THE EFFECT OF DIFFERENT N/P RATIOS IN THE NUTRITIVE MEDIA ON UPTAKE AND METABOLISM OF N AND P BY SWEET POTATO TUBER DISKS

By

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Summary

The effect of different N/P ratios in the culture media on uptake and metabolism of N and P by sweet potats tuber disks was investigated.

1. NH_4Cl treatments leading to increased NH_4-N level in the tissues stimulated the rate of P uptake than did equivalent increases in P concentration in the extermal medium. P uptake rates were highly correlated with the total-N level in the tissues indicating the close connection between uptake and metabolism of P and those of N. Absorption was dependent on respiration.

2. The connection between N-metabolism and P-metabolism manifests itself by the increase of N-intermediates, glutamine in particular, whose synthesis have processes in common.

3. Low concentrations of N or P in the nutritive media led to low accumulation of nucleic acids and proteins inside the tissues. The low concentration of N or P strongly reduced the RNA content while the rise of P or N dose at low level of N or P respectively caused a sharp decrease in that of DNA.

Introduction

In literature, there is a good deal of work devoted for the study of the dependence of nitrogen metabolism on N/P ratios (e.g. Thompson et al., 1960). All these authors confirm the fact that insufficient supply of N or P to plants leads to a reduction in the total nitrogen and protein-N. Excess of P at N deficiency leads to the same effect on N-metabolism. On the contrary, excess of N results in an increase of total-N and protein-N in different plant organs.

Cole et al. (1963) stated that the effects of N on P uptake by corn plants suggest a connection between P uptake and N metabolism, and that

the increases in P uptake rates may reflect higher levels of N-intermediates whose synthesis have processes common with those of P uptake. Nosseir and Spirldinov (1965) found that the progressive increase of N over P concentration in the nutritive media of phaseolus plants stimulated the uptake of P^{32}, thus emphasizing Cole's statement.

However, the problem of the influence of different ratios between N and P in the nutritive media on P metabolism is not yet thoroughly studied. Literature in this field mainly concerns with the study of the effect of N/P ratios in the nutritive media on the amount of nucleoproteins in plant tissues. The dificiency of N causes a depression in the quantity of nucleoprotcins, whereas excess of either one of them stimulates their formation (Zuev et al. 1962).

It should be recognized that researches concerning the effect of N and P in the nutritive media on nucleic acid metabolism are very few. Studies in this field began to appear only in the last few years and were mainly done on plant seedlings (Turkova, 1960 and 1963).

Accordingly, the aim of the present study is to investigate the effect of different N/P ratios in the nutritive media on uptake and metabolism of N and P hy sweet potato tuber disks.

Material, Methods and Experiments

The disks used in this study were taken from sweet-potato, variety «Balady» tubers. Such plant material was chosen for this work since it was already proved by El-Shishiny (1955) to contain both amides (glutamine and asparagine) and are able to synthesize them when placed under conditions leading to an increase in the NH_4-N level in the tissues. Also, sweet potato tubers contain sugars. These facts might help in the study of metabolic N and P reactions. Moreover, sweet-potato tubber disks taken from the core of the tuber contain no colouring material which might interfere, if present, in the colour reactions during analysis.

The technique of the disk culture experiments was the same as in the earlier work described by El-Shishiny (1955) and El-Shishiny and Nosseir (1957). A stock of disks was prepared from the plant material from which samples of about 20 gm. of disks each, taken at random were used for each treatment. Duplicate samples after being washed for 48 hours in aerated distilled water (El-Shishiny, 1955) were transferred into 400 ml. water or culture solution kept at 25°C in a constant temperature water-bath according to the following schema :

Distilled water

N_1P_0 = 0.005M NH_4Cl

N_0P_1 = 0.005M KH_2PO_4

N_1P_1 = 0.005M NH_4Cl + 0.005M KH_2PO_4

$N_1P_{1/2}$ = 0.005M NH_4Cl + 0.0025M KH_2PO_4

$N_{1/2}P_2$ = 0.0025M NH_4Cl + 0.01M KH_2PO_4

$N_2P_{1/2}$ = 0.01M NH_4Cl + 0.0025M KH_2PO_4

$N_{1/2}P_1$ = 0.0025M NH_4Cl + 0.005M KH_2PO_4

A current of air was passed through each vessel to serve for aeration and determination of CO_2 output. After a culturing period of 24 hours, one sample of each treatment was drained, washed several times with distilled water, extracted and analysed for the final distribution of the various nitrogen fractions. The second sample of the same treatment was air-dried until a constant weight and used for determination of nucleic acids. The analytical methods used for determination of nitrogen fractions were those described by El-Shishiny (1955) and El-Shishiny and Nosseir (1957) except that of amino-acids which was carried out using ninhydrin method (Nosseir, 1967). The extraction, separation, and determination of nucleic acids were carried out using the methods of Schmidt and Thanhauser (1945) as cited by Glick (1966). These methods briefly consist of treating finely powdered dry tissues first with 7% trichloroacetic acid on cold followed by centrifugation, then treating the residue with a mixture of alcohol and ether to extract lipids. The RNA in this residue was hydrolysed by N KOH at 37°C for 18 hours while the DNA was precipitated from the solution by adding perchloric acid. The P content of nucleic acids was digested by H_2SO_4 and H_2O_2 and then determined colorimetrically using the molybdinum reagent as described by the author (1967).

Two experiments were done using the same technique. The results were fully consistent with those summarized in tables (1), (2) and (3). The KH_2PO_4 solution was adjusted to pH 7 by KOH before supplied to the tissues.

Results and Discussion

Uptake of N and P : Table (1) shows that disks of sweet potato tubers cultured in 0.005M NH_4Cl absorbed 22.1 mgm. NH_4-N/100 gm. tissue in 24 sence of NH_4Cl. the NH_4-N uptake was increased progressively by the increase of P concentration in the nutritive media. These increases were 40% in presence of $P_{\frac{1}{2}}$ and more than 100% in presence of P_1. In the

same time P uptake rates increased progressively by the increase of concentration of that ion in the nutritive media containing NH_4-N the supply of which was the same for all treatments (more than 100% increase of P uptake from P_1 solution over that from $P_{½}$ solution in presence of N_1 concentration). Similarly, Williams (1948) working with oat plants found that with N, the supply of which was the same for all treatments, large stimulatory effects of P treatment on the rate of intake of P and N were obtained.

TABLE I

P uptake, NH_4-N uptake and CO_2 output by sweet potato tuber disks cultured for 24 hours in solutions containing different N/P ratios (Mgm./100 gm. fresh wt.)

Treatment	P—uptake	NH_4-N uptake	CO_2 output
Distilled water	—	—	259.2
N_1P_0	—	22.1	300.0
N_0P_1	40.7	—	400.8
N_1P_1	107.5	44.6	568.8
$N_1P_{½}$	52.5	30.9	432.0
$N_{½}P_1$	83.0	36.7	432.4
$N_2P_{½}$	150.5	60.9	732.0
$N_{½}P_2$	49.0	10.5	372.8

On the other hand, table (1) shows that sweet potato tuber disks cultured in 0.005M KH_2PO_4 absorbed 40.7 mgm. P/100 gm. tissue during 24 hours. The presence of NH_4-N together P in the culture media caused progressive increases in P uptake rates by the increase of NH_4-N in these media. These increases were more than 100% in case of $N_{½}$ solution and more than 164% in case of N_1 solution. Similarly stimulation of P uptake by N additions has been reported for a many of plant species (Reichman et al., 1959 ; Werkhoven et al., 1960 and many others). Simultaneously, the NH_4-N uptake rates increased progressively by the increase of concentration of that ion in the culture media containing P, the concentration of which was the same for all treatments (22% increase of NH_4-N uptake from N_1 solution over that from $N_{½}$ solution in presence of P_1 concentration) .

The increases of NH_4-N uptake rates accompanying those of P might show the possibility of any special companion effect of ammonium and phosphate ions on entry of each other into the root cells of sweet potato tubers as suggested by Arnon (1939) for barley roots, but argue against the finding of Cole et al. (1963) who showed that the presence of ammonium ions in the test solution during the uptake period had negligible effects on P uptake rates by corn plants.

Again, table (1) shows that when the culture media around sweet potato tuber disks contain a lower dose of P ($P_{1/2}$) together with higher doses of NH_4-N (N_1 or N_2), the NH_4-N uptake rate was 100% increase from N_2 solution while the P uptake rate increased by about 200% from the culture medium containing N_2 over that containing N_1 although the concentration of P in these cultures does not change ($P_{1/2}$). On the other hand, when the culture media around sweet potato tuber disks contain a lower dose of NH_4-N ($N_{1/2}$) together with higher doses of P (P_1 or P_2), both NH_4-N and P uptake rates from P_2 solution were 71% and 41% respectively below those from P_1 solution. In this respect, sweet potato tuber disks behave similar to some gramineous plants (Williams, 1948) in which the % P content of the stems, leaves and roots arrived values with a higher supply of P lower than with a low supply of P in presence of a constant supply of N. These results clearly show that when the concentration of P was 4 times that of NH_4-N in the culture media of sweet potato tuber disks, disturbances in the uptake rates of both N and P do occur in contrast to what happens when the concentration of N was 4 times that of P where the uptake rates of both N and P were increased although the P concentration in the culture medium was constant.

The major determinants of the rate of uptake of P by plants were described by Williams (1948) and Cole et al. (1963) as the demand set up by the growth and normal functioning of the various plant parts, and the concentration of P in the medium. They considered that the indirect effects of nutrient treatments on growth and hence on demand were more important than the direct effects of external concentration of P on the rates of intake of that element. These conclusions were supported by the present investigation in which NH_4-N stimulated the rate of P uptake by sweet potato tuber disks than did an equivalent increase in P concentration in the culture medium. From table (1), it is clear that sweet potato tuber disks cultured in $N_1P_{1/2}$ solution absorbed 52.5 mgm. P per 100 gm. fresh weight of tissue during 24 hours. By doubling the concentration of P in the culture medium (N_1P_1), P uptake was increased by more than 100% while doubling the concentration of NH_4-N in the culture solution ($N_2P_{1/2}$) stimulated further the P uptake where the increase of uptake of that element about 200%. Evenmore, tissues cultured in $N_{1/2}$ P_1 solution absorbed 83.0 mgm. P and by doubling the concentration of P in the culture solution ($N_{1/2}$ P_2), P uptake did not increase but decreased by about 41% in contrast to 30% increase in the absorption of that element when the concentration of NH_4-N was doubled around the tissues (N_1P_1).

Also from table (1), it is evident that the P uptake rates by sweet potato tuber tissues from the different culture media go parallel to those of NH_4-N indicating the close connection between the uptake of both N

and P. The absorption rates were also found dependent on respiration rates of the tissues, Humphries (1952).

Distribution of the nitrogenous Fractions

As has been already shown by El-Shishiny (1955), sweet potato tuber disks have assimilated NH_4-N and synthesized complex-N compounds (rest- and protein-N) in a large part, and glutamine and asparagine forming a small part. The amino-acid-N fraction decreased probably due to its participation in the formation of amides and proteins. Addition of P to the nutritive media containing the NH_4-N, caused marked increases in amides, particularly glutamine, and proteins but caused decreases in amino-acids and rest-N. With the low dose of P ($P_{1/2}$) in presence of N_1 solution, the increase in amides, mainly glutamine, were very pronounced while the high dose of P (P_1), the very pronounced increase was in protein-N.

Also from table (2), it is evident that when the P level of sweet potato tuber disks was increased, in absence of NH_4-N, both amides increased presumably at the expense of amino-acids while the increase in protein-N was at the expense of rest-N. Addition of different concentrations of N to the nutritive media containing P caused analogous changes in the N fractions as those taking place with similar concentrations of P added to the nutritive media containing NH_4-N. This means that with the low dose of NH_4-N ($N_{1/2}$) in presence of P_1 solution, the increases in amides mainly glutamine, were very pronounced while with the high dose of NH_4-N (N_1), the very pronounced increase was in proteins.

The rise of NH_4-N dose at low level of P caused marked increases in all nitrogen fractions especially amides and this was accompanied by the highest respiration rate (compare $N_1P_{1/2}$ with $N_2P_{1/2}$). In contrast, the rise of P dose at low level of NH_4-N caused marked decreases in all the nitrogen fractions and this was accompained by the lowest respiration rate (compare $N_{1/2}$ P_1 with $N_{1/2}P_2$).

The absorption rates of NH_4-N and P were accompanied by parallel increases in CO_2 production indicating the dependence of amide and protein syntheses on NH_4-N and energy production related to P uptake.

The effects of NH_4-N on P uptake as shown from tables (1) and (2) clearly elucidate the connection between P uptake and N metabolism. The increases in P uptake have reflected higher levels of N-intermediates whose synthesis have processes in common with those of P uptake.

Viets et al. (1946) observed large increases in the amounts of glutamine, asparagine, and amino-N in the roots of previously N-depleted corn plants absorbing N from nutrient solution containing NH_4-N. Chibnall (1939)

TABLE 2

Distribution of various nitrogen fractions in sweet potato tuber disks cultured for 24 hours in solutions containing different N/P ratios (Mgm-N/100 gm. fresh wt.)

Treatment	Nitrogen Fractions						
	Ammonia-N	Glutamine-N	Asparagine-N	Aminoacid-N	Rest-N	Protein-N	Total-N
H_2O	0.00	2.8	2.3	21.7	15.3	50.4	90.5
N_1P_0	1.00	5.1	4.5	15.8	27.7	58.2	112.3
N_0P_1	0.00	6.4	5.1	15.3	3.0	60.9	90.7
N_1P_1	0.00	15.0	10.0	5.5	5.0	99.0	134.5
$N_1P_{1/2}$	0.00	37.0	12.5	9.0	5.0	61.0	124.5
$N_{1/2}P_1$	0.00	34.0	11.0	5.5	5.0	77.5	133.0
$N_2P_{1/2}$	4.50	46.0	20.0	10.5	8.0	65.5	154.5
$N_{1/2}P_2$	0.00	25.0	8.5	5.0	3.5	63.0	105.0

infiltrated Lolium leaves with solutions of ammonium phosphate and found that it gave glutamine synthesis. Webster and Varner (1955) considered that the synthesis of amides in higher plants could proceed by coupling the corresponding amino-acid with A.T.P and NH_3 in presence of Mg^{++} and that the energy necessary for amide synthesis was derived from A.T.P. From the present investigation, table (2) shows considerable increases of N-intermediates following NH_4-N and P uptake by sweet potato tuber disks. The increases were mainly in the amides, glutamine in particular and the increases were accompained by high levels of total -N in the tissues together with high P uptake rates. Yemm and Willis (1956) demonstrated that a rapid and extensive synthesis of glutamine occurs when ammonium salts are supplied to barley roots and that this synthesis is coupled with high rate of respiration and considerable loss of sugars from the tissues. They point out the close relationship of the synthesis of glutamic acid and its amide to glycolysis and indicate that the reductive amination of α-keto-glutaric acid proceeds by means of electron transfer mediated by pyridine nucleotides, while the synthesis of the amide bond is promoted by phosphorylations involving A.T.P. Also the enzymic synthesis of glutamine is chiefly localised in the mitochondria of plant cells (Webster, 1953), and the mitochondria are the sites of P uptake (Jackson et al., 1962). The rate-limiting reactions in P uptake are those steps in oxidative phosphorylation that involve phosphate. These results have led Cole et al. (1963) to suggest that the stimulatory effects of N on P uptake by corn plants may involve synthesis of N-intermediates with corresponding increase in turnover of DPNH and ATP coupled to P-uptake reactions. Studies in hand of the level of N-intermediates following NH_4-N and P absorption and assimulation by sweet potato tuber tissues were found in harmony with the suggestion of Cole et al. (1963) since higher levels of amides, glutamine in particular, and amino-acids whose synthesis have processes in common with P uptake and metabolism were obtained. Also the results in hand show the possible operation of oxidative phosphorylation reactions in sweet potato tuber tissues similar to those operating in other plant tissues such as barley roots (Yemm and Willis, 1956), since synthesis of glutamine was also coupled with high rate of respiration.

Distribution of Nucleic Acids.

The results depicted in table (3) showed high nucleic acid contents of sweet potato tuber disks cultured in various culture media having different N/P ratios. Sugars present in such disks together with P absorbed from the nutritive media serve in the formation of phosphorylated sugars which are precursors of RNA synthesis.

TABLE 3

Distribution of nucleic acids in sweet potato tuber disks cultured for 24 hours in solutions containing different N/P ratios. Mgm P of nucleic acid / 100 gm dry wt. of tissue.

Treatment	Total	RNA	DNA	RNA / DNA
Distilled H_2O	90.0	40.0	50.0	0.80
N_1P_0	103.0	60.0	43.0	1.40
N_0P_1	105.0	65.0	40.0	1.60
N_1P_1	500.1	322.5	177.6	1.80
$N_1P_{1/2}$	165.5	78.4	87.1	0.90
$N_{1/2}P_1$	279.6	158.1	121.5	1.30
$N_2P_{1/2}$	207.0	151.0	56.0	2.70
$N_{1/2}P_2$	211.9	148.3	63.6	2.20

Also table (3) showed that different N/P ratios in the nutritive media of sweet potato tuber disks did not exert the same effect on RNA and DNA contents of the tissues. While the deficiency of N or P strongly reduced the RNA content, the surplus of either one of them showed a sharp decrease in DNA content. Such decrease is an indication for the depression of DNA synthesis in the cells.

P-deficient tissues showed a lower content of RNA and DNA. As in the case of N deficiency, P deficiency led to a low total nucleic acid content as well as a low RNA/DNA ratio (compare $N_1P_{1/2}$ or $N_{1/2}P_1$ with N_1P_1). The deficiency in the nutritive media slows down the rate of synthesis of nucleic acids since P is a precursor for their building. P is also considered to be an essential factor for the maintenance of high energy level which by turn determines the speed of synthetic processes in the roots. It is also known that P plays an essential role in phosphorylation reactions during respiration and other processes. These reactions are disturbed at the conditions of its deficiency. As a result of these disturbances in the energetic processes and the depression of nucleic acid synthesis at conditions of P deficiency, the protein synthesis also decreases (table 2).

Again excess of P at conditions of N deficiency led to the reduction of nucleic acid content of the tissue s(table 3). A sharp reduction of DNA content was observed, while the RNA content clearly did not change as a result of which the ratio RNA/DNA increased (compare $N_{1/2}P_1$ with $N_{1/2}P_2$) It may be mentioned, here, that disturbance of nucleic acid metabolism occurring when P was in excess in N-deficient media, can be considered one of the reasons for the low protein content of the root cells. On the

other hand, our experiments showed that at surplus of N supply, the DNA content decreased. On the contrary, the RNA content increased resulting in a high RNA/DNA ratio (compare $N_1P_{1/2}$ with $N_2P_{1/2}$).

Dickens (1957) stated that, while the predominance of glycolytic type of respiration favours the DNA synthesis, the predominance of hexosemonophosphate pathway activates the RNA synthesis due to the formation of considerable amounts of ribose at these conditions. Shaw et al. (1958) indicated that indolacetic acid favours the hexosemonophosphate pathway, compared to the glycolytic type. These results give a base to suppose that excess of N leading to a high auxin content of sweet potato tuber tissues under investigation, favours the hexosemonophosphate breakdown of glucose, which unfavourably affects the DNA synthesis in cells, but favourably affects that of RNA. Relevantly, Nooden (1968) stated that indolacetic acid has a very large and very rapid stimulatory effect on incorporation of P^{32} orthophosphate into RNA in disks of artichoke tuber, and that the promotive effect could be accounted for through increased uptake of P^{32}.

From the above mentioned, it can be concluded that conditions developed in potato tuber disks due to high auxin content such as high level of hexosemonophosphate berakdown of glucose might have led to the depression of DNA synthesis and the rise of RNA/DNA ratio. Such effect was noticed by Silberger et al. (1954) in tobacco tissues. Consequently, this can explain the observed rise of RNA/DNA ratio in our results at conditions of excess N over P in the culture medium of sweet potato tuber disks.

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