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Specific effects of certain salts on nitrogen metabolism of young corn seedlings

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Abstract

The effect of sodium and magnesium chlorides and sulphates on nitrogen metabolism of corn seedlings and their constituent parts have been studied. Treatment with all salts led to a decrease in the nitrogen content of the seedling as a whole, and the decrease became more pronounced with the increase of salt concentration, though these concentrations were too low to induce any osmotic action. The same trend of changes was noticed as regards nonprotein-N, whereas the opposite was recorded in reference to the changes of protein-N. Higher concentrations of the salt solutions led to leaching out of more nonprotein-N than did lower concentrations. The study of the distribution of nitrogenous constituents among the different organs of the seedling showed that while the total-N content of the whole seedling decreased with the increase of salt concentration, the total-N content of the roots decreased markedly, and the total-N content of the tops decreased also but less whereas, the total-N content of the grains increased with the increase of salt concentration as compared with that in the control. As a result of disturbances of nitrogen metabolism under salinization, more ammonia and amides were accumulated in all seedling organs.

INTRODUCTION

The nitrogen metabolism of plants reflects the physiology of the whole plant as well as its interaction with the surroundings. Although the nitrogen metabolism of plants under normal conditions is known fairly well, the knowledge of nitrogen metabolism of plants exposed to the effect of salts is far from complete. There is no doubt that the nitrogen metabolism of plants grown under conditions of salinisation, differs from that of normal plants. First of all it must be stressed that in such plants, there are disturbances in the breakdown and synthesis of proteins (Kahane and Poljakoff-Mayber 1968, Huffaker and Peterson 1974).

Studying protein synthesis in leaf discs of *Nicotiana rustica*, Ben-Zioni et al. (1967) found that salt stress reduced the uptake of L-leucine and its incorporation into protein. Lupin (1963) found in protein synthesizing cell-free systems obtained from *Escherichia coli* that an addition of Na^+ in a 175 mM concentration considerably reduced the rate of incorporation of ^{14}C-phenylalanine into protein. In the light of these findings the object of the investigation here described was to test the effect of some single salt solutions on the nitrogen metabolism of young corn seedlings.

MATERIAL AND METHODS

The conditions of culture of double hybrid (186) corn (*Zea mays* L.) for this investigation have already been fully described by Hatata and Farah (1982). The different nitrogenous fractions of air-dry grains as well as 54- and 126-hour-old whole seedlings were determined. On the other hand, 174-hour-old seedlings were dissected into grains, roots and tops, and the nitrogenous constituents were determined for these organs separately. The procedures used were as follows.

The soluble nonprotein fraction of fresh samples was extracted with hot distilled water, after homogenization, the soluble protein was precipitated with 10% acetic acid as described by El-Shishiny (1966). The extract was then centrifuged, the clear solution was made to up the previous volume and used immediately to determine the different soluble nitrogenous fractions. The precipitate from centrifugation was dried at 100-110°C and finally powdered and used for determination of protein-N content.

Ammonia-N was estimated by distillation under reduced pressure using a borax-NaOH mixture at 40°C (Vickery et al. 1935b) using the apparatus described by El-Shishiny (1966). The distillate was received in 1/70 N HCl. The excess acid was titrated with 1/70 N CO_2-free NaOH using Tashiror's indicator.

Glutamine amide-N was estimated by the method of Vickery et al. (1935a) involving hydrolysis at pH 6.5 for 2 hours in boiling water bath. The increase of free ammonia after distillation being estimated as above.

The stable amide-N (asparagine amide-N) was determined from the amount of ammonia formed after hydrolysis with 2 N H_2SO_4 at 100°C for 3 hours. Ammonia-N, in excess of the free ammonia of the extract and ammonia from unstable amide-N, was considered as asparagine amine-N.

The α-amino nitrogen content of the water extract was determined by the method described by Yemm and Cocking (1955).

Total-N, protein-N and nonprotein-N were determined by a micro-Kjeldahl technique as described by Jackson (1958).

RESULTS

The results in Table 1 give an idea of the changes in various nitrogenous constituents at different stages of growth of the corn seedlings grown in distilled water. The dry grains contained 1120 mg nitrogen per 100 grains, of this amount 12 per cent was in the form of nonprotein-N and the rest was protein-N. After germination in water for 54 hours there was a decrease in protein-N amounting to 231 mg, and a simultaneous increase in nonprotein-N of 200 mg per batch. The total-N content of the grains, however, decreased during this period of 54 hours by 30.5 mg only. Also protein-N as percent of total-N decreased by 19 per cent and the nonprotein-N increased by the same value. These features indicate that as a result of germination in water for 54 hours, 19 per cent of the insoluble-N was transformed to the soluble form,

Table 1

Changes in the nitrogenous fractions of corn seeds during germination and growth in distilled water. Values are means of four replicates of 48 seedlings and expressed as mg nitrogen per 100 seedlings. The percental error did not exceed 5%

Form of nitrogen	Time after soaking, h			
	0	54	126	174
Total	1119.78	1089.28	968.84	959.11
Protein	989.97	758 83	546.33	604.12
Nonprotein	129.81	330.45	422.51	354.99
Ammonia	5.32	14.78	20.01	20.31
Amino	76.79	223.24	251.97	233.01
Glutamineamide	1.06	9.13	15.53	12.68
Asparagine amide	23.40	32.89	32.00	23.82
Residual	23.24	50.41	103.00	65.17

with a simultaneous loss — probably by leaching out — of a very small fraction of the nitrogenous content, not exceeding 2.7 per cent of total-N present in dry grains.

After the next period of growth in water (72 hours) the amount of protein-N in the seedlings decreased, the total-N decreased also by

120 mg/batch, this being 11 per cent of the initial value. On the other hand, the nonprotein-N increased during the same period by 92 mg/batch. In other words, the second period of germination in water was marked by active hydrolysis of protein-N, accompanied by a distinct loss of nitrogen from the seedlings, probably due to leaching out.

After another two days the total-N content of the seedlings decreased only slightly, the drop was 10 mg/batch as compared with 120 mg during the previous 3 days, and this corresponds to a further drop of only one per cent of the initial value. This slight drop was accompanied by an increase in protein-N and decrease in nonprotein-N.

The changes in the various fractions of nonprotein-N in seedlings grown up to 174 hours in water are given also in Table 1. The amino-N was the major fraction of nonprotein-N at all stages, and it increased considerably 54 hours after soaking. Relative to the total nonprotein-N, the amino-N content amounted to 59.1, 67.6, 59.6, and 65.6 per cent at 0, 54, 126 and 174 hours, respectively. Ammonia-N, glutamine and asparagine (as amides) were found at all stages in relatively small amounts; they account for jointly 22.9 per cent of the total nonprotein-N in the dry grains and decreased to 17.2 and 16.0 per cent at 54 and 126 hours, remaining constant till 174 hours after soaking. Other nonprotein-N fractions referred to here as residual-N, were found in noticeable amounts at all stages and increased with lapse of time after soaking till 126 hours decreasing thereafter markedly.

In the organs of 174-hour-old corn seedlings raised in water (Fig. 2) 44.1 per cent of the total-N of whole seedlings was found in the tops, 30.0 per cent in the roots and 25.9 per cent in the grains.

Referring now to the effects of salts on the nitrogenous constituents. In the seedling as a whole it would appear from Fig. 1, that after 72 hours in salt solution (126 hour seedlings) the total-N decreased with increasing salt concentrations from 0 to 20 meq/l. The same trend, but with differences much wider was noticed for nonprotein-N, but the protein-N, on the other hand, shows an opposite picture, increasing with rising of salt concentration. Sodium chloride had a greater effect, while magnesium sulphate had the least one and the higher concentration was more detrimental than the lower one. Total-N of seedlings rose in higher concentrations of salt solution to 86.9, 90.8, 92.5 and 94.8 per cent of the control in water in 20 meq/l of NaCl, Na_2SO_4, $MgCl_2$ and $MgSO_4$, respectively. Nonprotein-N was 47.6, 54.5, 67.7 and 75.1 per cent, while protein-N was 117.2, 118.8, 111.7 and 110.5 per cent in the same concentration of the salts.

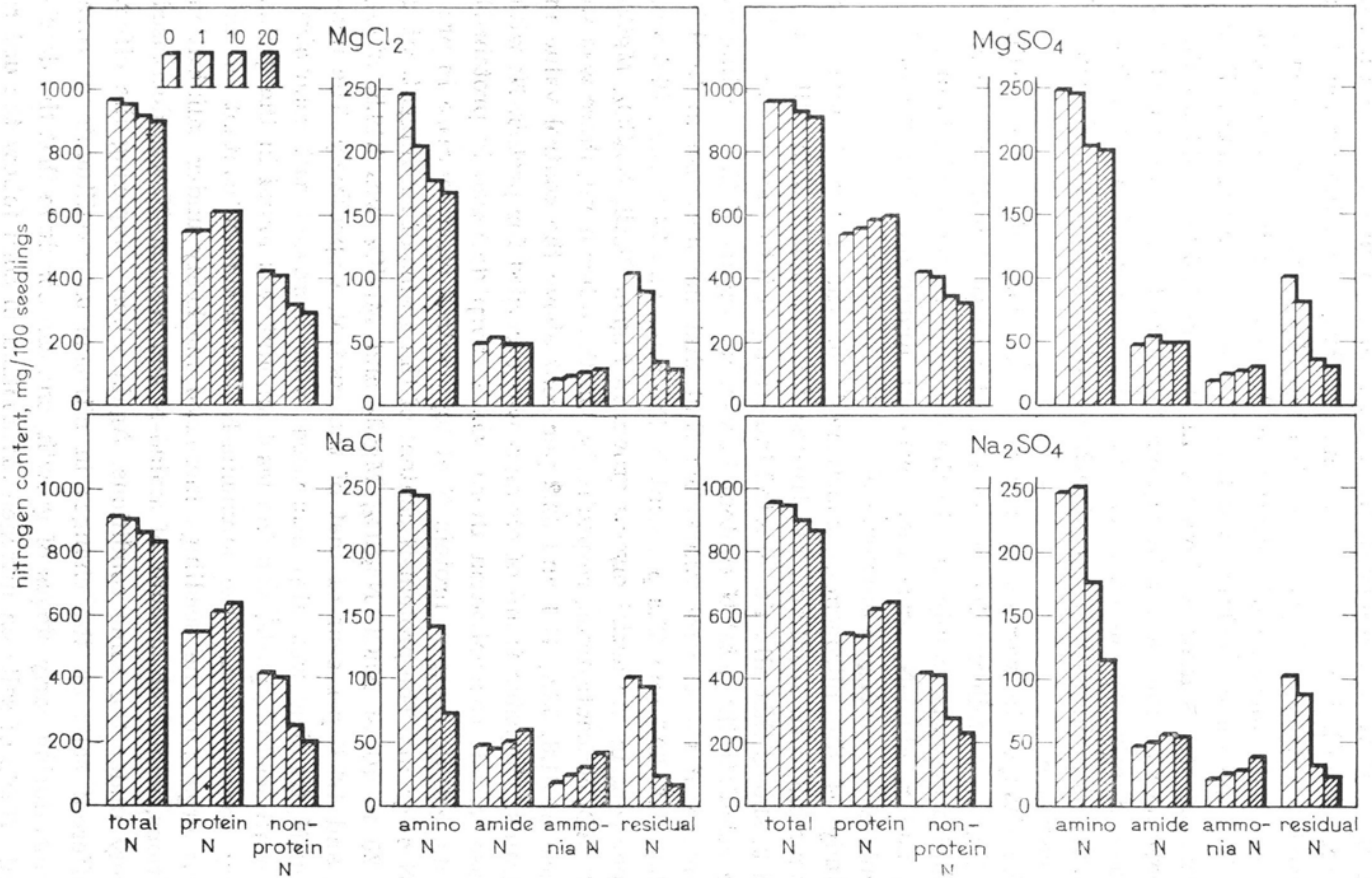

Fig. 1. Effects of different concentrations of salts (meq/l) on the nitrogenous constituents of corn seedlings. Salt treatment 72 hours. Values are means of 4 replicates of 48 seedlings. The error did not exceed 5%

After 120 hours in salt solution (174-hour-old seedlings) the same trend of changes was observed: the total-N and nonprotein-N continued to decrease with increase of salt concentration, while the protein-N of salinized plants was higher than that in the control (in water) and increased with increase of salinization. However, total-N and nonprotein-N of the same seedlings, whether in water or in various salt concentrations, was somewhat lower than that in 126-hour-old seedlings, the reverse was the case with protein-N. Since total-N decreased with increase of salt concentration one is obliged to conclude that higher salt concentrations led to more leaching out of nonprotein-N than did the lower ones.

The changes in various nonprotein-N fractions of whole seedlings (Fig. 1) were as follows: the decrease in total nonprotein-N with increase of salinization was mainly due to the decrease in the contents of amino-N and residual-N, since there was an appreciable increase in the contents of amide-N and ammonia-N.

Study of the nitrogenous constituents of the different organs of 174-hour-old corn seedlings, i.e. treated for 120 hours with salt (Figs. 2 and 3) shows that all salts exerted the strangest influence on the roots. If we calculate the total-N content in various organs of the seedling as percentage of the control values we find that the decrease in total-N was 50, 49,3, 29.7 and 33.8 per cent in the roots and 34.9, 33.6, 26.6 and 22.8 per cent in the aerial organs grown in higher NaCl, Na_2SO_4, $MgCl_2$ and $MgSO_4$ concentrations, respectively. On the other hand, there was an increase of 61.8, 58.4, 41.1 and 32.2 per cent above the control value in the grains. The same trend of changes was recorded for protein-N: with increase in salt concentration there was a sharp decrease in protein-N of roots, less decrease in protein-N of tops with a clear increase in protein-N of grains. The protein-N content of the grains of seedlings treated with 20 meq/l of NaCl, Na_2SO_4, $MgCl_2$ and $MgSO_4$ was 249.5, 251.4, 206.1 and 195.6 per cent of that of the control, respectively. The nonprotein-N fraction was also decreased with increased salt concentration in all seedling parts, and the least amount was found in the grains at any concentration. Free ammonia-N and amide-N content of the various organs of the seedlings increased markedly under salinization conditions, while the amount of amino-N and residual-N was decreased severely under the same conditions. Again when comparing the effect of different salts on the changes in nitrogenous constituents of corn seedlings during early stages of growth, one can conclude that the detrimental effects of salts on nitrogen metabolism in corn follow the order: $NaCl > Na_2SO_4 > MgCl_2 > MgSO_4$.

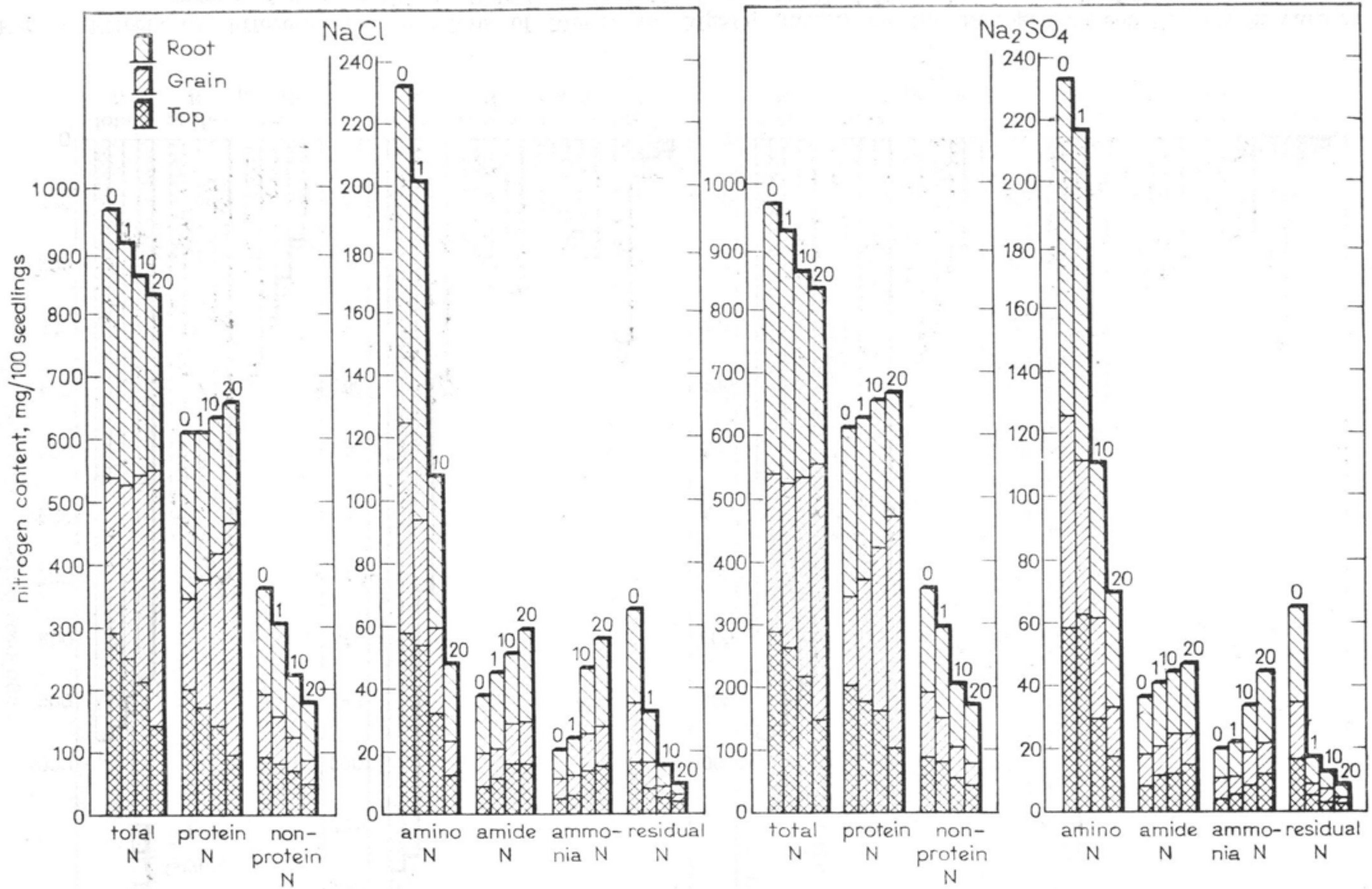

Fig. 2. Effects of different concentrations of NaCl and Na_2SO_4 (meq/l) on the nitrogenous constituents in various organs of corn seedlings. Salt treatment 120 hours. Vertical lines denote standard error

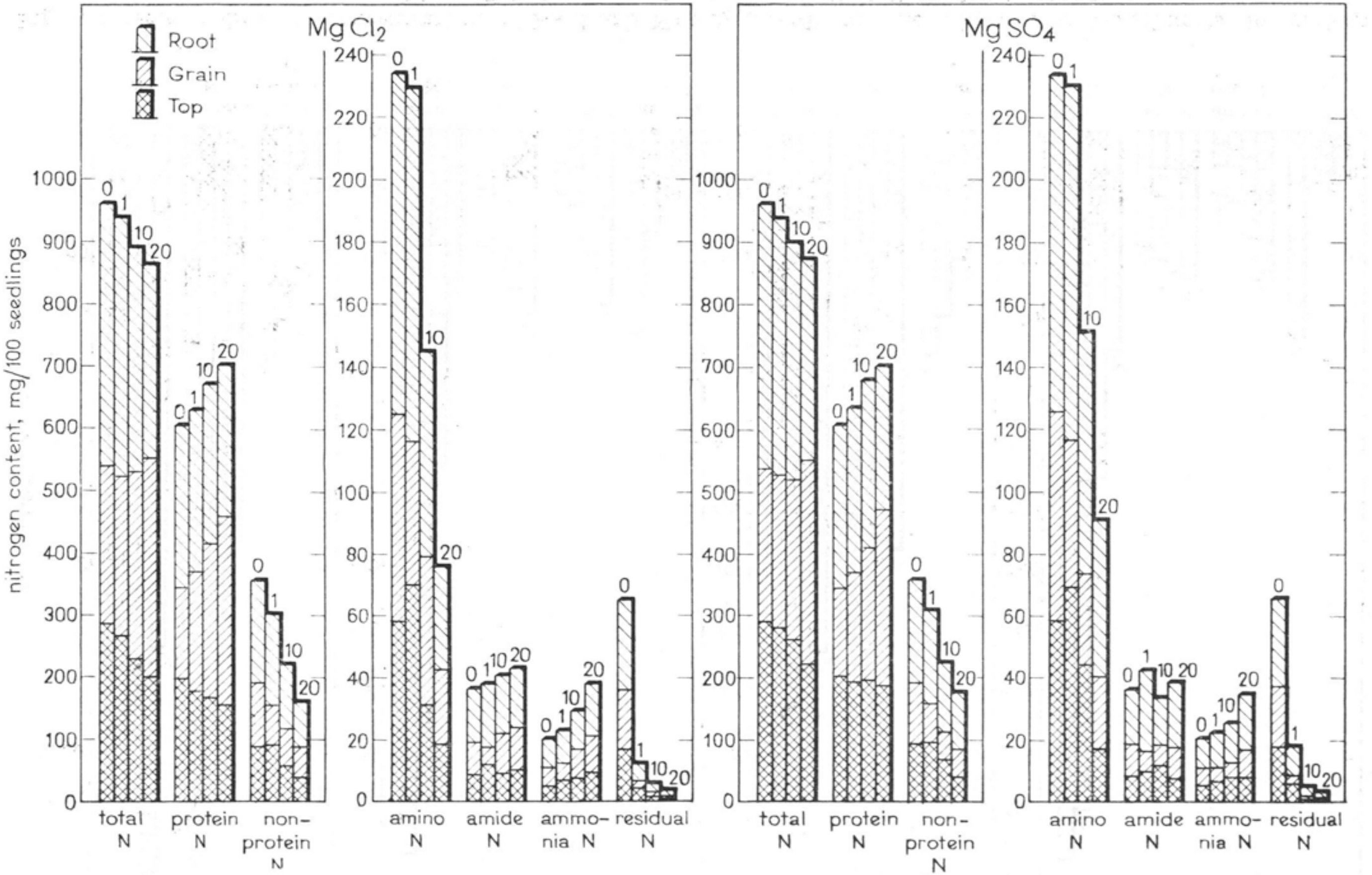

Fig. 3. Effects of different concentrations of $MgCl_2$ and $MgSO_4$ (meq/l) on the nitrogenous constituents in various organs of corn seedlings. Salt treatment 120 hours. Vertical lines denote standard error

DISCUSSION

The experimental results, presented here, provide evidences that the investigated salts affect nitrogen metabolism of young corn plants. It could be concluded that with advance of time, whether in water or in salt solutions, more of the protein-N is converted into nonprotein-N, and that higher concentrations of salts lead to more leaching out of nonprotein-N than did the lower concentrations. However, leaching was more intensive during the first 120-hour period of germination than during the later period, a conclusion which agrees with that of Brown (1943), Folkes (1959), Sircar and Datta (1962).

In conformity with our present results, Saakyan and Petrosyan (1964) detected a sharp drop in the amount of total-N and protein-N in grape-fruits under soda salinization, and noted small changes in the amount of nonprotein-N. They attributed growth inhibition to disturbances in protein and nucleic acid metabolism. Lupin (1963), Ben-Zioni et al. (1967), Kahane and Poljakoff-Mayber (1968), Hall and Flowers (1973) also found that incorporation of labelled amino acids into protein was impaired by salt stress.

The relative increase in protein-N of grains should be considered as a sign of reduction in the rate of protein hydrolysis as the result of salt treatment. This leads naturally to a reduction in the amino acids transported to the roots and tops, which would be resynthesized there into proteins. In this connection, Strogonov (1964) wrote that amino acids may accumulate in plants under saline conditions owing to disturbances in breakdown and synthesis of proteins. Free amino acids may be metabolized and consequently some amino acids may disappear while others accumulate in excessive amounts.

On the other hand, Kessler and Snir (1969) found in a salt-resistant citrus variety that the salt stress improved N metabolism. Also Langdale et al. (1973) reported that *Cynodon plectostachyus* showed reduced growth rates, but enhanced protein contents, when exposed to NaCl salinity. Similar observations have been reported by Prisco and O'Leary (1973). Wiemberg (1975) found that soluble protein concentration was not effected in the leaves of highly salinized (100 mM NaCl) pea seedlings. As the experimental conditions of these authors differ in several respects from ours, different findings and conclusions do not represent an actual contradiction. In this respect the degree of salt stress seems to be of particular importance. Thus Hsiao (1973) suggests that only a strong water or salt stress will affect protein synthesis. Under such conditions a breakdown of polysomes has been observed by Nir et al. (1970). Another explanation was offered by Helal and Mengel (1979) who showed that the metabolic response to salinity is probably dependent on some other factors like growth rate, age of the plant, availability of some nutrients (especially K, Ca and N) and the level of salinity applied.

The results presented here show also that there was accumulation of free ammonia and amides in all seedling organs grown under saline conditions and their amounts increased with increase of salt concentration. This may be due to disturbances in nitrogen metabolism under saline conditions. In this connection Strogonov (1964) showed that disturbances in the nitrogen metabolism of cotton, under saline conditions, were accompanied by accumulation of ammonia. This accumulation may be due either to extensive breakdown of proteins or it may be a result of metabolism of such substances as amines and diamines. It is natural that accumulation of toxic substances, such as ammonia, will have an adverse effect on physiological processes in the plant. Lapina (1966) also noticed an accumulation of ammonia, amino acids and amides in corn seedlings under the effect of NaCl salinization. Coleman and Richards (1956) showed that the necrotic spots found in leaves as a result of salinity and potassium deficiency were due to the toxic effect of putrescine which is a diamino acid which results from disturbances in amino acid metabolism. The deficiency of potassium, however, may be the outcome of other treatments such as unbalance due to excess Na or Mg.

In conclusion, it is clear from the work here presented that the effects of salt were mainly on the mobilization of metabolites and accumulation of some toxic substances such as ammonia and amides as a result of disturbances in nitrogen metabolism under such conditions. Sodium proved to be more suppressive to the mobilization of these metabolites than magnesium, and chloride was more so than sulphate; the role of these salts being due to both cations and anions.

REFERENCES

Ben-Zioni A., Itai C., Vaadia Y., 1967. Plant Physiol. 42: 361-365.

Brown R., 1943. Ann. Bot. 7: 275-296.

Coleman R. G., Richards F. Y., 1956. Ann. Bot. 20: 393-409.

El-Shishiny E. D. H., 1966. Bull. Fac. Sci. Alexandria Univ., 7: 127-136.

Folkes B. F., 1959. Utilization of nitrogen and its compounds by plants. XIII. Symposis of the Society for Experimental Biology. Academic Press Inc., New York. pp. 126-147.

Hall T. L., Flowers T. J., 1973. Planta (Berl.) 110: 361-368.

Hatata M., Farah M., 1982. Acta Soc. Bot. Pol. 51: 241-250.

Helal H. M., Mengel K., 1979. Plant and Soil 51: 457-462.

Hsiao T. C., 1973. Ann. Rev. Plant Physiol. 24: 519-570.

Huffaker R. C., Peterson L., 1974. Ann. Rev. Plant Physiol. 25: 363-392.

Jackson M. L., 1958. Nitrogen determinations for soils and plant tissues. In: Soil Chemical Analysis. M. L., Jackson (ed.). Prentice-Hall, New Jersey, pp. 183-204.

Kahane I., Poljakoff-Mayber A., 1968. Plant Physiol. 43: 1115-1119.

Kessler B., Snir I., 1969. Proc. Ist Int. Citrus Symp. 1: 381-386.

Langdale G. W., Thomas J. R., Littleton T. G., 1973. Agron. J. 65: 468-470.

Lapina L. P., 1966. Soviet Plant Physiol. 13: 1029-1040.

Lupin M., 1963. Cell potassium and the regulation of protein synthesis. In: The cellular functions of membrane transport. J. E. Hoffman (ed.), Prentice-Hall, New Jersey. pp. 193-211.

Nir I., Poljakoff-Mayber A., Klein S., 1970. Israel J. Bot. 19: 451-462.

Prisco J. T., O Leary J. W., 1973. Plant and Soil 37: 263-276.

Saakyan R. C., Petrosyan G. P., 1964. Fiziol. Rast. 11: 681-688 (C. F. Biol. Abst., 1965, p. 12).

Sircar S. M., Datta S. C., 1962. J. Exp. Bot. 13: 61-74.

Strogonov B. P., 1964. Physiological basis of salt tolerance in plants. (English translation from Russian). A. Poljakoff-Mayber and A. M. Mayer (eds.). (Israel Program for Scientific Translations), Jerusalem.

Vickery H. B., Pucher G. W., Clark H. E., Chibnall A. G., Westall L. G. 1935a. Biochem. J. 29: 2710-2720.

Vickery H. B., Pucher G. W., Leavenworth C. S. 1935b. Indus. Engin. Chem. Anal. Ed. 7: 152-156.

Weimberg R., 1975. Plant Physiol. 56: 8-12.

Yemm E. W., Cocking E. C., 1955. Analyst 80: 209-213.

Specyficzny wpływ niektórych soli na metabolizm azotowy młodych siewek kukurydzy

Streszczenie

Badano wpływ chlorków i siarczanów sodu i magnezu na metabolizm azotowy siewek kukurydzy i ich części. Wszystkie sole powodowały spadek zawartości azotu całkowitego siewek. Ten efekt był coraz wyraźniejszy w miarę wzrostu stężenia soli, pomimo że użyte stężenia były za małe aby powodować efekt osmotyczny. Ten sam kierunek zmian obserwowano w odniesieniu do azotu niebiałkowego, natomiast zmiany w zawartości azotu białkowego przebiegały w odwrotnym kierunku. Stosowanie wyższych stężeń soli powodowało większe „wymywanie" azotu niebiałkowego w porównaniu z niższymi stężeniami. Badania rozdziału składników azotowych do różnych organów siewek wykazały, że zawartość azotu całkowitego: w całych siewkach zmniejszała się wraz z wzrostem stężenia soli, w korzeniach wyraźnie spadała, w pędach spadała ale mniej wyraźnie, podczas gdy w ziarnach wzrastała wraz ze stężeniem soli. W wyniku zaburzeń metabolizmu azotowego w warunkach zasolenia, we wszystkich organach siewek gromadziło się więcej amoniaku i amidów.