide. The test tube was placed in the boiling water bath for 15 minutes and neutralised the solution with 1 N hydrochloric acid. Volume was made to 10 ml with distilled water. 2 ml of this solution was taken in pyrex test tube with 1 ml of ninhydrin reagent and was kept in boiling water bath for 15 minutes. It was cooled by keeping in cold water and volume made to 5 ml with 50 per cent alcohol. The colour in the tube was read in a colorimeter, at 580 m μ filter. With every set of tubes, a five point standard curve of L-leucine was plotted for comparing the sample with unknown.

RESULTS AND DISCUSSION. — The samples of seed, seed cover and leaves taken after 17, 27, 37 and 47 days after tagging of soybean flowers and samples of cotyledons, roots, shoots, leaves and testa taken after 0, 2, 4, 6 and 8 days of germination of soybean, were analysed quantitatively for different nitrogen fractions. The results obtained are tabulated in Table 1 and 2 and shown graphically in Fig. 1 to 12.

Nitrogen metabolism during germination (Table 1). — During germination, the total nitrogen content decreased in cotyledons and leaves, while an increase in content of total nitrogen was observed in roots and shoots. Similar results were reported by PATEL *et al.* (1961) and LAWRENCE *et al.* (1959) in pea seedlings.

There is a break down of proteins in cotyledons on one hand and appearance of new proteins in the other parts of the seedlings

TABLE 1. — *Changes in nitrogen fractions during germination of soybean.*
gm/100 gm (on dry wt. basis)

Plant part	Time (days)	Total-N	Total Soluble-N	Protein-N (total-N- total sol. N)	Amino-N	Ammoniacal-N	Amide-N	Nitrate-N	Soluble org. N (total sol. N- nitrate-N)
Cotyledons	0	7.70	3.14	4.56	0.16	0.12	0.35	0.10	4.45
	2	7.88	2.98	4.90	0.10	0.05	0.18	0.12	2.86
	4	7.53	2.28	5.25	0.12	0.07	0.31	0.10	2.18
	6	7.00	3.50	3.50	0.17	0.98	0.66	0.09	3.41
	8	6.48	3.60	2.98	0.13	0.81	0.74	0.11	3.40
Testa (1)	2	3.68	1.05	2.63	0.07	0.04	0.13	0.10	0.96
Roots	2	4.03	1.93	2.10	0.07	0.04	0.26	0.11	1.84
	4	5.78	2.98	2.80	0.08	0.21	0.29	0.14	2.84
	6	6.13	2.80	3.33	0.10	0.46	0.70	0.08	2.72
	8	6.30	2.60	4.03	0.11	0.56	0.76	0.13	2.15
Shoots	2 (2)	—	—	—	—	—	—	—	—
	4	7.00	5.60	1.40	0.07	0.35	0.74	0.10	5.50
	6	7.53	4.38	3.15	0.08	0.60	1.05	0.12	4.25
	8	7.68	3.68	4.20	0.10	0.81	1.71	0.13	3.56
Leaves	6	7.63	3.68	3.85	0.14	0.14	0.44	0.12	3.56
	8	5.78	5.25	0.53	0.15	0.32	0.57	0.11	5.14

(1) Testa degraded after 2 days of germination.

(2) The plumule portion was too small and taken together with root at 2 days germination of soybean.

TABLE 2. — *Changes in nitrogen fractions during ripening of soybean.*
gm/100 gm (on dry wt. basis)

Plant part	Time (days)	Total-N	Total Soluble-N	Protein-N (total-N- total sol. N)	Amino-N	Ammoniacal-N	Amide-N	Nitrate-N	Soluble org. N (total sol. N- nitrate-N)
Seed	70	5.78	3.50	2.28	0.14	0.35	0.14	0.08	3.42
	80	6.48	2.45	4.03	0.12	0.07	0.19	0.09	2.36
	90	7.00	2.63	4.38	0.10	0.06	0.23	0.10	2.52
	100	7.53	2.63	4.90	0.13	0.04	0.13	0.07	2.55
Seed cover	70	3.68	2.45	1.23	0.16	0.25	0.13	0.09	2.36
	80	2.30	1.93	0.88	0.14	0.12	0.17	0.08	1.84
	90	2.28	1.58	0.70	0.17	0.07	0.22	0.09	1.49
	100	1.75	1.05	0.60	0.18	0.04	0.15	0.07	1.69
Leaves	70	5.95	2.10	3.85	0.17	0.04	0.05	0.09	2.01
	80	3.50	1.58	1.93	0.10	0.07	0.09	0.10	1.48
	90	2.98	1.05	1.93	0.12	0.07	0.13	0.10	0.95
	100	2.30	1.75	1.05	0.13	0.11	0.05	0.14	1.61

Changes in various nitrogen fractions during growth and germination of soybean.

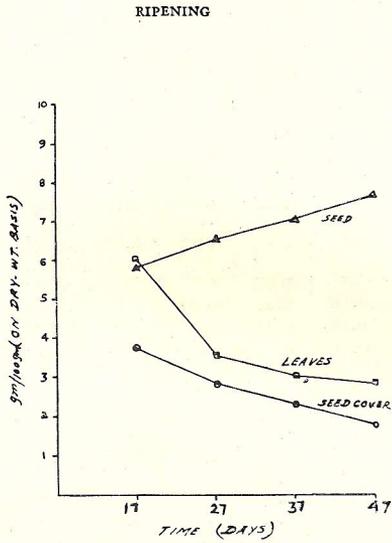


FIG. 1. Total-N

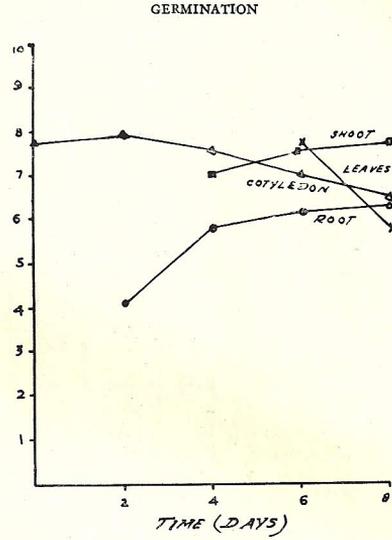


FIG. 2. Total-N

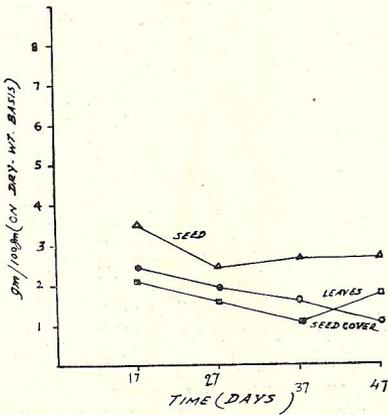


FIG. 3. Total soluble-N

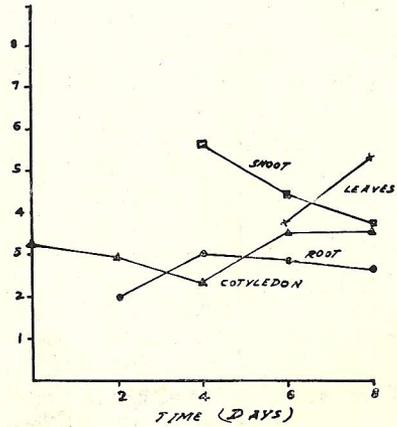


FIG. 4. Total soluble-N

RIPENING

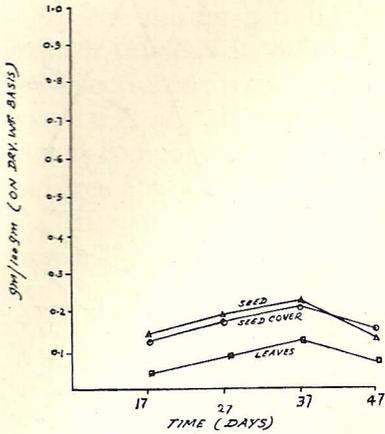


FIG. 5. Amide-N

GERMINATION

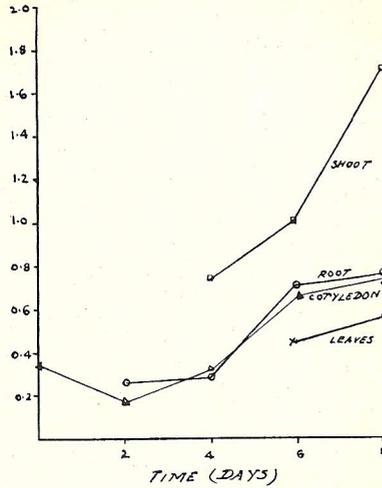


FIG. 6. Amide-N

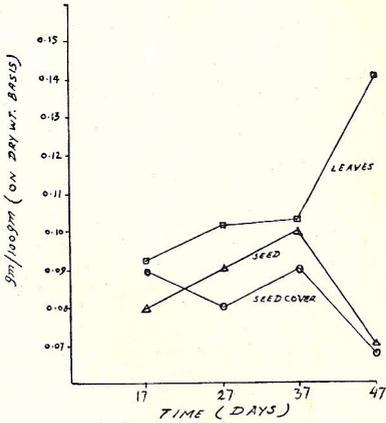


FIG. 7. Nitrate-N

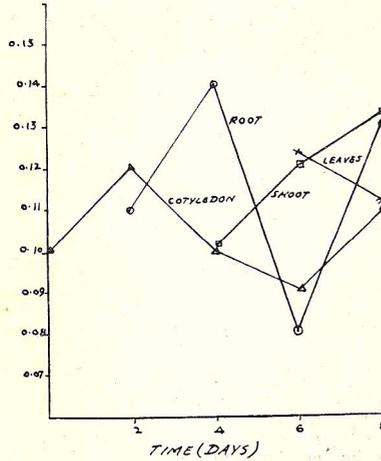


FIG. 8. Nitrate-N

on the other. Other nitrogenous compounds also appear as germination proceeds. Thus there is a little change in the total nitrogen content of the seedling during germination. The observation that the total-N in the cotyledons decreases more rapidly than the con-

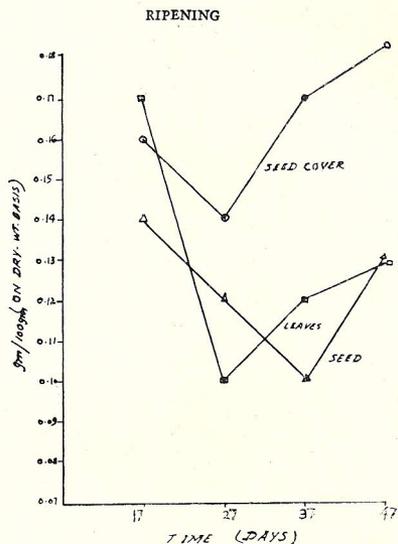


FIG. 9. Amino-N

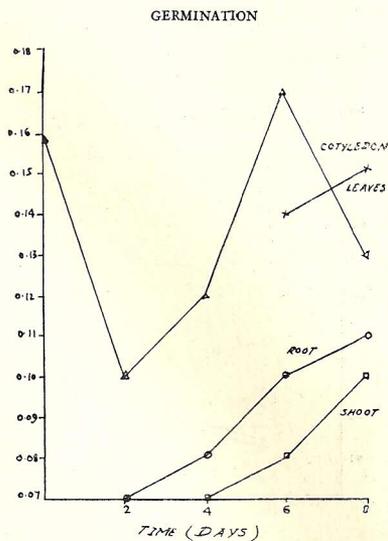


FIG. 10. Amino-N

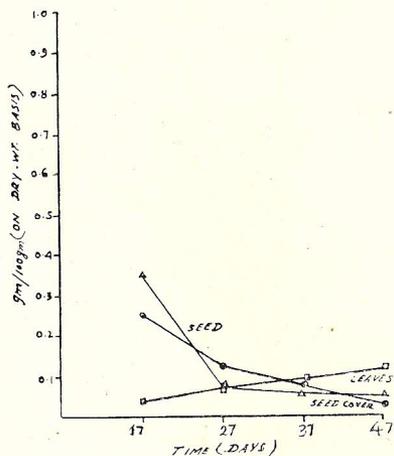


FIG. 11. Ammoniacal-N

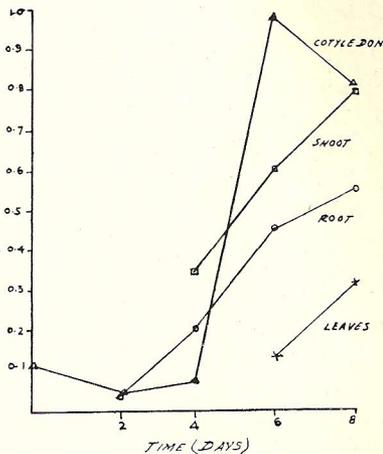


FIG. 12. Ammoniacal-N

comitant build up of soluble nitrogen, indicates that the products of the reserve protein hydrolysis are readily translocated to the developing axis (BEEWERS & GUARNSEY, 1966).

During germination of soybean, total soluble-N decreased (4 days) followed by an increase during later stages in cotyledons.

Protein-N on the other hand increased upto 4 days and then decreased gradually. Amino-N increased during the early stage followed by a decline in the later stage. Ammoniacal-N showed a sharp rise during germination on day 6 followed by a slight decrease. Amide-N increased with the germination time while nitrate-N decreased in the beginning and increased in later stage. LAWRENCE *et al.* (1959) also observed an increase in different nitrogenous compounds i.e. alcohol soluble-N, amide-N, ammoniacal-N and free amino-N in the cotyledon of germinating pea.

In roots (Table 1) the contents of protein-N, amino-N, ammoniacal-N, and amide-N were found to increase over germination time whereas an increase in total soluble-N content was followed by a gradual decrease. In shoots total soluble-N, was found to decrease while the content of protein-N, amino-N, ammoniacal-N, amide-N and nitrate-N were found to increase with germination time. In leaves, total soluble-N, amino-N, ammoniacal-N, and amide-N were found to increase whereas protein-N and nitrate-N were found to decrease with germination time. The accumulation of total soluble-N, primarily amino-N, in the cotyledons and axis during germination indicates that the mobilisation of nitrogen is facilitated by proteolysis and translocation of products.

LEBLOVA (1966) also reported a slight increase of ammoniacal, amide-N and of free amino acids both in the reserve and in other parts of germinating pea plants.

Nitrogen metabolism during ripening (Table 2). — During seed formation, the total nitrogen content increased, while in seed covers and leaves, it decreased. The nitrogen content was low during early stages of pod formation, increased to high level in subsequent stages.

PICKET (1950) also reported that in developing peanut seed, protein increased from low level in very immature seeds to high level in mature seeds and remained almost constant in latter stages of maturity.

In the seed covers, although the synthesis is also occurring, the breakdown of protein is predominant. Nitrogenous materials from the seed covers are gradually absorbed by developing embryo thus corroborating chemically the long known histological observations. On the other hand, the balance of metabolic activi-

ties lies overwhelmingly on the synthetic side, as is shown by the rapid increase in protein, in seeds. RAACKE (1957, a) observed similar results for ripening pea seeds.

The decrease in total-N in seed covers may be attributed to the fact that nitrogen compounds were moving from the seed covers to the seeds at a more rapid rate than they were entering the seed covers, thereby causing a drain on the reserve nitrogen compounds in the seed covers. The rate on drain was greatest during the early growth. The results are in conformity with the findings of BISSON and JONES (1932). The total-N content in leaves decrease throughout the ripening, with concurrent accumulation of total-N in seeds. HAY, EARLEY and DE TURK (1953) observed similar trend of total-N in maize leaf during growth.

During seed formation, total soluble-N, decreased during initial stages followed by an increase in the latter stage and remained constant thereafter whereas in seed cover and leaves, it decreases. Protein-N increased in seeds whereas in seed cover and leaves, it decreased markedly. Amino-N in seed and seed covers remains more or less constant during ripening while in leaves, it decreases. Ammoniacal-N decreased in seeds and seed covers and in leaves it slightly increases. The content of amide nitrogen decreases during later stages after an initial increase in all the parts. Nitrate-N increased slightly in seeds and leaves while in case of seeds cover, it remained almost constant.

WOODMAN and ENGLEADOW (1924) and ZALESKI (1905) reported that in ripening seeds, soluble nitrogen compounds passed from the leaves to the fruit and disappeared there with the formation of protein. SCHULZE and coworkers (1910 and 1911) reported that during ripening, there was a breakdown of protein in the pod, and soluble nitrogen compounds pass from pods to seed.

KHAVKIN *et al.* (1964) analysed mature and young leaves of *Faba vulgaris* and reported that the mature leaves contained lower nitrogen content than the young leaves. In the seeds, the combined effects of, rapidly increasing cell size and increased rate of protein synthesis, with increasing protein concentration, resulted in a decrease in the concentration of soluble nitrogen constituents. About this time the cocentration of all amino acids decreased. The seed covers showed a distinct contract to the seeds. The soluble nitrogen concentration was never as high as in seeds, and drifted slowly down.

The decrease in soluble nitrogen and protein nitrogen must be attributed largely to export from the seed covers. Since the soluble nitrogen did not change upto 27 days after tagging but the protein nitrogen increases so the grain in seed might have come from the seed cover. The increase in the rate of protein synthesis coincided with a decrease in soluble nitrogenous compounds, which entered the seed at a lower rate than they were used in protein synthesis, as is well known that there is a translocation of nutrients from the seed covers of the leguminosae to the developing seeds. As is seen from Table 1, the decrease in total-N concentration is closely paralleled by a decrease in protein concentration whereas the concentration of amino and amide nitrogen remains almost constant throughout the ripening process. These data (Table 2), illustrate very clearly that protein is first synthesized in the seed covers and then broken down and transported to developing seeds.

Since, however, there is a little change in amide in seed cover protein, the large amounts of free amide must be formed by secondary reactions from amino acids in the built up of soybean proteins. It was suggested by RAACKE (1957) that amide metabolism in the plant does not follow a single pathway, and that in normally synthesizing tissues which are not subjected to any stress such as etiolation or unbalanced supply of nutrients, which call upon the defence mechanism of the plant, at least part of amide nitrogen does not suffer a fate different from that of amino nitrogen.

ACKNOWLEDGEMENT — The Authors are grateful to the Director of Research, Punjab Agricultural University for providing necessary research facilities and to Prof. I. S. BHATIA, Department of Chemistry and Biochemistry for encouragement.

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SUMMARY — Nitrogen metabolism during germination and ripening of soybean is reported in this paper. The nitrogen content increased throughout the ripening of the soybean seeds where as it decreased in seeds covers and leaves. The content of amino-N and ammoniacal-N decreased during ripening. whereas amide-N and nitrate-N increased. In case of seed cover the content of amino-N and amide-N increased along with nitrate-N. Ammoniacal-N decreased.

The nitrogen content decreased slowly throughout the germination in the cotyledons and increased slightly in the developing roots and shoots. The amino-N and ammoniacal-N increased whereas protein-N and nitrate-N decreased in cotyledons. In case of roots and shoots, amino-N, ammoniacal-N and nitrate-N increased. The amide-N increased throughout the germination.

RÉSUMÉ — On a cherché de connaître le métabolisme azoté de la soya pendant la germination et la maturation.

Le contenu en azote augmente dans les graines, pendant la germination, tandis qu'il diminue dans les téguments et dans les feuilles. Le contenu en N-ammoniacales et Naminique diminue pendant la maturation, tandis que l'N-amidic et nitrique augmente. Dans les téguments le contenu en N-amini- que, amidique et nitrique augmente, tandis que l'ammoniacale diminue.

Le contenu en azote baisse lentement pendant la germination des coty- ledons, et augmente légèrement dans les racines et les bourgeons.

Dans les cotylédons le N-aminic et ammoniacale augmente tandis que le N-protéique et nitrique diminuent.

Dans les racines et les poussés le N-diminue pendant la germination.

ZUSAMMENFASSUNG — Man hat den stickstoffhaltigen Metabolismus der Soja, während der Keimung ausgeforscht.

Die Enthaltung an Stickstoff nimt in den keimenden Samen zu, während sie in den Hülsen und in den Blättern abnimmt. Die Enthaltung an ammoniakhaltig und aminic-N, nimmt während der Reife ab, und nimmt, in gegen teil zu an amidic und Nitric-N. In den Hülsen nimt die Enthaltung an aminic-N, amidic-N und nitric-N zu, während die ammoniakhaltige abnimt.

Die Enthaltung an Stickstoff wird langsam weniger während der Keimung der cotiledoni, und nimt ein wenig zu, in den Wurzeln und in den Knospen. In den cotiledoni nehmen zu aminic-N, das ammoniakhaltige, während eiweiss haltige und nitric-N abnehmen.

In den Wurzeln und in den Sprösslingen nimt das aminic, amoniakale und nitric-N zu.

Das amidic-N nimt während der Keimung ab.

RESUMEN — Ha sido estudiado el metabolismo nitrogenado en la soja durante la germinación y la maduración.

En contenido en nitrógeno aumenta en las semillas germinantes, mientras que va disminuyendo en los tugmentos y en las hojas. El contenido en N-amoniacal y amínico disminuye durante la maduración, pero va aumentando el N-amídico y nítrico. En los tugmentos aumenta el contenido en N-amínico, amídico y nítrico, mientras que va disminuyendo el amoniacal

El tenor de nitrógeno se baja lentamente durante la ferminación en los cotiledones y crece levemente en las yemas. En los cotiledones aumentan el N-amínico y el N-amínico y el amoniacal, mientras que disminuye el N proteico y nítrico. En las raices y en las gérmenes aumenta el N-amínico, amoniacal y nítrico. El N-amídico disminuye durante la germinación.

RIASSUNTO — È stato indagato il metabolismo azotato della soia durante la germinazione e la maturazione.

Il contenuto in azoto aumenta nei semi germinanti, mentre diminuisce nei tegumenti e nelle foglie. Il contenuto in N-ammoniacaale e aminico decresce durante la maturazione, mentre cresce l'N-amidico e nitrico. Nei tegumenti aumenta il contenuto in N-aminico, amidico e nitrico, mentre l'ammoniacaale diminuisce.

Il tenore in azoto si abbassa lentamente durante la germinazione nei cotiledoni e cresce leggermente nelle radici e nelle gemme. Nei cotiledoni aumentano l'N-aminico e l'ammoniacaale mentre diminuiscono l'N proteico e nitrico. Nelle radici e nei germogli aumenta l'N-aminico, ammoniacaale e nitrico. L'N-amidico decresce durante la germinazione.