

Plant Nitrogen Metabolism and Calcium or Potassium Deficiency

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Abstract. The content and distribution of nitrogen substances in wheat, pumpkin and pea seedlings in complete (NS) or deficient ($-Ca^{2+}$ or $-K^{+}$) nutrient solutions were studied with the aim of establishing whether impairments in the synthesis of nitrogen substances in vegetative tissues due to the deficiencies may be compensated for by storage nitrogenous substances. Pumpkin and pea seedlings were found to accumulate nitrogen mainly from depots of the seed stores. Both the accumulation and the synthesis of nitrogen substances were inhibited by the cation deficiencies, especially by that of calcium. Concomitant morphological deformations were probably the direct cause of the impairments in the mobilization and hence also in the utilization of the substances. With wheat, on the other hand, no morphological changes resulted from the deficiencies and nitrogen was accumulated intensively even from the cultivation solution. Still, the rate of the synthesis of the nitrogenous substances was significantly reduced. Under the conditions of calcium or potassium deficiency in the outer nutrient solution the possibility or utilizing the ions from the storage tissues according to the requirements of the plant appeared to be decisive. The extent of the disproportion of the two factors was the original cause of the early morphological changes which, in their turn, impaired the natural circulation of substances inside the organism, including the substances containing nitrogen.

Conspicuous growth defects resulting from calcium or potassium nutrient deficiency in wheat, pumpkin and pea, as described by us earlier (PICHL and FIALOVÁ 1972) led us to study the content and distribution of nitrogenous substances. Our main interest was to find out which factors are of importance in the utilization of reserve nitrogenous substances and in what extent a certain store of *e.g.* proteins may compensate for disturbances in the synthesis of nitrogenous substances in vegetative tissues of calcium or potassium deficient plants. The three plant species used in the experiments differed not only in the extent of nitrogen, calcium and potassium reserves but also in their localization (endosperm, epigeic or hypogeic cotyledons) and in the requirements for these nutrients. The present communication is a continuation of a series of papers from this laboratory (DVOŘÁK and VAŇOUSOVÁ 1966, VAŇOUSOVÁ 1968, VRZÁKOVÁ 1968, ŠINDELÁŘOVÁ 1969).

It is known from the literature that calcium is an important factor in preserving the integrity of subcellular membranous structures and that the destruction of these membranes due to calcium deficiency is the primary cause of metabolic disturbances. Potassium, on the other hand, is known to catalyze a number of important metabolic reactions. All the same, the quantitative requirement for potassium, probably due to its further functions, is proportional to the metab-

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olism intensity. Unlike with calcium deficiency, potassium deficiency brings about metabolic disturbances immediately. Further importance of the two cations is in their neutralization effect and specially in the colloid hydration. There the physiological concentration of calcium usually already decreases the colloid hydration, whereas the physiological concentration of potassium still increases it. The mobility of potassium in tissues is high; calcium, on the other hand, forms insoluble salts and chelates and the two ions are used by the plant accordingly.

Materials and Methods

Triticum aestivum L. cv. Fanal, *Cucurbita pepo* L. cv. Kveta and *Pisum sativum* L. cv. Zázrak z Kelvedonu were germinated and cultivated in Knop's nutrient solution which was either complete or calcium- or potassium-deficient (see PICHL and FIALOVÁ 1972 for details). Nitrogen content was determined using the standard Kjeldahl technique. Proteins from vegetative tissues were isolated from frozen homogenized tissue with a buffer solution according to Staples and Stahmann as modified by DVOŘÁK and ČERNOHORSKÁ (1967): 0.1 M TRIS, 17% sucrose, 0.1% ascorbic acid, pH 8; 1 part of the buffer solution was used for 1 part of the tissue homogenate. After 1 hour extraction at the laboratory temperature, supernatant was separated from the sediment using a centrifuge and the proteins in the supernatant were precipitated with TCA in the final concentration of 10% and determined according to LOWRY *et al.* (1951). Proteins from storage tissues of dry seeds and their rests in seedlings were isolated by the standard technique of Barnstein (see JANÍČEK *et al.* 1962) and their amount estimated from their nitrogen content by multiplication with 6.25. The technique was chosen in view of the poor solubility of the reserve proteins. The isolation is more effective than extraction with buffer according to Staples and Stahmann and leads to values which are by about 30% higher. This difference was taken into account when evaluating the experimental results.

Determined values of nitrogen and protein contents were expressed per one plant or per content of dry matter. The usual t-test was used when evaluating the results statistically.

Results and Discussion

Rapid destruction of storage proteins during early stages of germination is a characteristic of cereals (BISHOP 1929). According to our data the decrease of nitrogen and protein content in wheat grains proceeded at the highest rate between the 3rd and the 7th day of germination (Fig. 1) and was significantly slowed down in deficient, especially calcium-deficient nutrient solutions (Table 1). During subsequent days of germination the content of storage nitrogen substances decreased to about one-tenth of the original value.

Intensive increase in the nitrogen content of vegetative tissues (Fig. 1) — the content on about the 13th day of plant age is approximately three times higher than the original content in the grain — suggests that wheat accumulates intensively also the nitrogen from the nutrient solution. Hence an effective functioning of plant roots is to be assumed although the deficient nutrition affected both their fresh weight and dry matter (PICHL and FIALOVÁ 1972) as well as the nitrogen content in dry matter (Fig. 3). The increase in the nitrogen content was continuous and was slowed down by the deficiencies (Fig. 1). The content of synthesized proteins in vegetative tissues reached at most one-half of their content in storage tissue (Fig. 1). In complete and calcium-deficient nutrient solutions their content at first increased at a higher rate; on about the 11th day, however, their total amount started to decrease. On about the 13th day, it reached approximately the same value as in the potassium-deficient solution, where the initial rate of protein accumulation was lower already from the start of germination.

TABLE 1
SIGNIFICANCES OF DIFFERENCES in Figures 1 and 2

Plant	Tissues	Age [d]	Nitrogen		Protein	
			NS/-Ca	NS/-K	NS/-Ca	NS/-K
Wheat	Endosperm	5	+++	+	-	-
		7	++	+	+++	++
		9	+++	-	++	+
	Shoot	5	+	-	+	-
		7	+	++	-	-
		9	-	-	+	+
		11	-	-	-	++
	Root	5	+	-	-	-
		7	+	++	-	-
		9	-	++	-	-
		11	-	-	-	-
		13	-	+	-	++
Pumpkin	Cotyledons	5	-	-	-	++
		7	-	++	-	-
		9	-	+++	-	-
		11	-	+++	-	-
		13	-	++	-	-
	Hypocotyl	5	-	-	+	-
		7	-	-	-	+
		9	++	+++	++	+
		11	++	+++	-	+
		13	+++	-	-	-
	Root	5	-	-	++	++
		7	+	++	+++	-
		9	+++	+++	+++	++
		11	+++	+++	+++	++
		13	-	+++	-	-
Pea	Cotyledons	16	+++	+	+++	-
		22	+++	-	+++	-
	Epicotyl	16	+++	-	+++	++
		22	+++	-	-	++
		28	-	-	-	++
	Root	22	+++	++	-	-

- = $P > 5\%$; + = $P < 5\%$; ++ = $P < 1\%$; +++ = $P < 0.1\%$

The results point not only to the different function of calcium and potassium in plant metabolism (reviewed by MENGEL 1961, pp. 229, 241) but also to different requirements for the two. According to KAHN and HANSON (1957) requirements of grasses for potassium are high and hence the seed reserve (0.54% of dry matter according to VRZÁKOVÁ 1968), relatively low when compared, *e.g.*, with pumpkin; is not quite sufficient, although its mobility is high. Findings concerning the importance of potassium in a number of enzymatic reactions, among others in transamination reactions (KURSAKOV *et al.* 1965, MENGEL 1961, p. 234 *etc.*) make understandable the unfavourable effects of potassium deficiency on the synthesis of nitrogenous substances. Retardation in the accumulation of proteins at a time when the accumulation of nitrogen is intensive (*cf.* Fig. 2) may be explained by formation of other, especially low-molecular-weight, substances which may be either due to an increase in the activity of destructive enzymes, or due to disturbances in the synthesis, starting already with the phosphorylation (MENGEL 1961, p. 235).

Although the calcium deficiency has unfavourable effects on proteinsynthesis, be this due to affected nitrate uptake (BURSTRÖM 1952) or their affected reduction (ECKERSON 1932) or due to energy metabolism disturbances in destroyed mitochondria (FLORELL 1956), it seems probable that with wheat the low requirement in a deficient cultivation solution is satisfied by the relatively high reserve in grains (0.08% of dry matter according to VRZÁKOVÁ 1968).

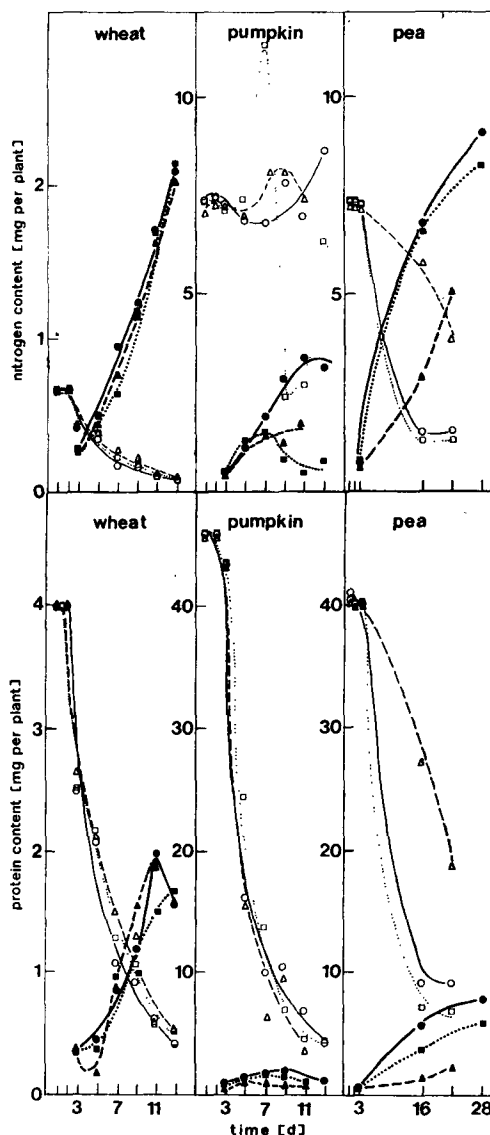
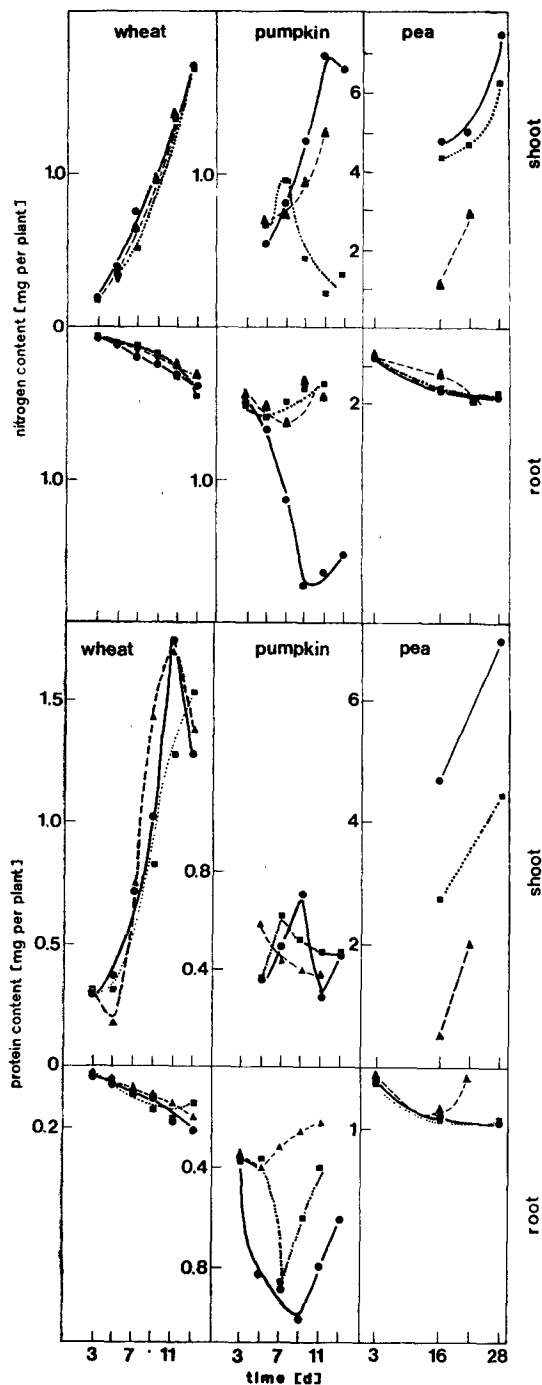


Fig. 1. Content of nitrogen and of protein in vegetative tissues (solid symbols) and storage tissues (open symbols) in plants growing in complete nutritive solution (—), in calcium deficiency (---) or potassium deficiency (.....).



Relative accessibility of calcium and potassium cations in the endosperm of the wheat grain (as compared with cotyledons of pumpkin or of pea) is probably the reason for which the deficiency of these ions in the cultivation solution has less serious metabolic, distributional or morphological consequences in wheat than in pumpkin and pea.

The content of nitrogen in the pumpkin seed is about ten times higher than in the wheat grain (Fig. 1). During 13-day-cultivation with complete nutrition the content after a mild initial decrease increased continuously in proportion to transformation of cotyledons in metabolically (photosynthetically) active organ. In comparison with newly formed vegetative organs (hypocotyl, roots) the content of nitrogen in cotyledons is twice as high. From this it is obvious that on complete nutrition plants accumulate nitrogen also from the external medium. In the absence of calcium the nitrogen uptake came soon to an end. There was a decrease in the nitrogen content of cotyledons and roots at about 11 days of plant age. The calcium reserve in pumpkin seed is relatively low (0.04% of dry matter according to VAŇOUSOVÁ 1968), half of that in wheat grain and the requirement for calcium is high, as may be judged from

Fig. 2. Distribution of nitrogen and of protein in plants growing in complete nutritive solution (—●—), in calcium deficiency (---▲---) or potassium deficiency (....■....).

serious metabolic and morphological consequences (PICHLE and FIALOVÁ 1972). From this it follows that the compensation of calcium deficiency from cotyledons, which are the metabolically active organ binding the little mobile calcium in their structure is minimal. Under potassium deficiency, the uptake of nitrogen by the whole plant was very intensive, however, only during first days of germination. Judging from the healthy habitus of plants it may be concluded that during that period the reserve in seeds (0.62% of dry solids according to VAŇOUSOVÁ 1968) was sufficient to cover the requirement. A sudden fall in the nitrogen content of the whole plant follows, suggesting that the potassium reserve is exhausted to the degree at which destructive enzymes are activated (MENGEL 1961, p. 235) releasing nitrogen as the ammonium ion, which may be secreted by roots (PRYANISHNIKOV 1952).

The content of proteins in the seed of pumpkin was approximately one order of magnitude higher than in vegetative tissues of the whole seedlings after 13 days of cultivation (Fig. 1). Dynamics of the protein decrease in cotyledons and of the total protein increase in newly formed vegetative tissues was about the same both with complete and deficient nutrition. The content of proteins in hypocotyl and in roots, however, was influenced by the deficiencies, especially by the calcium one, unfavourably (Fig. 2). Nitrogen content is high at the same time, suggesting a considerable accumulation of other nitrogenous substances, probably of low molecular weight, in cotyledons; they may result either from disturbances in synthetic processes or from decomposition of substances of high molecular weight, as mentioned before. Some of them, as putrescine (SMITH 1972) which may amount to about 10% of the total nitrogen content when the potassium deficiency is extreme, are liable to cause serious metabolic disturbances, so conspicuous in growth and habitus of pumpkin.

The content of nitrogen in pea seeds was almost the same as in the seeds of pumpkin, the protein content was only slightly lower (Fig. 1). On complete nutrition, the content of nitrogen in vegetative tissues increased about three times as rapidly as with pumpkin (*cf.* variants NS Fig. 1) and after initial rapid exhaustion of a considerable part of the content (of about 80%)

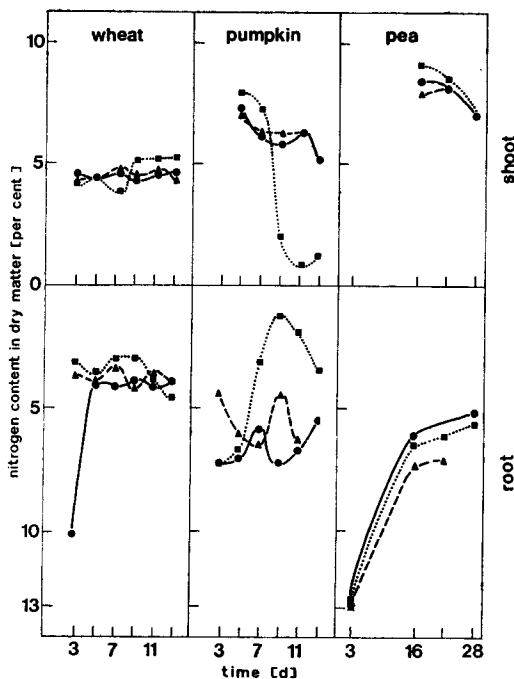


Fig. 3. Content of nitrogen in dry matter of plants growing in complete nutritive solution (—○—), in calcium deficiency (---△---) or potassium deficiency (.....■.....).

after some 16 days of cultivation nitrogen was still taken up intensively from the nutrient solution. In parallel with that an intensive proteosynthesis took place (Fig. 1). Potassium deficiency influenced the nitrogen metabolism unfavourably only in the rate of proteinsynthesis in vegetative tissues, which was reduced by about one-third as compared with complete nutrition Fig. 2). Irrespective of our ignorance of potassium content in pea seeds, it may be concluded from the data of KAHN and HANSON (1957) concerning the low requirement of *Viciaceae* for potassium that the potassium content of pea seedlings was not so unfavourable as to cause more important disturbances in synthetic processes. This is suggested both by intensity of growth and plant habitus, not differing from that of plants cultivated in the complete solution. Considerable retardation of shifts of nitrogenous substances between storage and vegetative tissues under calcium deficiency in view of high requirements of *Viciaceae* for calcium in nutrition (KAHN and HANSON 1957) suggests that the situation is like that in the pumpkin. It may be concluded that the calcium reserve in pea seeds, if there is one, cannot be mobilized for the sake of newly formed tissues. Early metabolic disturbances in these tissues were caused by the calcium deficiency, already when the reserve proteins were decomposed. Slow growth and serious morphological deformations brought about, similarly as with pumpkin, an early decay of the plants.

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Sledovali jsme obsah a distribuci dusíkatých látek při úplné (NS) nebo deficitní ($-Ca^{2+}$ nebo $-K^{+}$) výživě u mladých rostlin pšenice, tykve a hrachu se záměrem, zda určitý obsah zásobních dusíkatých látek může kompenzovat poruchy jejich syntézy ve vegetativních tkáních při deficitní výživě.

Klíčení rostlinky tykve a hrachu čerpaly dusík hlavně ze zásob semene. Čerpání i syntéza dusíkatých látek ve vegetativních tkáních byly deficiencí kationtů, zvl. vápníku zbrzděny. Průvodní morfologické deformace byly pravděpodobně přímou příčinou poruch mobilisace a tím i nevyužití zásob. Naproti tomu pšenice nevykazovala vlivem deficiencie morfologické změny a čerpala dusík intenzivně i z kultivačního roztoku. Avšak syntéza dusíkatých látek byla deficiencí průkazně zpomalena.

Při deficitu Ca^{2+} nebo K^{+} ve vnějším živném prostředí byla zřejmě rozhodující možnost využití těchto iontů ze zásobní tkáně úměrně potřebě rostliny. Míra disproporce těchto dvou faktorů byla pak prvotní příčinou časnosti morfologických změn, které znesnadnily přirozený pohyb látek v organismu, včetně látek dusíkatých.