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Mineral Nutrition of Cultured Chlorophyllous Cells of Tobacco (XIV) Upper Limits of NH_4^+/NO_3^- Metabolisms and of Cell Growth Rates

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Shifting in growth rates of subcultured NG, cell cultures of *Nicotiana glutinosa* on media changing NO_3^- levels at a fixed NH_4^+ level was examined, and the following was elucidated. The theoretical equation was given to $RCOO_p^-$, the maximum capacity of producible carboxylates induced by NO_3^- and SO_4^{2-} metabolisms. In NG on media 1 and 2 having lower NO_3^- levels, $RCOO_p^-$ was completely exhausted by NH_4^+ and NO_3^- assimilations. $Res=RCOO_p^- - RCOO_m^-$, the utilizable residual capacity of $RCOO_p^-$ ($RCOO_m^-$, carboxylates utilized in inorganic nitrogen assimilation), $C-A$, the difference between inorganic cation and anion concentrations in NG, and $-Ex$, the amount of $OH^-+HCO_3^-+RCOO^-$ excreted into the medium, were all turned into negative values in NG on media 1 and 2, and caused NG growth rates to reduce due to physiological stresses. Media 4 to 6 with higher NO_3^- levels raised NG growth rates with increasing $RCOO_p^-$, Res, $C-A$, $-Ex$, $Res/RCOO_p^-$, $(C-A)/RCOO_p^-$ and $-Ex/RCOO_p^-$, and with decreasing metabolized molar ratios of $NH_{4\cdot m}^+/NO_{3\cdot m}^-$, and $N_m/RCOO_p^-$ or $RCOO_m^-/RCOO_p^-$, where $N_m=NH_{4\cdot m}^+ + NO_{3\cdot m}^-$. It seemed that among these parameters, $C-A$, $(C-A)/RCOO_p^-$ and $(C-A)/N_m$ were closely correlated with NG growth rates. Furthermore, from the equation of $RCOO_p^-$, another equation was also derived, which showed the upper limit of $NH_{4\cdot m}^+/NO_{3\cdot m}^-$ in NG with high growth rates, and it approximated 2.3 to 2.7. On media 1 and 2, however, the values in NG were 3.2 and 2.8, respectively, which exceeded considerably the critical value, and NG growth rates were depressed. In NG on media 1 and 2, the relations of $N_m > RCOO_p^-$ and $RCOO_m^- > RCOO_p^-$ were found, so that NG utilized carboxylates originated from other sources or routes besides $RCOO_p^-$ in assimilating NH_4^+, and such additional carboxylates $RCOOH_m$ amounted to 6 to 19% of total carboxylates utilized to assimilate inorganic nitrogen sources.

The growth of cell cultures is generally inhibited by physiological injuries of free NH_4^+ and basic nitrogenous compounds accumulated in cells on media having too high NH_4^+/NO_3^- molar ratios, bacause metabolized NO_3^- induces most of carboxylates $RCOO^-$ utilized to assimilate NH_4^+ absorbed by cells.[1,9]

The present paper, using subcultured tobacco cells, intended to demonstrate theoretically how much metabolized NO_3^- may be required to assimilate a certain amout of NH_4^+ in cells.

For the purpose, the theoretical equation 2 was given to $RCOO_p^-$ producible in NO_3^- and SO_4^{2-} metabolisms by NG. $RCOO_p^-$ consisted of four components: (1) $RCOO_m^-$; (2) $A_{neu}^- = N_m - RCOO_m^-$ corresponding to the amount of anions $OH^-+HCO_3^-+RCOO^-$ required to neutralize the excess H^+ caused by more than one NH_4^+ assimilation per $RCOO^-$; (3) $|C-A|$[1,2] corresponding to carboxylates $RCOO^-$ or basic nitrogenous compounds RNH_3^+ retained in NG; and (4) $|Ex|$,[3,4] the amount of $OH^-+HCO_3^-+RCOO^-$ or $H^++RNH_3^+$ excreted into the medium to maintain the electroneutrality in and outside the cells. The latter two components were changed from positive to

Table 1. Compositions of major ions in a series of media used to examine effects of NH_4^+/NO_3^- ratios on growths of tobacco cell cultures NG.

Med. no.	π^a	ΣM^{n+}	K^+	Mg^{2+}	Ca^{2+}	$^{15}NH_4^+$	NO_3^-	SO_4^{2-}	$H_2PO_4^-$	Cl^-	Growth rate
1	2.2	50	15	5	5	25	10	10	15	15	39
2	2.4	55	15	10	5	25	15	10	15	15	50
3	2.6	60	15	15	5	25	20	10	15	15	63
4	2.7	65	15	20	5	25	25	10	15	15	120
5	2.9	70	15	25	5	25	30	10	15	15	133
6	3.1	75	15	30	5	25	35	10	15	15	134
MS[b]	2.8	50	20	3	6	21	40	3	1	6	100

Unit: meq/*l*. pH in media: 5.1±0.1.
[a] π=CRT (atm at 25°C by salts according to van't Hoff's equation).
[b] Murashige and Skoog medium.[6)]

negative values with rising NH_4^+/NO_3^- molar ratios in the media.

The upper limit of $NH_{4\cdot m}^+/NO_{3\cdot m}^-$ in NG with high growth rates was estimated using the theoretical equation 8.

Furthermore, the cases were discussed, where the relations of $N_m > RCOO_p^-$ and also $RCOO_m^- > RCOO_p^-$ occurred in NG having lowered growth rates, and where accordingly NG inevitably utilized carboxylic acids and carboxylates originated from other sources or routes besides $RCOO_p^-$ in assimilating NH_4^+.

Materials and Methods

Materials. The material used in the agar culture was NG, chlorophyllous tobacco cell cultures derived from the pith of *Nicotiana glutinosa.*[5)]

MS medium[6)] was used to produce stock cells for the experiment and also as a referential medium (**Table 1**). Concentrations of organic and inorganic constituents other than major mineral nutrients were always common to all media.[5)]

Fresh NG was inoculated in 100-ml Erlenmeyer flasks containing 50 ml of agar medium, and grown in sterile conditions at 25°C under continuous illumination from day-light fluorescent lamps at an intensity of about 4,000 lux all day.

Experimental methods. Table 1 indicates the compositions of major inorganic ions in media used to examine uptakes and metabolisms of NH_4^+ and NO_3^- by NG in relation to its growth rate. The series of media had different NO_3^- concentrations of 10 to 35 meq/*l* with a fixed NH_4^+ concentration of 25 meq/*l*.

In this series of media, NH_4^+ was labelled by ^{15}N, and NH_4^+/NO_3^- molar ratios were changed from 2.5 to 0.7. The salt osmotic pressure, π_{salts} (atm, π=CRT at 25°C, according to van't Hoff's equation, where C represents moles of total ions, R=0.082 and T=298) increased 1.4-fold from 2.2 to 3.1 atm. The π_{salts} changes were within the range which did not much affect NG growth rates. Media pH was adjusted to 5.1±0.1.

Three cell clumps of ca. 0.1 g each were separately inoculated on 50 ml of agar medium and then incubated aseptically by the method used to produce stock cells. When ca. 0.3 g of cells had proliferated to ca. 3 g, samples of ca. 0.1×3 g were again subcultured to the new medium, or they were harvested as described above to prevent changes of pH, concentrations and compositions of media, which also induce concentration changes of constituents in NG. Subculture was carried out at least 3 times.

At the end of incubation, NG on all media were harvested and prepared for analyses. The harvest time in this experiment, however, became somewhat late, because of the higher growth rates on media 4 to 6 and MS. MS-1 and MS-2 in **Figs. 1** to **5** indicated the analytical results of normal[5)] and late harvests NG on MS media, respectively.

The NG growth rate was represented as the ratio of the increase in the dry weight of NG prolif-

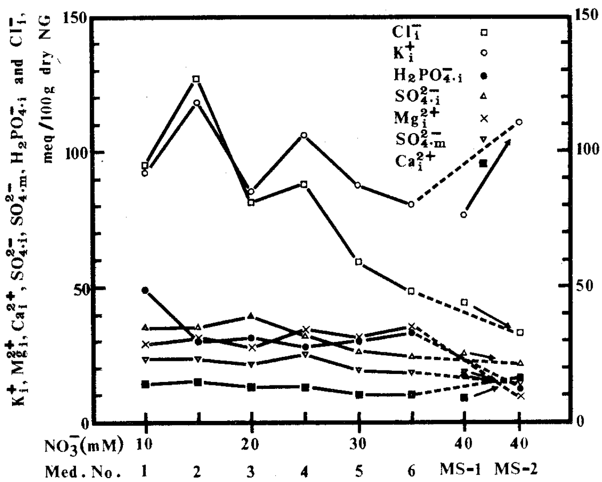

Fig. 1. Chemical analyses of K_i^+, Mg_i^{2+}, Ca_i^{2+}, $SO_{4.i}^{2-}$, $SO_{4.m}^{2-}$, $H_2PO_{4.i}^-$ and Cl_i^- in NG cultured on $NH_4^+ + NO_3^-$ media changing NO_3^- at the fixed NH_4^+ concentration, and on MS medium. MS-1 : normal harvest. MS-2 : late harvest.

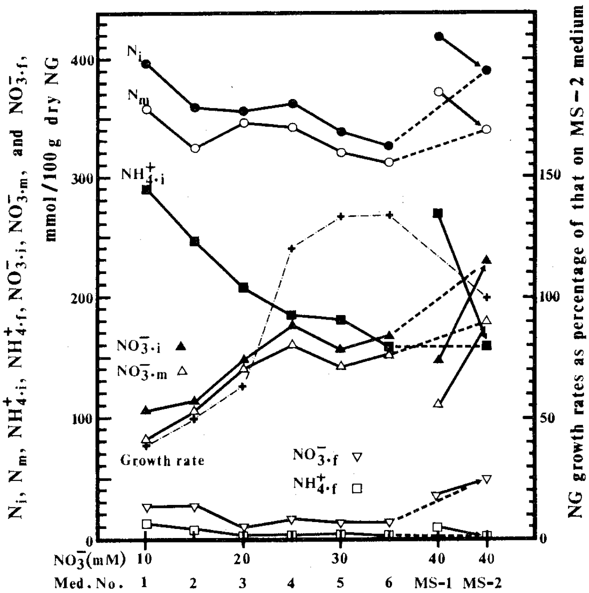

Fig. 2. Relations of N_i, N_m, $NH_{4.i}^+$, $NH_{4.f}^+$, $NO_{3.i}^-$, $NO_{3.m}^-$ and $NO_{3\cdot f}^-$ in NG to NG growth rates on $NH_4^+ + NO_3^-$ media changing NO_3^- at the fixed NH_4^+ concentration, and on MS medium. MS-1 : normal harvest. MS-2 : late harvest.

erated for ca. 20 days to the initial dry weight. The comparative growth rates were indicated as percentages based on NG on MS medium.

Chemical symbols and analyses of constituents in NG. Subscripts i, f and m, attached to ionic or elementary symbols signify 'taken in,' 'free' and 'metabolized,' respectively. They have the interrelation of i=f+m such as $NH_{4\cdot i}^+ = NH_{4\cdot f}^+ + NH_{4\cdot m}^+$.

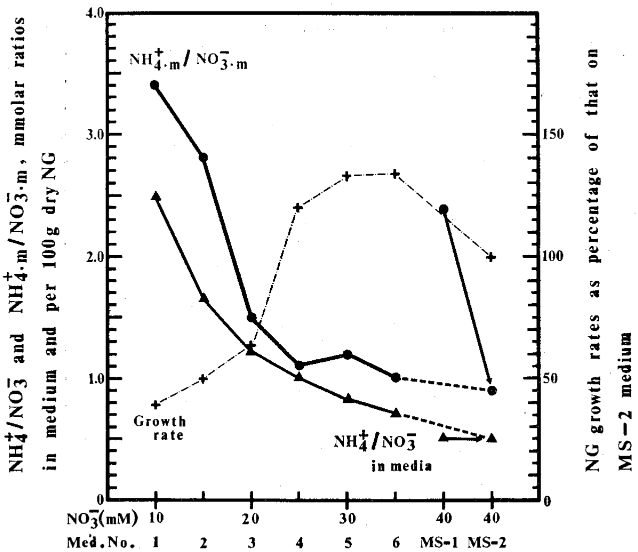

Fig. 3. Relations of molar ratios, NH_4^+/NO_3^- in media and $NH_{4\cdot m}^+/NO_{3\cdot m}^-$ in NG to NG growth rates. MS-1: normal harvest. MS-2: late harvest.

Concentrations of K_i^+, Mg_i^{2+}, Ca_i^{2+}, N_i ($={}^{15}NH_{4\cdot i}^+ + NO_{3\cdot i}^-$), ${}^{15}NH_{4\cdot i}^+$, $NH_{4\cdot f}^+$, $NO_{3\cdot f}^-$, $SO_{4\cdot i}^{2-}$, $SO_{4\cdot f}^{2-}$, $H_2PO_{4\cdot i}^-$ and Cl_i^- in NG were determined as described previously.[5)]

Concentrations of the following constituents were calculated according to the equations $NH_{4\cdot m}^+ = NH_{4\cdot i}^+ - NH_{4\cdot f}^+$, $NO_{3\cdot i}^- = N_i - {}^{15}NH_{4\cdot i}^+$, $NO_{3\cdot m}^- = NO_{3\cdot i}^- - NO_{3\cdot f}^-$, $N_m = NH_{4\cdot m}^+ + NO_{3\cdot m}^-$ and $SO_{4\cdot m}^{2-} = SO_{4\cdot i}^{2-} - SO_{4\cdot f}^{2-}$.

The difference between inorganic cation and anion concentrations in NG was calculated according to the formula, $C-A=(K_i^+ + Mg_i^{2+} + Ca_i^{2+} + NH_{4\cdot f}^+) - (NO_{3\cdot f}^- + SO_{4\cdot f}^{2-} + H_2PO_{4\cdot i}^- + Cl_i^-)$ after determining each mineral content. Organic and inorganic phosphates are mainly monovalent at the common cell pH, *i.e.* $H_2PO_{4\cdot i}^- = H_2PO_{4\cdot f}^- + H_2PO_{4\cdot m}^- = H_2PO_{4\cdot f}^- + RHPO_4^-$, where $RHPO_4^-$ denotes organic phosphates.

The excess anion or cation uptake was calculated according to the formula,

$$Ex = \sum M_i^{n+} - \sum A_i^{n-} = (K_i^+ + Mg_i^{2+} + Ca_i^{2+} + NH_{4\cdot i}^+) - (NO_{3\cdot i}^- + SO_{4\cdot i}^{2-} + H_2PO_{4\cdot i}^- + Cl_i^-). \quad (1)$$

In chemical formulas proposed here, all parameters are given in units of meq (in cases of ions) or mmol (in cases of elements such as N and S) per 100 g dry NG except for a few well-known metabolic formulas based on stoichiometric molar relations.

Results and Discussion

Carboxylates producible in NG to metabolize NH_4^+ and NO_3^-

The excess anion or cation uptake $|Ex|$ may be excreted into the medium as $OH^- + HCO_3^- + RCOO^-$, when $Ex<0$, or as $H^+ + RNH_3^+$, when $Ex>0$ to maintain the electroneutrality in and outside cells.[3,5,7-10)]

Equation 1 can be rewritten as follows.

$$\begin{aligned} Ex &= (K_i^+ + Mg_i^{2+} + Ca_i^{2+} + NH_{4\cdot f}^+) - (NO_{3\cdot f}^- + SO_{4\cdot f}^{2-} + H_2PO_{4\cdot i}^- + Cl_i^-) \\ &\quad + NH_{4\cdot m}^+ - (NO_{3\cdot m}^- + SO_{4\cdot m}^{2-}) \\ &= (C-A) + NH_{4\cdot m}^+ - (NO_{3\cdot m}^- + SO_{4\cdot m}^{2-}). \end{aligned}$$

Then, $NO_{3\cdot m}^- + SO_{4\cdot m}^{2-} = NH_{4\cdot m}^+ + (C-A) - Ex$.

Adding $NO_{3\cdot m}^-$ to both sides of this equation, equation 2 can be obtained:

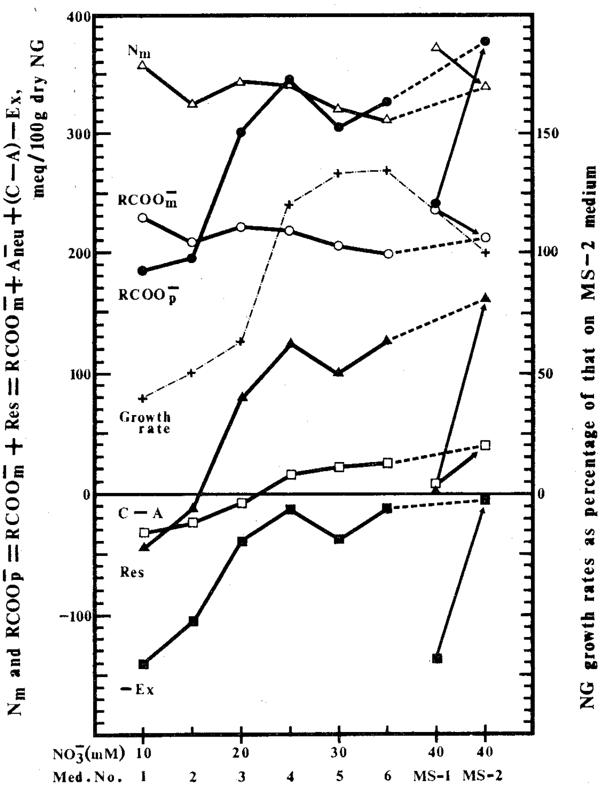

Fig. 4. Shifting in N_m, $RCOO_p^-$, $RCOO_m^-$, Res, (C−A), −Ex and growth rates of NG cultured on $NH_4^+ + NO_3^-$ media changing NO_3^- at the fixed NH_4^+ concentration, and on MS medium. MS-1 : normal harvest. MS-2 : late harvest.

$$
\begin{aligned}
RCOO_p^- = 2\,NO_{3\cdot m}^- + SO_{4\cdot m}^{2-} &= (NH_{4\cdot m}^+ + NO_{3\cdot m}^-) + (C-A) - Ex, \\
&= N_m + (C-A) - Ex \\
&= (1/x)N_m + (1-1/x)N_m + (C-A) - Ex \\
\text{or} \quad &= RCOO_m^- + A_{neu}^- + (C-A) - Ex = RCOO_m^- + Res. \qquad (2)
\end{aligned}
$$

The metabolic transition of the maximum capacity of carboxylates $RCOO_p^-$ produced in cells by an arbitrary amount of $NO_{3\cdot m}^-$ plus $SO_{4\cdot m}^{2-}$ initiall metabolized can be expressed by equation 2.

In this equation, $2NO_{3\cdot m}^- + SO_{4\cdot m}^{2-}$ would be equivalent to $RCOO_p^-$, according to the following stoichiometric metabolic equations of NO_3^- and SO_4^{2-} :

$$NO_3^- + 8\,H = NH_4^+ + 2\,OH^- + H_2O \qquad (3\,a)$$

$$SO_4^{2-} + 8\,H = (SH_2) + 2\,OH^- + 2\,H_2O \qquad (3\,b)$$

$$2\,OH^- + 2\,CO_2 = 2\,HCO_3^- \qquad (3\,c)$$

$$2\,HCO_3^- + 2\,RH = 2\,RCOO^- + 2\,H_2O. \qquad (3\,d)$$

Here, RH denotes some metabolite such as pyruvate subjected to carboxylation, and (SH_2) organic sulfur.[1,5,9,10]

According to equations 3 a to 3 d, the reduction of 1 eq NO_3^- to NH_4^+ induces 2 eq $RCOO^-$ in maximum, while that of 1 eq SO_4^{2-} to organic sulfur induces only 1 eq $RCOO^-$ in maximum.

It has been also reported that the metabolic conversion of absorbed NO_3^- and SO_4^{2-} into organic compounds is the main source of carboxylates and a major route of their synthesis in plant cells.[1]

On the other hand, it has been suggested that the carboxylate content in NH_4^+-fed plants depends mainly on HCO_3^- absorbed with metallic cations from the medium, and that consequently its content

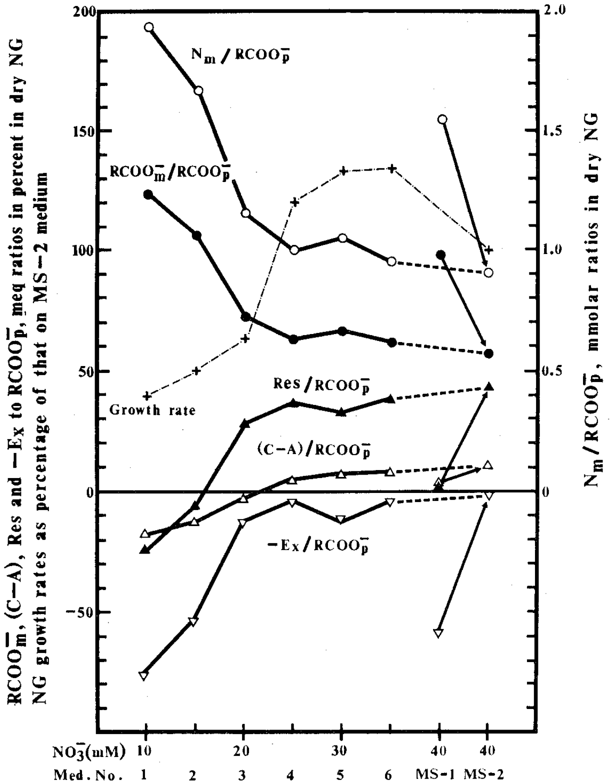

Fig. 5. Shifting in meq ratios of N_m, $RCOO_m^-$, (C−A), Res and −Ex to $RCOO_p^-$ and in growth rates of NG cultured on $NH_4^+ + NO_3^-$ media changing NO_3^- and fixing NH_4^+ concentrations, and on MS medium. MS-1: normal harvest. MS-2: late harvest.

becomes low.[1]

Not approximately the half, but all of $RCOO^-$ induced in such a way could be utilized by cells on $NH_4^+ + NO_3^-$ media, if necessary, to lead to the production of amino acids.

Consideration, however, has been attempted only in the case, where only the half of $RCOO^-$ inducible via OH^- and HCO_3^- from the metabolized NO_3^- is utilized to produce amino acids by cells on NO_3^- media, as expressed by the equation $NO_3^- + 8H + RH + CO_2 = (NH_3) + RCOO^- + 3H_2O$, where (NH_3) denotes nitrogen metabolites.

$RCOO_m^-$ in equation 2 was calculated by $RCOO_m^- = (1/x)N_m$ as mmol of metabolized carboxylates or meq of their primary monoanions.

The next assumption was set up to find out an approximate value of x.

Carboxylates are the precursors in synthesizing amino acids in cells. Furthermore, amino acids are located in the central position as the precursors in metabolisms of organic nitrogenous compounds. In addition, a large part of nitrogen metabolites is amino acids and proteins.

Therefore, $N_m/RCOO_m^- = x$ may attain to a certain upper critical value $n > 1.0$, before cells accumulate more nitrogen metabolites such as free basic amino acids and amines containing more nitrogen per molecule, and accumulate even free NH_4^+ with rising uptake ratios $NH_{4 \cdot i}^+/NO_{3 \cdot i}^-$ from media, beginning to reduce its growth rate due to the physiological injury by these compounds.

Thus, free and protein-bound amino acids (18 species) were analyzed in NG cultured on NO_3^- and $NH_4^+ + NO_3^-$ type media to obtain x and n values.[5]

The mmolar ratios of total N to total amino acids, contained in free plus protein-bound amino acids in NG were 1.42 and 1.56, respectively, on both types of media.[5] The latter value was adopted as that of x in the present experiment.

$A_{neu}^- = (1-1/x)N_m = N_m - RCOO_m^-$ in equation 2 corresponds to the amount of anions $OH^- + HCO_3^- + RCOO^-$ required to neutralize $(1-1/x)N_m$ mmol of the excess H^+ caused by the assimilation of N_m mmol of NH_4^+ with $(1/x)N_m$ mmol of monoanion $RCOO^-$.

(C−A) in equation 2 is the difference between inorganic cation and anion concentrations in cells corresponding approximately to the amount of free carboxylates as $C-A = RCOO^- - RNH_3^+ \simeq RCOO^-$ in the case of intact plants.[1,2,9]

However, $RCOO^-$ in the cell cultures may be expressed more generally as

$$RCOO^- = (C-A) + RNH_3^+. \quad (4)$$

C−A may be shifted to negative values, *i.e.*, $A-C = RNH_3^+ - RCOO^- \simeq RNH_3^+$, when NH_4^+/NO_3^- molar ratios rise in media.[1,2]

$Res = RCOO_p^- - RCOO_m^- = A_{neu}^- + (C-A) - Ex$ in equation 2 is the maximum capacity of carboxylates left producible for assimilating NH_4^+.

$RCOO_p^-$, $RCOO_m^-$, Res, C−A and −Ex were calculated using the analytical results shown in **Figs. 1** and **2** and $x=1.56$ (see **Fig. 4**).

N_m or $RCOO_m^- = N_m/1.56$ did not change appreciably among NG on media 1 to 6 and MS-2.

$RCOO_p^-$, Res, C−A, −Ex, $Res/RCOO_p^-$, $(C-A)/RCOO_p^-$, $-Ex/RCOO_p^-$, Res/N_m, $(C-A)/N_m$[3,11] and $-Ex/N_m$ increased, whereas $NH_{4 \cdot m}^+/NO_{3 \cdot m}^-$, and $N_m/RCOO_p^-$ or $RCOO_m^-/RCOO_p^-$ decreased in NG with rising NG growth rates in increasing NO_3^- concentrations in media 1 to 6 (**Figs. 3, 4 and 5**).

In addition, $N_m/RCOO_p^-$ and $RCOO_m^-/RCOO_p^-$ were over 1 to 1.56 and 100%, respectively, in NG on media 1 to 2 or 3. These results are discussed in the following section.

However, Res, C−A and −Ex may be high and $N_m/RCOO_p^-$ or $RCOO_m^-/RCOO_p^-$ low in NG on NO_3^- media generally having lower growth rates.[5,12]

Accordingly, the NG growth rate was lower on media with too high NH_4^+/NO_3^- ratios and also on NO_3 media, while it was higher on media having adequate NH_4^+/NO_3^- ratios. In other words, *e.g.*, as to C−A, which is retained in cells, the NG growth rate was lower, when C−A became too low or negative, and too high, whereas it was higher, when C−A became adequate in NG on this kind of a media series.

It seemed that among these parameters, C−A, $(C-A)/RCOO_p^-$ and $(C-A)/N_m$ were closely correlated with NG growth rates (**Figs. 4** and **5**), although Res/N_m may be more reasonable than $(C-A)/N_m$ from the wider point of view as a measure of evaluating the extent of the NH_4^+ nutrition in cells.

Consequently, cells may have to maintain high levels of $RCOO_p^-$ and N_m, and also a certain level of C−A in order to keep high growth rates.

The reason for the lower NG growth rates on media 1 and 2 than on media 4 to 6 having 1.0 to 0.7 of NH_4^+/NO_3^- ratios may be that NG on the former media had lower $RCOO_p^-$ and all $RCOO_p^-$ was exhausted, inducing negative values of Res, C−A and −Ex, which caused physiological injuries to NG, whereas NG on the latter media still had Res, utilizable residual capacities of $RCOO_p^-$.

The upper limit of $NH_{4 \cdot m}^+/NO_{3 \cdot m}^-$ in NG related to high growth rates

From equation 2,

$$RCOO_p^- - RCOO_m^- = (2NO_{3 \cdot m}^- + SO_{4 \cdot m}^{2-}) - (1/x)(NH_{4 \cdot m}^+ + NO_{3 \cdot m}^-) = Res \geqq 0.$$

Then,

$$2NO_{3 \cdot m}^- + SO_{4 \cdot m}^{2-} \geqq (1/x)(NH_{4 \cdot m}^+ + NO_{3 \cdot m}^-) \quad (5)$$

is derived, and formula 5 can be rewritten as follows:

$$NH_{4 \cdot m}^+/NO_{3 \cdot m}^- \leqq 2x - 1 + x(SO_{4 \cdot m}^{2-}/NO_{3 \cdot m}^-). \quad (6)$$

In the case of $SO_{4 \cdot m}^{2-} \ll NO_{3 \cdot m}^-$, formula 6 becomes

$$NH_{4 \cdot m}^+/NO_{3 \cdot m}^- \leqq 2x - 1. \quad (7)$$

When the right side is going to be equal to the left side in formula 5, all $RCOO_p^-$ produced in cells is exhaused to metabolize NH_4^+ and NO_3^-, so that $RCOO_m^- = RCOO_p^-$ is attained. In such a case, the result of $C-A=RCOO^- - RNH_3^+ = -RNH_3^+ < 0$, *i. e.*, $A-C=RNH_3^+$, is obtained. Thus, cells accumulate basic nitrogen metabolites and even free NH_4^+, and their growth rates begin to decline.[12]

When Res is nearing zero, x may approach n, an upper critical value in formulas 6 and 7 to attain to

$$NH_{4\cdot m}^+/NO_{3\cdot m}^- \simeq 2n-1+n(SO_{4\cdot m}^{2-}/NO_{3\cdot m}^-) \quad (8)$$

and $NH_{4\cdot m}^+/NO_{3\cdot m}^- \simeq 2n-1$, as $SO_{4\cdot m}^{2-} \ll NO_{3\cdot m}^-$ (9)

The n value can be obtained by calculating it from equation 8, using experimental values of $NH_{4\cdot m}^+$, $NO_{3\cdot m}^-$ and $SO_{4\cdot m}^{2-}$ just before cells begin to accumulate free organic cations and NH_4^+ to their toxic levels, and to reduce growth rates with rising NH_4^+/NO_3^- molar ratios in media.

The n value was estimated to be about 1.6 for NG.[5] Accordingly, an upper critical $NH_{4\cdot m}^+/NO_{3\cdot m}^-$ value was 2.3 to 2.7 from equation 8, as $SO_{4\cdot m}^{2-}/NO_{3\cdot m}^- = 0.06$ to 0.30 in normal cases.

In this experiment, $NH_{4\cdot m}^+/NO_{3\cdot m}^-$ values in NG were 3.2 and 2.8, far exceeding the critical value, with lower growth rates on media 1 and 2, and were 1.1, 1.2 and 1.0 on media 4 to 6, respectively, much lower than the critical value (**Fig. 3**).

The $NH_{4\cdot m}^+/NO_{3\cdot m}^-$ value in NG was 2.4 on medium MS-1 harvested normally,[5] which was near the upper critical value, and became 0.9 lower on medium MS-2 harvested late.

The reason would be due to a lower NH_4^+ uptake from medium MS-2, in which the NH_4^+ concentration was diluted by the high NH_4^+ uptake of NG at the early stage in the last subculture period, and also due to the higher NO_3^- uptake of NG less inhibited by the lower NH_4^+ concentration in the medium.[13,14] The shifting pattern of $NH_{4\cdot m}^+/NO_{3\cdot m}^-$ values in NG on media 4 to 6 was also supposed to be similar to that on MS medium.

The $NH_{4\cdot m}^+/NO_{3\cdot m}^-$ value in NG on medium 3 also seemed to be as high as that on media 1 and 2, exceeding the upper critical value at the early stage in the last subculture period, inhibiting the NG growth rate.

The cell growth rate could be improved further, if $RCOO_p^-$ is raised with increasing N_m by reforming compositions of media, because N_m is, to some extent, positively correlated to the growth rate.

On the contrary, the relations, $N_m > RCOO_p^-$ and $RCOO_m^- = (1/x)N_m = (1/1.56)N_m > RCOO_p^-$ occurred in NG having lower growth rates on media 1 and 2. In these cases, the excess H^+, caused by the NH_4^+ metabolism and equal to $N_m-(2NO_{3\cdot m}^- + SO_{4\cdot m}^{2-}) = (A-C)+Ex$ from equation 2, may not be neutralized by any $(OH^- + HCO_3^- + RCOO^-)$, because there is no more available $(OH^- + HCO_3^- + RCOO^-)$. Further, the latter relation signifies that there is already no more $RCOO_p^-$ utilizable to assimilate NH_4^+.

In this case, however, if electrically neutral RCOOH produced in cells and also $RCOO^-$ produced from RH in cells and HCO_3^- absorbed from the medium are not negligible as compared with $RCOO_p^-$ induced by NO_3^- and SO_4^{2-} metabolisms,[15] part of N_m may be induced by the metabolism of NH_4^+ with such RCOOH and $RCOO^-$ symbolized as $RCOOH_m$, generating and leaving H^+ in cells and media.

$RCOOH_m$ originated from the sources other than $RCOO_p^-$ is expressed as $RCOOH_m = -Res = RCOO_m^- - RCOO_p^- = (1/x)N_m - RCOO_p^-$.

$RCOOH_m$ in NG on media 1 and 2 was 44 and 12 mmol/100 g dry NG, and percentages of $RCOOH_m$ to $RCOO_m^- = RCOO_p^- + RCOOH_m$ were about 19 and 6%, respectively.

Consequently, it is concluded that the normal and maximum, growth and assimilation of inorganic nitrogen sources are carried out by using the carboxylates induced by NO_3^- and SO_4^{2-} metabolisms in NG.

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≪和文要約≫

タバコ培養緑色細胞の無機栄養（XIV）
NH_4^+/NO_3^- 代謝と細胞成長速度との上限について

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一定濃度の NH_4^+ に異なる濃度の NO_3^- を加えた一連の培地におけるタバコ（*Nicotiana glutinosa*）培養細胞 NG の成長速度の変動を試験し，次の結果を得た．

NO_3^- と SO_4^{2-} の代謝によって誘引される生産可能なカルボン酸イオンの最大容量 $RCOO_p^-$ は，低濃度の NO_3^- を含む培地1と2の NG においては，NH_4^+ と NO_3^- の同化によって完全に消費された．$RCOO_p^-$ の利用可能な残余容量 $Res=RCOO_p^- - RCOO_m^-$（$RCOO_m^-$ は無機態窒素の同化に利用されたカルボン酸イオン量），NG 内の無機の陽イオンと陰イオンの濃度差 C－A，および培地へ排出される $OH^- + HCO_3^- + RCOO^-$ の量 －Ex，はすべて培地1と2の NG では負の値となり，NG の成長速度は生理的ストレスによって低下した．

高い NO_3^- 濃度の培地 4～6 では，NG は，高い成長速度を持ち，C－A，$(C-A)/RCOO_p^-$，$(C-A)/N_m$，$(N_m = NH_{4 \cdot m}^+ + NO_{3 \cdot m}^-)$ などが NG の成長速度によく相関したようにみえた．

高い成長速度を持つ NG の $NH_{4 \cdot m}^+/NO_{3 \cdot m}^-$ の上限値 2.3～2.7 を，培地1と2の NG ではかなり越え，3.2 および 2.8 となり，成長速度も低下した．

培地1と2の NG では，$N_m > RCOO_p^-$ および $RCOO_m^- > RCOO_p^-$ の関係が生じ，NH_4^+ を同化するために $RCOO_p^-$ のほかに，他の由来のカルボン酸も利用し，このような追加のカルボン酸 $RCOOH_m$ は，無機態窒素を同化するために利用された全カルボン酸の 6～19% であった．