

Increase in respiratory cost at high growth temperature is attributed to high protein turnover cost in *Petunia × hybrida* petals

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ABSTRACT

It is widely believed that turnover of nitrogenous (N) compounds (especially proteins) incurs a high respiratory cost. Thus, if protein turnover costs change with temperature, this would influence the dependence of respiration rate on growth temperature. Here, we examined the extent to which protein turnover cost explained differences in N-utilization costs (nitrate uptake/reduction, ammonium assimilation, amino acid and protein syntheses, protein turnover and amino acid export) and in respiration rate with changes in growth temperature. By measurements and literature data, we evaluated each N-utilization cost in *Petunia × hybrida* petals grown at 20, 25 or 35 °C throughout their whole lifespans. Protein turnover cost accounted for 73% of the integrated N-utilization cost on a whole-petal basis at 35 °C. The difference in this cost on a dry weight basis between 25 and 35 °C accounted for 75% of the difference in N-utilization cost and 45% of the difference in respiratory cost. The cost of nitrate uptake/reduction was high at low growth temperatures. We concluded that respiratory cost in petals was strongly influenced by protein turnover and nitrate uptake/reduction, and on the shoot basis, C investment in biomass was highest at 25 °C.

Key-words: nitrate reduction.

INTRODUCTION

Nitrogen (N) utilization processes, including nitrate/ammonium uptake, nitrate reduction, ammonium assimilation, amino acid synthesis, protein synthesis, protein turnover and amino acid export, incur large respiratory costs in plants (Penning de Vries, Brunsting & van Laar 1974; Zerihun, McKenzie & Morton 1998). Among these processes, nitrate reduction and ammonium assimilation are thought to consume high respiratory costs. In fact, the form of N source (e.g. nitrate or ammonium) greatly influences estimates of construction respiration (McDermitt & Loomis 1981; Williams *et al.* 1987). On the other hand,

maintenance respiration rates are often correlated with protein content, suggesting that protein turnover cost dominates maintenance respiration (Penning de Vries 1975; Amthor 1989; Lambers, Chapin & Pons 1998). The cost of amino acid export during senescence is also claimed to be substantial (Dangl, Dietrich & Thomas 2000). Because these processes consume large respiratory costs, these processes would influence plant growth and production. Thus, it is important to assess the costs of these respective N-utilization processes and their responses to environmental factors, including growth temperature.

On the basis of data from the literature, Zerihun *et al.* (1998) calculated the total respiratory cost of N-utilization processes (N-utilization cost) in bean leaves. They suggested that protein turnover cost dominates the N-utilization cost. However, their calculation was based on data on protein turnover rates in several plant species grown under different conditions. Moreover, these plant materials were at various developmental stages, and protein turnover rate is known to differ markedly depending on the developmental stage (Mae, Makino & Ohira 1983; Barneix *et al.* 1988; Bouma *et al.* 1994). Therefore, their calculation would have been inaccurate, although the approach was valid.

Growth temperature greatly influences plant respiration. This is because growth temperature changes substrate availability and/or demand for ATP (Bunce 2004; Atkin, Scheurwater & Pons 2006). Generally, the specific maintenance respiration rate increases with increasing growth temperature, whereas specific construction respiration is affected little (Amthor 1989; Marcelis & Baan Hofman-Eijer 1995; Van Iersel 2003, 2006). Interestingly, the half-life of protein in wheat roots decreases with increasing temperature because of activity of the ubiquitin proteolytic pathway (Ferguson, Guikema & Paulsen 1990). This indicates that the protein turnover rate increases with temperature.

All of these studies indicate that protein turnover cost is a major component of the N-utilization cost and is likely to increase with growth temperature. Our aims here were to estimate the costs of the respective N-utilization processes – especially that of protein turnover – and to examine the effects of growth temperature on these costs. The quantitative understanding of these components of respiratory costs

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will provide us the information to improve plant growth and productivity.

Growth temperature generally changes not only the specific maintenance respiration rate, but the lifespans of plant organs (Kikuzawa 1995; Forbes, Black & Hooker 1997). Therefore, it is difficult to estimate the protein turnover cost from data on one developmental stage of an organ, and analyses that cover the whole lifespan of the organ are preferable. We estimated each of the N-utilization costs in petals of *Petunia × hybrida* grown at one of three temperatures throughout their lifespans. Petals are useful materials, because they have shorter lifespans than leaves and it is easy to determine their developmental stages. Moreover, most petals are non-photosynthetic, and therefore ATP and reducing equivalents are supplied mainly from the respiratory pathway.

To calculate the costs of nitrate/ammonium uptake, nitrate reduction, ammonium assimilation, amino acid and protein syntheses, and amino acid transport, we used the specific costs of these respective processes in the literature (De Visser, Spitters & Bouma 1992; Boorer & Fischer 1997; Zerihun *et al.* 1998; Amthor 2000; Dangl *et al.* 2000), and multiplied these specific costs by actual changes in the amounts of amino acids and proteins. The cost of protein turnover was estimated by using cycloheximide (CHI), an inhibitor of cytosolic protein synthesis, according to Bouma *et al.* (1994). On the basis of the data obtained, we discuss the contribution of protein turnover cost to N-utilization cost and respiration rate. We also evaluated the effects of different N sources on estimates of N-utilization cost. Furthermore, we calculated the biomass C, N-utilization cost and respiratory cost to the petals on one shoot over 90 d, and we discuss the changes in N-utilization cost and in C investment in petal biomass with growth temperature. Our precise estimations clarified that the costs of nitrate reduction/uptake and protein turnover were large, and that the growth temperature intensively influenced these costs.

MATERIALS AND METHODS

Plant materials and growth conditions

We used *Petunia × hybrida* cv. Surfinia 'White Vein' (Suntry flowers, Tokyo, Japan). We purchased seedlings and removed the shoots other than the main one. We then cultivated the seedlings in vermiculite in pots (diameter, 10.5 cm; depth, 17.5 cm; one seedling per pot). We placed the pots in a growth chamber (Biotron, Nippon Medical & Chemical Instruments Co., Osaka, Japan). Light was supplied by a bank of cool-white fluorescent tubes. The photosynthetically active photon flux density (PPFD) was ca. $450 \mu\text{mol m}^{-2} \text{s}^{-1}$. The air temperature was 20, 25 or 35 °C, relative humidity was 55%, and day length was 16 h (from 1000 to 0200 h). Twice a week, the plants received 200 mL per pot of a nutrient solution containing 2 mM KNO₃, 2 mM Ca(NO₃)₂, 0.75 mM MgSO₄, 0.665 mM KH₂PO₄, 25 μM ethylenediaminetetraacetic acid (EDTA)-Fe, 5 μM MnSO₄, 0.5 μM ZnSO₄, 0.5 μM CuSO₄, 25 μM H₃BO₃,

0.25 μM Na₂MoO₄, 50 μM NaCl and 0.1 μM CoSO₄. We cultivated the plants for approximately 1 month. For measurements, we used the new petals on shoots newly developed in the growth chamber at 20, 25 or 35 °C. We measured and sampled the petals between 1400 and 0000 h.

Respiration rate

Respiratory O₂ uptake rates of detached petals were measured polarographically with a gas-phase oxygen electrode system (LD2; Hansatech Instruments Ltd, Kings Lynn, Norfolk, UK). The respiratory quotient (RQ) was assumed to be 1.

Protein and amino acid contents

Proteins were extracted from samples frozen in liquid N₂ and powdered using a mortar and pestle with a buffer containing 62.5 mM Tris-HCl (pH 6.8), 2% sodium dodecyl sulphate [w/v], 7.5% glycerol [v/v], 0.01% bromophenol blue [w/v], 50 mM 1,4-dithiothreitol, and 1 tablet/50 mL Complete proteinase inhibitor cocktail (Roche Diagnostics GmbH, Mannheim, Germany). The ratio of the sample dry weight (DW) to the sample buffer was 10 to 40 mg/mL. Extracts were incubated at 100 °C for 5 min and centrifuged at 12 000 g. The supernatants were used for protein quantification by the modified Lowry method (Peterson 1977).

Amino acids were extracted from the powdered samples with 80% ethanol by the method of Ono, Terashima & Watanabe (1996). Amino acids in the supernatant were quantified by the modified ninhydrin method (Rosen 1957).

Inhibition of protein synthesis with CHI

To estimate the protein turnover cost, we incubated the petals in 40 mM Hepes-KOH buffer (pH 7.2) in the presence or absence of 500 μM CHI for 20 min in the dark at the growth temperature, and then measured the O₂ uptake rates.

Definition of petal age and lifespan

Day 0 denoted the day when the petal started to unfold. Negative days were those on which the petals were still folded. We measured the folded petal lengths of *P. × hybrida* everyday at each temperature ($n \geq 30$ on each day at each growth temperature) and plotted length against the day number. The age of the folded petal (in days) was estimated from the folded petal length by polynomial approximation ($R^2 \geq 0.99$). We defined the petal lifespan as the number of days for which the size and respiratory activity of a single detached petal were sufficient for us to measure the respiration rate (see Fig. 1).

Definition of respiratory cost

Respiratory cost was taken to include not only C in the CO₂ emitted by the respiratory pathway to supply ATP and

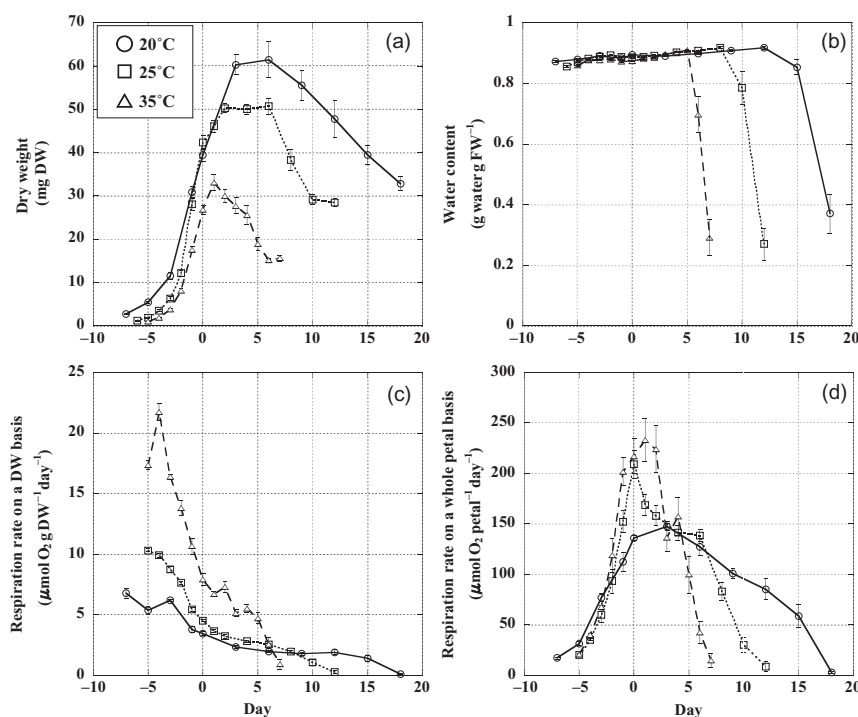


Figure 1. Changes in dry weight (DW) (a), water content on a fresh weight (FW) basis (b), respiration rate on a DW basis (c) and respiration rate on whole-petal basis (d) during development of *Petunia × hybrida* petals at each growth temperature (20 °C, circles; 25 °C, squares; 35 °C, triangles). Day 0 was the first day on which the petal began to unfold. Vertical bars represent standard error of the mean (SEM) ($n \geq 4$).

reducing equivalents, but also the C in CO₂ emitted in other biosynthetic pathways (see Amthor 2000).

Efficiency of respiratory ATP production

ATP production efficiencies from sucrose and reducing equivalents were estimated according to Amthor (1994). He assumed that oxidation of 1 mol of sucrose via pyruvate produces 58 mol of ATP, and that all sucrose is degraded by invertase (for details, see Amthor 1994). The number of ATP produced via mitochondrial oxidation of reducing equivalents (P_{ATP} ; mol ATP) was estimated as

$$P_{ATP} = \frac{[H^+_{III,IV}] \times ([NADH_{cyt}] + [NADPH_{cyt}] + [FADH_2]) + [H^+_{I,III,IV}] \times [NADH_{mit}]}{(1 + [H^+_{ATP}])} \quad (1)$$

where $[H^+_{III,IV}]$ and $[H^+_{I,III,IV}]$ are the numbers of protons pumped into the inter-membrane space when a pair of electrons flows through Complex III/IV and Complex I/III/IV, respectively. We adopted 6 for $[H^+_{III,IV}]$ and 10 for $[H^+_{I,III,IV}]$ (Amthor 2000). $[NADH_{cyt}]$, $[NADPH_{cyt}]$, $[FADH_2]$ and $[NADH_{mit}]$ are the concentrations of the corresponding reductants, and the subscripts 'cyt' and 'mit' indicate the cytosol and mitochondrial matrix, respectively, and indicate the compartments where these reductants are produced. In the respiratory oxidation of sucrose via pyruvate, $[NADH_{cyt}]$, $[NADPH_{cyt}]$, $[FADH_2]$ and $[NADH_{mit}]$ are given the values 4, 0, 4 and 16, respectively (Amthor 2000). $[H^+_{ATP}]$ is the number of protons moving through H⁺-ATPase per ADP that undergoes phosphorylation; a value of 3 was used for the calculation (Amthor

2000). The 1 in the denominator is the number of protons co-transported with phosphate into the matrix to compensate for the deficit in phosphate.

Respiratory costs of nitrate/ammonium uptake, nitrate reduction, ammonium assimilation, amino acid synthesis and protein synthesis

Nitrate and ammonium ions are taken up from the xylem into the symplast across the plasma membrane, with stoichiometries of $2H^+/NO_3^-$ and H^+/NH_4^+ , respectively (Cannell & Thornley 2000; Kronzucker *et al.* 2001). We assumed that the number of membranes to be crossed was one (Kurimoto *et al.* 2004). The uptake of nitrate and ammonium as N sources requires additional specific respiratory costs of proton motive force formation, at 1.24 and 0.62 mol C mol⁻¹ amino acid residue, respectively (Table 1). The processes of unloading of sucrose and amide into the petal are assumed to occur only via the symplastic pathway and thus require no respiratory costs (Cannell & Thornley 2000).

The respiratory costs of nitrate reduction, ammonium assimilation [glutamine (Gln) synthesis], and synthesis of amino acids other than Gln were calculated from Zerihun *et al.* (1998) and the AraCyc metabolic map (Zhang *et al.* 2005). The elemental composition of amino acid residues was considered to be the average of the 20 amino acids, with a molecular weight of 118.9 g mol⁻¹ (C_{5.35}H_{7.95}O_{1.45}N_{1.45}S_{0.10}). Carbon skeletons and reducing equivalents for amino acid synthesis were assumed to be supplied by Gln and sucrose. The pathway for each amino acid synthesis (see Appendix) was based on the AraCyc metabolic pathway database for

Table 1. Specific respiratory costs of nitrate reduction, ammonium assimilation, other amino acid synthesis and nitrate/ammonium uptake

Process basis	Equation	Specific cost on amino acid residues [mol C mol ⁻¹ amino acid residues]
(1) Nitrate reduction	NO ₃ ⁻ + NADH _{cyt} + 3NADPH _{cyt} → NH ₃ + OH ⁻ + H ₂ O ^a (a) 0.05Sucrose → 0.6CO ₂ + NADH _{cyt} + 0.2FADH _{2cyt} + 0.4ATP (b) 0.125Sucrose + 0.25ATP → 1.5CO ₂ + 3NADPH _{cyt}	3.05
(2) Ammonium assimilation	2NH ₃ + 0.5Sucrose + ATP → L-glutamine + CO ₂ + NADH _{cyt} + 2NADH _{mit} ^b (c) α-ketoglutarate + 2NH ₃ + 2ATP + NADH _{cyt} → L-glutamine (d) 0.5Sucrose → α-ketoglutarate + 2NADH _{mit} + 2NADH _{cyt} + ATP + CO ₂	0.73
(3) Other amino acid synthesis	14.5L-glutamine + 9.95Sucrose + 2SO ₄ ²⁻ + 8NADPH _{cyt} + 19ATP ^c → 20C _{5.35} H _{7.95} O _{1.45} N _{1.45} S _{0.10} + D-glyceraldehyde-3-phosphate + 10.5α-ketoglutarate + Succinate + 2Fumarate + 2Acetate + 12.21CO ₂ + 25.5NADH _{cyt} + 7NADH _{mit}	0.81
(4) NO ₃ ⁻ uptake		1.24
(5) NH ₄ ⁺ uptake		0.62

Each specific cost is estimated on the basis of the equations shown.

^aEquations (a) and (b) denote pathways from which NADH and NADPH are supplied, respectively. NADH and NADPH are produced via the glycolysis and tricarboxylic acid cycle, and the pentose phosphate pathway, respectively.

^bEquation (c) denotes L-Gln synthesis via glutamine synthetase and glutamine: 2-oxoglutarate amidotransferase reactions. α-ketoglutarate is produced from pathway (d). ATP is produced via the oxidation of additional NADH_{cyt} or NADH_{mit} in mitochondrial electron transport.

^cAverage amino acid (C_{5.35}H_{7.95}O_{1.45}N_{1.45}S_{0.10}) is assumed to be the average of 20 amino acids. Pathways for 20 amino acids are summarized in the Appendix. NADPH is supplied from the pentose phosphate pathway, as shown in Eqn (b). ATP is produced via the oxidation of additional NADH_{cyt} or NADH_{mit} in mitochondrial electron transport.

plant research (Zhang *et al.* 2005). The specific respiratory costs of nitrate reduction, ammonium assimilation and other amino acid synthesis are listed in Table 1. The respiratory cost of pH regulation was neglected, because little information was available (Zerihun *et al.* 1998). To estimate the respiratory costs of nitrate/ammonium uptake, nitrate reduction, ammonium assimilation and other amino acid synthesis, we multiplied the rate of increase in amino acid residues on a DW basis (ΔA ; mol amino acid residue g DW⁻¹ d⁻¹) by the specific respiratory costs in Table 1. The rate of increase in amino acid residues was estimated as follows:

$$\Delta A = \frac{(A_n - A_{n-1})}{(T_n - T_{n-1})} \times \frac{1}{DW_{n-1}} \quad (2)$$

where A_n is the amount of amino acid residues in amino acids and proteins on a whole-petal basis on day n (mol amino acid residues petal⁻¹), T_n is the sampling time (day n), and DW_{n-1} is the dry weight on day $n-1$.

The specific respiratory costs of protein synthesis processes are listed in Table 2. The respiratory cost of protein synthesis (R_{cpro} ; mol C g DW⁻¹ d⁻¹) was calculated according to the following equations:

$$R_{\text{cpro}} = \Delta P \times E_s \times \frac{1}{(1 - F_{\text{org}})} \quad (3)$$

and

$$\Delta P = \frac{(P_n - P_{n-1})}{(T_n - T_{n-1})} \times \frac{1}{DW_{n-1}} \times \frac{1}{118.9} \quad (4)$$

where ΔP is the rate of increase in amount of amino acid residues in the proteins on a DW basis (mol amino acid residues g DW⁻¹ d⁻¹), E_s is the specific respiratory cost of protein synthesis (mol C mol⁻¹ amino acid residue) in Table 2 (we used the mean value in Table 2 for the calculation), and F_{org} is the amount of protein synthesis or turnover in the organelles (e.g. mitochondria and plastids) as a ratio of that in the whole cell. F_{org} was assumed to be 0%, because (1) fewer than 10% of all mitochondrial proteins are encoded by mitochondrial DNA and synthesized in the mitochondria and (2) the petals of *P. × hybrida* do not have green tissues and include little ribulose 1-5-bisphosphate carboxylase/oxygenase (Rubisco), which accounts for a large proportion of the proteins synthesized in the leaf chloroplasts. P_n is the amount of protein on a whole-petal basis on day n (g petal⁻¹). T_n is the sampling time (on day n). DW_{n-1} is the dry weight on day $n-1$, and 118.9 (g mol⁻¹) is the average molecular weight of the amino acid residues as noted.

Respiratory cost of protein turnover

From the information provided by De Visser *et al.* (1992), Bouma *et al.* (1994), Zerihun *et al.* (1998) and Noguchi *et al.* (2001), we assumed that the total specific respiratory cost of protein turnover included the costs of protein biodegradation and protein biosynthesis (see Table 2). The expected effects of CHI on protein turnover and the specific respiratory costs of these processes are also shown in Table 2. The respiratory cost of protein turnover (R_{mpro} ; mol C g DW⁻¹ d⁻¹) was as follows:

Table 2. Specific respiratory costs for each process of protein synthesis, protein turnover and amino acid export on the basis of amino acid residues

Process	Specific cost on an amino acid residues basis [mol C mol ⁻¹ amino acid residues]			
	Min	Max	Mean	CHI effect
Protein biodegradation				
(1) Protein biodegradation (E_{deg}) (a)	0.21	0.21	0.21	–
Protein biosynthesis				
(2) Amino acid activation (b)	0.41	0.41	0.41	+
(3) Editing for misaminoacylation of tRNA (c)	0.00	0.03	0.02	+
(4) Peptide bond formation and translocation (a)	0.41	0.41	0.41	+
(5) Signal sequences (c)	0.04	0.21	0.13	+
Tool maintenance				
(6) Amino acid turnover ^a (a,b)	0.36	0.50	0.43	–
(7) mRNA turnover (c)	0.03	0.07	0.05	–
Amino acid export				
(8) Gln synthesis (E_{syn}) ^b (d)	0.21	0.21	0.21	–
(9) Membrane transport (E_{tra}) (e)	0.21	0.21	0.21	–
Protein synthesis [E_S : (2) + (3) + (4) + (5) + (6)]	0.86	1.06	0.96	
Protein turnover [E_{SP} : E_S + (7) + (8)]	1.46	1.84	1.65	
Expected CHI effect ($E_{CHI} = E_S$)	0.86	1.06	0.96	

‘CHI effect’ denotes the expected effect of cycloheximide (CHI) on the process. It was assumed that oxidation of 1 mol sucrose produced 58 mol ATP. Letters denote references for estimation of the specific cost of each process (a: Zerihun *et al.* 1998, b: De Visser *et al.* 1992, c: Anthor 2000, d: Dangl *et al.* 2000, e: Boorer & Fischer 1997).

^aSpecific respiratory cost of amino acid turnover was estimated on the assumption that 30 to 50% of amino acids are recycled.

^bGln was assumed to be transported across one membrane for its export.

$$R_{mpro} = (R_{CHI} - R_{cpro}) \times \frac{E_{SP}}{E_{CHI}} \times \frac{1}{(1 - F_{org})} \quad (5)$$

where R_{CHI} is the decrease in respiration rate (mol C g DW⁻¹ d⁻¹) in response to CHI application, E_{SP} is the specific respiratory cost of protein turnover (mol C mol⁻¹ amino acid residue) and E_{CHI} is the specific respiratory cost of the processes blocked by CHI (mol C mol⁻¹ amino acid residue). We used the mean value in Table 2 for the estimation.

Respiratory cost of amino acid export from the petal

We defined amino acid export as a series of processes from the biodegradation of proteins into amino acids to the loading of amino acids into the phloem. We assumed that amino acids were transported in the form of Gln. ATP-requiring processes were assumed to be protein biodegradation, Gln synthesis and transport across the plasma membrane.

In protein biodegradation, amino acids are assumed to be converted to Glu by specific transaminases (Dangl *et al.* 2000). The specific cost of Gln synthesis from Glu by Gln synthetase is 1 mol of ATP per mol Gln produced (Table 2). A certain fraction of amino acids from the protein degradation is considered to be degraded into NH₄⁺ and α -keto acids by deaminase or Glu dehydrogenase (Dangl *et al.* 2000). If NH₄⁺ for Gln synthesis is derived from the

deamination of amino acids, 2 mol of amino acids will be required for 1 mol of Gln synthesis. We assumed that Gln is exported from the petal via the apoplastic pathway. The export of 1 mol of protons is coupled to that of the equivalent mole of amino acid, in accordance with the stoichiometry of the *Arabidopsis thaliana* amino acid transporters AAP1 and AAP5 (Boorer & Fischer 1997; Table 2).

The minimum and maximum respiratory costs ($R_{amino,min}$ and $R_{amino,max}$; mol C g DW⁻¹ d⁻¹) of amino acid export from the petal were calculated using Eqns 6 and 7. To calculate the minimum cost, we assumed that half the NH₄⁺ was derived from the pool in the same cell and half was derived from the deamination of amino acids. To calculate the maximum cost, we assumed that all NH₄⁺ was derived from that pool:

$$R_{amino,min} = \frac{12}{58} \times \left(E_{deg} \times NPDR + \frac{1}{2} \times E_{syn} \times NPDR + E_{tra} \times NAER_{min} \right) \quad (6)$$

and

$$R_{amino,max} = \frac{12}{58} \times (E_{deg} \times NPDR + E_{syn} \times NPDR + E_{tra} \times NAER_{max}) \quad (7)$$

where E_{deg} , E_{syn} and E_{tra} denote the specific respiratory costs of protein degradation, Gln synthesis and membrane transport, respectively (Table 2). $NPDR$ (mol amino acid residues g DW⁻¹ d⁻¹) and $NAER$ (mol amino acids g DW⁻¹ d⁻¹)

are the net protein decrease rate and net amino acid export rate, respectively. *NAER* was calculated according to the following equations:

$$NAER_{\min} = \frac{1}{2} \times NPDR + NADR \quad (8)$$

and

$$NAER_{\max} = NPDR + NADR \quad (9)$$

where *NADR* (mol amino acid g DW⁻¹ d⁻¹) is the net amino acid decrease rate. We assumed that the amino acids from the protein degradation were immediately used for Gln synthesis. *NPDR* and *NADR* correspond to negative values of the rates of increase of proteins and amino acids, respectively (see Results).

Statistical analyses

All statistical analyses were conducted with STATVIEW (ver. 5.0 J; SAS, Cary, NC, USA). The difference between respiration rates in the absence and presence of CHI was determined using Student's *t*-test. For the other empirical data, we conducted two-way analysis of variance; the interaction between factors (petal age and growth temperature) was statistically significant in all analyses (*P* < 0.01).

RESULTS

Effects of growth temperature on growth and respiration rate in *P. × hybrida* petals

The DW of the *P. × hybrida* petal increased as the flower developed (Fig. 1a). At peak petal DW, the petal was fully

expanded. The maximum DW of the petal at 35 °C was half that of the petal at 20 °C. At any growth temperature, the water content (WC) of the petal on a fresh weight (FW) basis gradually increased from about 0.85 to above 0.90 and then steeply decreased (Fig. 1b). By the time the petals had attained their maximum WC, visible senescence or wilting had already occurred. Respiration rates on a DW basis consistently declined with time (Fig. 1c). In contrast, respiration rates on a whole-petal basis increased as the petals developed, peaked and then decreased (Fig. 1d).

Changes in the amounts, synthesis, and degradation of proteins and amino acids

The amounts of protein on a DW basis decreased with time (Fig. 2a) and did not differ markedly among growth temperatures. At 20 and 25 °C, the amino acid amounts on a DW basis also decreased with time (Fig. 2b) and were strongly correlated with the protein amounts (at 20 °C, *r* = 0.875, *P* < 0.0001; at 25 °C, *r* = 0.941, *P* < 0.0001). On the other hand, at 35 °C, the amount of amino acid started to increase just before full petal expansion (Fig. 2b) and by day 2 had reached 30% of the amount of protein. The amounts of proteins and amino acids are expressed on a whole-petal basis in Fig. 2c,d. At 35 °C, the amount of amino acid on a whole-petal basis peaked on day 2; this peak amount was much higher than the amounts at lower temperatures (Fig. 2d).

The rates of increase in the amounts of protein and amino acids in Fig. 3 were calculated from the changes in protein and amino acid amounts in Fig. 2c,d (see Materials and Methods). Negative values denote decreases in the amounts of protein or amino acids. The minimum

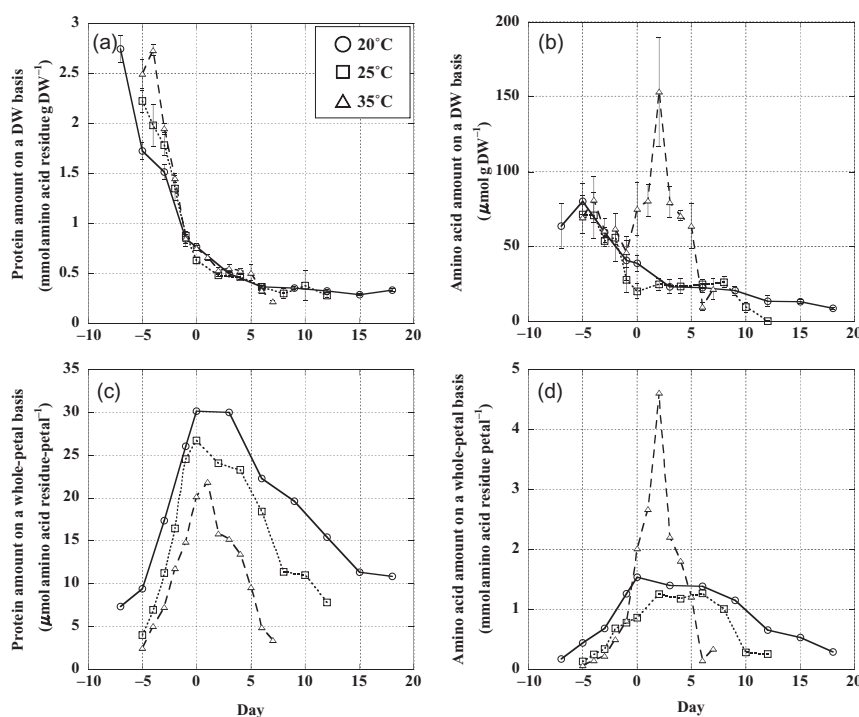


Figure 2. Changes in amounts of protein (a) and amino acids (b) on a dry weight (DW) basis, and in amounts of protein (c) and amino acids (d) on a whole-petal basis during development of *Petunia × hybrida* petals at each growth temperature (20 °C, circles; 25 °C, squares; 35 °C, triangles). Vertical bars represent standard error of the mean (SEM) [*n* ≥ 3 except on day -3 at 20 °C and days 6 and 7 at 35 °C (*n* = 2)]. Amounts of protein and amino acids on a whole-petal basis were calculated by multiplying the amounts of protein and amino acids on a DW basis by average values of DW.

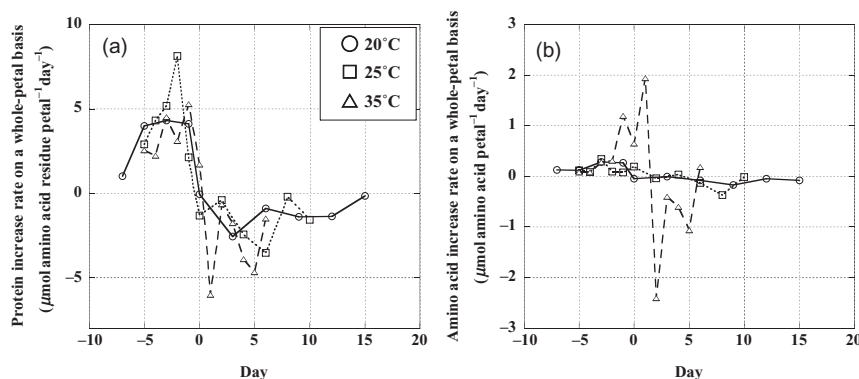


Figure 3. Changes in protein increase rate (a) and amino acid increase rate (b) on a whole-petal basis during development of *Petunia x hybrida* petals at each growth temperature (20 °C, circles; 25 °C, squares; 35 °C, triangles).

($NAER_{\min}$) and maximum ($NAER_{\max}$) net amino acid export rates were calculated using these negative values (Fig. 3a,b; see Materials and Methods).

Effects of CHI on respiration rates and respiratory costs of protein turnover

We measured the effects of CHI on respiration rates (Fig. 4). Only FW-based results are shown, because the buffer incubation interfered with the measurement of DW. At 20 °C, the effects of CHI on respiration rate were greater during the initial developmental stages. At 20 °C, the decrease in respiration rate in response to CHI ranged from 9.4% (day 0) to 35.5% (day -3) (Fig. 4a). At 25 °C, the decrease in response to CHI treatment ranged from 7.8% (day -1) to 32.8% (day -4) (Fig. 4b). In contrast, at 35 °C, marked CHI effects were also observed after petal unfolding (Fig. 4c). For example, CHI treatment decreased the respiration rate by 33.3% on day 2 (Fig. 4c).

The respiratory costs of protein turnover on a DW basis were calculated from the data in Fig. 4 and the theoretical values in Table 2. Maximum values were observed on day -3 at 20 °C and on day -4 at 25 and 35 °C (Fig. 5). At 35 °C, there was another peak on day 2. It is noteworthy that the cost at 35 °C was approximately twice those at the lower temperatures at most times during development.

N-utilization costs and respiratory costs during petal development

We summarized the changes in N-utilization costs during petal development on a DW basis (Fig. 6a–c) and on a whole-petal basis (Fig. 6d–f). The negative values in Fig. 5 were regarded as zero for the calculation of protein turnover costs. To calculate the costs in Fig. 6, the N source was assumed to be solely nitrate. Respiratory costs on a DW basis were used for comparisons among growth temperatures, because in this way petal size did not affect the cost estimations. In contrast, costs on a whole-petal basis were used to evaluate the contribution of each N-utilization cost to the respiratory cost at each growth temperature.

The costs of nitrate uptake/reduction ($\text{NO}_3^- \text{--} \text{NH}_4^+$), ammonium assimilation ($\text{NH}_4^+ \text{Gln}$), other amino acid synthesis (Gln--Amino), protein synthesis (Amino--Protein) and protein turnover were large before petal unfolding at all temperatures. The contribution of nitrate uptake/reduction ($\text{NO}_3^- \text{--} \text{NH}_4^+$) was largest. At 35 °C, the cost of protein turnover accounted for a large part of the cost after petal unfolding (Fig. 6c,f). The costs of amino acid export (Amino export) arose mainly after petal unfolding and accounted for a minimal fraction of the N-utilization cost.

By using the data in Fig. 6, we integrated the N-utilization costs on a DW basis (Fig. 7a) and on a whole-petal basis (Fig. 7b) throughout the petal's whole lifespan. The integrated N-utilization cost on a DW basis was greatest at 35 °C, and those at 20 and 25 °C were similar to each other (Fig. 7a). The integrated protein turnover cost on a DW basis at 35 °C was more than four times that at 25 °C (Fig. 7a). The integrated protein turnover cost on a whole-petal basis at 35 °C was the largest component of the N-utilization cost, followed by the nitrate uptake/reduction cost (Fig. 7b). The integrated cost of nitrate uptake/reduction was similar to the sum of costs of the subsequent three processes from ammonium to protein synthesis at all temperatures.

We calculated the integrated respiratory cost, including various processes other than N-utilization processes, by integrating the respiration rates throughout the petal's lifespan (Fig. 1c,d). The integrated respiratory cost on a DW basis at 35 °C was higher than those at lower temperatures (Fig. 7c), but, on a whole-petal basis, the integrated respiratory cost at 35 °C was lowest (Fig. 7d).

Costs of net amino acid export

We assumed that net amino acid export started on the day when the value of $NAER$ became positive. The integrated costs of amino acid export on a whole-petal basis during petal development are shown in Fig. 7b and Table 3. These costs accounted for the smallest fraction of the N-utilization cost at all temperatures (Fig. 7b). The costs of amino acid export per amino acid were estimated by dividing the integrated cost of amino acid export by the integrated amount of amino acid exported (Table 3).

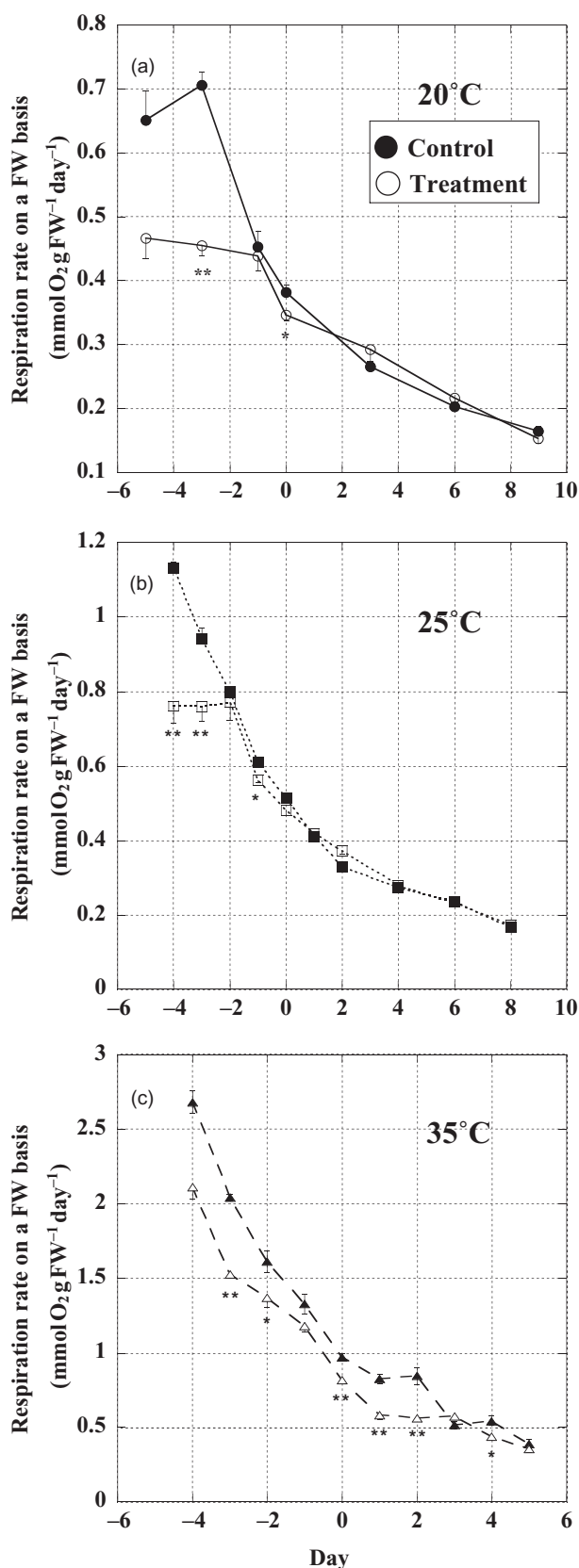


Figure 4. Effects of cycloheximide (CHI) treatment on respiration rates on a fresh weight (FW) basis of *Petunia x hybrida* petals during development at each growth temperature: (a) 20 °C, (b) 25 °C and (c) 35 °C. Open and closed symbols are the treatment and control, respectively. Vertical bars represent standard error of the mean (SEM) at each developmental stage ($n = 5$). * and ** denote statistically significant difference between treatment and control ($P < 0.05$ and $P < 0.01$, respectively) by Student's t -test.

Investment of respiratory costs and biomass in *P. x hybrida* petals

Growth temperature influenced the turnover of flowers and the petal lifespan. We estimated the respiratory cost and biomass of petal per shoot over 90 d to determine how growth temperature influences C investment in biomass at the shoot level. The intervals between flower bud initiations per shoot were 5.66 ± 0.28 d, 3.19 ± 0.11 d and 2.57 ± 0.13 d at 20, 25 and 35 °C, respectively (mean $\pm$ standard error of the mean, $n \geq 28$). The numbers of flowers on one shoot during 90 d were calculated to be 12, 23 and 31 at 20, 25 and 35 °C, assuming the petal lifespans to be 25, 18 and 12 d, respectively.

Investments of substrate C in the petal biomass and respiratory costs (N-utilization and residual respiratory costs) on a whole-petal basis decreased with increasing growth temperature (Fig. 8a). More than half the C investment in petals was consumed by respiration at any growth temperature. When expressed on a shoot basis, the C investment in petals was similar at 25 and 35 °C (Fig. 8b). On the other hand, the relative C investment in biomass (the C investment in biomass as a proportion of the C investment in biomass plus in respiration) was greatest at 25 °C (36%)

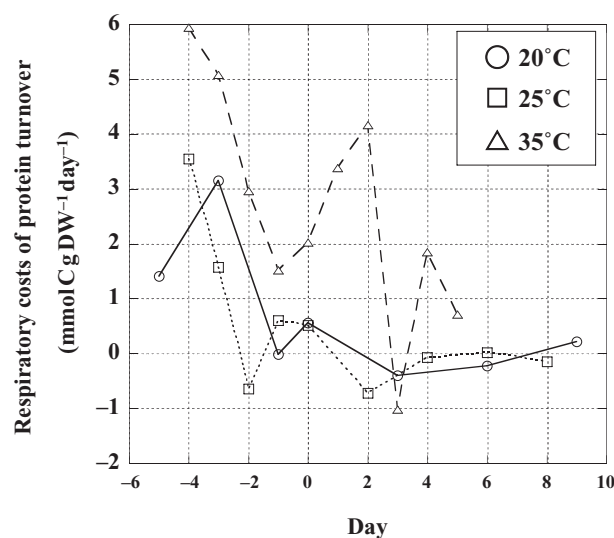


Figure 5. Changes in respiratory costs of protein turnover on a dry weight (DW) basis during development of *Petunia x hybrida* petals at each growth temperature (20 °C, circles; 25 °C, squares; 35 °C, triangles). Calculations of costs were based on Fig. 4 and Table 2.

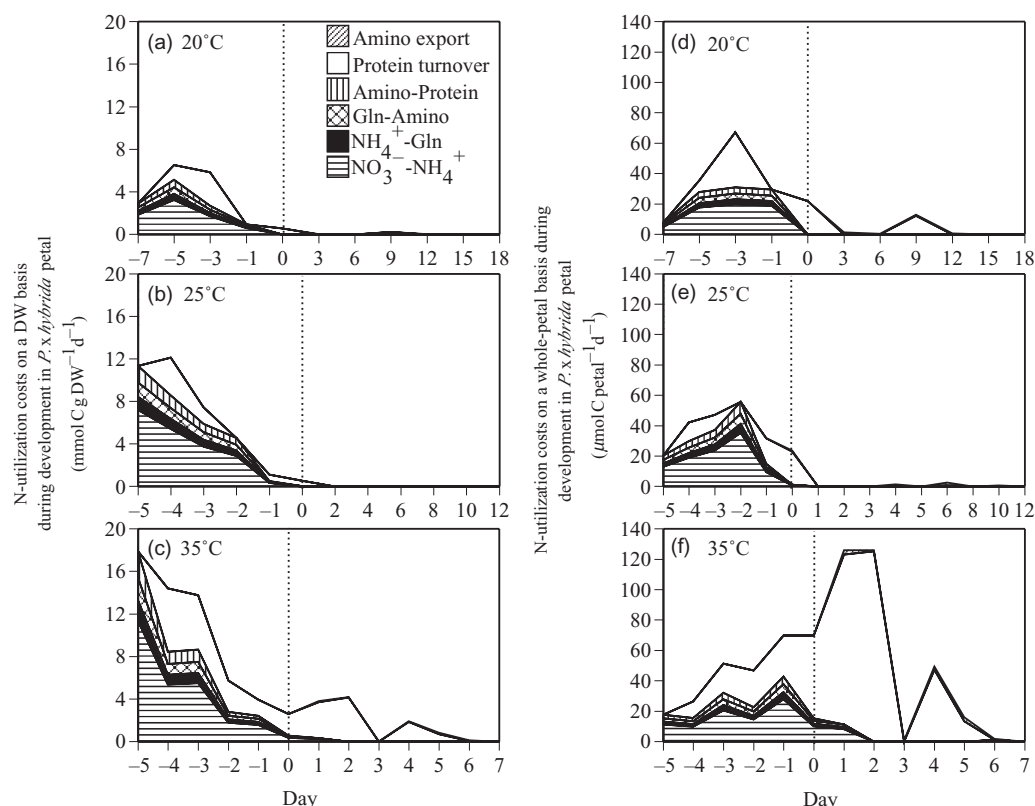


Figure 6. Changes in N-utilization costs on a dry weight (DW) basis during development of *Petunia × hybrida* petals at 20 °C (a), 25 °C (b) and 35 °C (c), and on a whole-petal basis at 20 °C (d), 25 °C (e) and 35 °C (f). ‘NO₃⁻-NH₄⁺’, ‘NH₄⁺-Gln’ and ‘Gln-Amino’ denote the respiratory costs of nitrate uptake and nitrate reduction, ammonium assimilation and other amino acid synthesis, respectively. We assumed that the elemental composition of amino acid residues was the average of the 20 amino acids (C_{5.35}H_{7.95}O_{1.45}N_{1.45}S_{0.10}). ‘Amino-protein’, ‘Amino export’ and ‘Protein turnover’ denote protein synthesis from amino acids, amino acid export and protein turnover, respectively. Nitrate was assumed to be the sole N source.

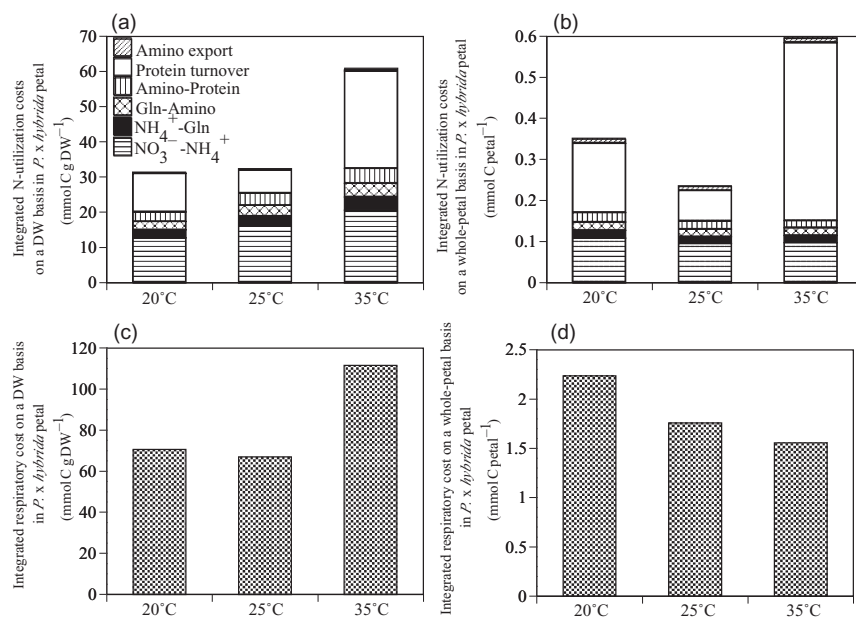


Figure 7. Integrated N-utilization costs on a dry weight (DW) basis (a) and on a whole-petal basis (b), and integrated respiratory costs on a DW basis (c) and on a whole-petal basis (d) during development in *Petunia × hybrida* petals at each growth temperature. These costs were integrated from the data in Fig. 6.

Table 3. Respiratory cost of amino acid export in *Petunia × hybrida* petals

		20 °C	25 °C	35 °C
Integrated amount of amino acids exported (μmol amino acid petal ⁻¹)	Min	10.6	8.8	10.8
	Max	19.8	16.8	18.1
Integrated amount of proteins degraded (μmol amino acid petal ⁻¹)	Min	19.0	16.0	14.7
	Max			
Integrated cost of amino acid export (μmol C petal ⁻¹)	Min	8.1	6.8	6.8
	Max	12.0	10.1	9.8
Cost of amino acid export per amino acid exported (mol C mol ⁻¹ amino acid)	Min	0.61	0.60	0.54
	Max	0.76	0.77	0.63
ATP cost of amino acid export per amino acid exported (mol ATP mol ⁻¹ amino acid)	Min	2.9	2.9	2.6
	Max	3.7	3.7	3.1

Data were calculated on the assumption that only glutamine (Gln) is transported via the apoplastic pathway from the petal. The cost of amino acid export per amino acid exported (Gln) was estimated by dividing the integrated cost of amino acid export by the integrated amounts of exported amino acid.

and lowest at 35 °C (25%). The integrated N-utilization cost accounted for 29% of the C investment at 35 °C, whereas the cost was 10% at 20 °C and 8% at 25 °C (Fig. 8a,b).

Changes in N sources among growth temperatures during petal development

The form of N source (nitrate, ammonium or Gln) greatly influenced the estimation of N-utilization costs. Particularly, the nitrate uptake/reduction cost was substantially affected (Fig. 7, Table 4). The integrated N-utilization cost was greatest at 35 °C, irrespective of the N source.

DISCUSSION

Protein turnover cost was high at high growth temperature

The integrated protein turnover costs on a whole-petal basis were 32 and 73% of the integrated N-utilization costs at 25 and 35 °C, respectively (Fig. 7b). The difference between 25 and 35 °C in terms of the integrated protein turnover cost on a DW basis accounted for 75% of the difference in the integrated N-utilization cost and 45% of the difference in the integrated respiratory cost (Fig. 7a,c). On the other hand, the differences between 20 and 25 °C in terms of these integrated costs were much smaller (Fig. 7a,c). Thus, we concluded that the high protein turnover cost at high temperature strongly increased the N-utilization cost and respiratory cost.

The lifespan of the petal decreased with increasing growth temperature (Fig. 1a,c). However, the integrated protein turnover cost on a whole-petal basis at 35 °C was greater than at the lower temperatures (Fig. 7b). Thus, the longer lifespan of the petal would not necessarily result in an increase in protein turnover cost. This result differed from the suggestion by Zerihun *et al.* (1998). Furthermore, the petal DW at 35 °C was considerably smaller than at the two lower temperatures (Fig. 1a). These results indicate that the protein turnover cost strongly depends on the growth temperature, rather than on the petal lifespan or size.

Effect of form of N source on estimation of integrated N-utilization cost

Whether the N source was assumed to be solely nitrate or solely ammonium, the integrated N-utilization costs at 20 and 25 °C were similar (Table 4). On the other hand, the integrated N-utilization cost was highest at 35 °C, irrespective of the N source. This was due to the expensive protein turnover cost at 35 °C (Fig. 7, Table 4). The proportion of the integrated protein turnover cost in the integrated N-utilization cost increased with the increase in level of reduction of N sources from nitrate to Gln.

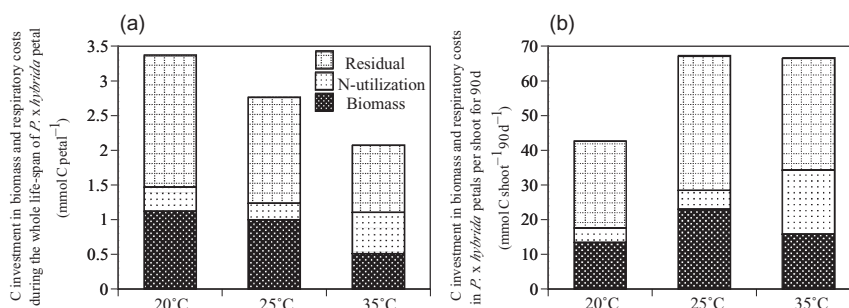


Figure 8. C investment in biomass and respiratory costs on a whole-petal basis during the whole petal lifespan (a), and C investment of petals on a shoot basis over 90 d (b) at each growth temperature. 'Biomass' and 'Residual' denote C investment in biomass and respiratory costs other than N-utilization costs, respectively. These values were calculated from the integrated N-utilization costs and integrated respiratory costs on a whole-petal basis in Fig. 7.

Table 4. Integrated N-utilization cost in *Petunia × hybrida* petal in the case of either nitrate, ammonium or glutamine (Gln) as the N source

	20 °C	25 °C	35 °C
Integrated N-utilization cost from nitrate (mmol C g DW ⁻¹)	31.1	32.1	60.5
Integrated N-utilization cost from ammonium (mmol C g DW ⁻¹)	20.2	18.3	42.8
Integrated N-utilization cost from Gln (mmol C g DW ⁻¹)	16.2	13.3	36.3

'Integrated N-utilization cost from ammonium' includes ammonium uptake cost. Specific cost of Gln uptake was assumed to be zero. DW, dry weight.

The integrated amounts of amino acids and proteins on a DW basis, which were estimated by integrating the protein and amino acid increase rates on a DW basis, were 3.0, 3.8 and 4.8 mmol amino acid residue g DW⁻¹ at 20, 25 and 35 °C, respectively. These amounts were not correlated with the data on integrated N-utilization costs (Table 4). This indicates that the integrated N-utilization cost depends on not only the amounts of proteins and amino acids synthesized, but more on the form of the N source and the protein turnover rate.

Respiratory cost of amino acid export and its significance

Surprisingly, the cost of amino acid export (2.6 to 3.7 mol ATP mol⁻¹ amino acid; Table 3) was almost equal to the cost of sucrose export through the apoplastic pathway (2.4 to 4.0 mol ATP mol⁻¹ sucrose) estimated by Bouma *et al.* (1994) and Noguchi *et al.* (2001). The respiratory cost per mole of amino acid synthesis was markedly higher than that per mole of sucrose synthesis – three times higher with ammonium as an N source and nine times with nitrate as a source. Moreover, in the *P. × hybrida* petal, 71 to 73% of N in the petal was recovered (unpublished data). This value was much higher than that of C recovery, ranging from 2 to 7% (unpublished data). Thus, the preferential export of amino acids would be economical on a whole-plant level.

Relationship among biomass, N-utilization cost and respiratory cost

Walker & Thornley (1977) reported that, in tomato fruits, there is an optimum temperature at which the ratio of C investment in biomass to C investment in biomass plus respiratory cost is maximized. We observed that not only this fraction, but also the amount of C investment in biomass per shoot, peaked at 25 °C (Figs 7c & 8a,b).

Little difference in the fraction of biomass C per petal was observed between 20 °C (33%) and 25 °C (36%) (Fig. 8a). C investment in the petals per shoot was lower at

20 °C (Fig. 8b). The amount of substrate C supply would be lower at 20 °C because of the decreased photosynthesis in the leaves or decreased rate of phloem loading to the petals from the leaves.

The fraction of biomass C at 35 °C (25%) was markedly lower than that at 25 °C (36%) (Fig. 8b). On the other hand, C investment per shoot at 35 °C was similar to that at 25 °C (Fig. 8b). Consequently, it is probable that, at 35 °C, the fraction of biomass C was limited by the increase in respiratory cost, and especially in the protein turnover cost. Loveys *et al.* (2002) examined the effect of growth temperature on biomass allocation and growth components in inherently fast- and slow-growing plant species. They observed that a greater portion of daily fixed C was respired at higher growth temperatures. Their result corresponds with our results. On the other hand, Marcelis & Baan Hofman-Eijer (1995) found no differences in the fraction of respiratory losses to C investment during the development of cucumber fruit at growth temperatures of 20, 25 or 30 °C. It appears that growth temperature above a certain limit causes an increase in the respiratory cost fraction. This would be mainly due to the increase in protein turnover cost.

Sensitivity analysis of alternative oxidase (AOX) and errors in evaluation of respiratory costs

Engagement of AOX in the mitochondrial electron transport chain is not coupled with H⁺ extrusion. This engagement decreases the ATP synthesis per substrate consumed – that is, the AOX engagement causes additional respiratory costs in terms of C (Noguchi *et al.* 2001). We recalculated the integrated N-utilization cost on a DW basis, assuming that 25 or 50% of electrons passed through AOX. The AOX engagement of 25% increased the integrated N-utilization costs by 10, 8 and 11% at 20, 25 and 35 °C, respectively. The engagement of 50% increased the costs by 25, 21 and 28% at 20, 25 and 35 °C, respectively. However, the magnitude of the increase depended on each N-utilization process. The costs of protein synthesis, protein turnover and amino acid export increased by 18 and 45% for AOX engagements of 25 and 50%, respectively. This is because these costs required only respiratory ATP. On the other hand, the nitrate uptake/reduction cost increased to lesser extents – by 5 and 13% for AOX engagements of 25 and 50%, respectively. This is because nitrate reduction requires large amounts of reducing equivalents. Consequently, the increase in AOX engagement increased the contribution of protein turnover cost to the N-utilization cost.

We measured cyanide-resistant respiration to estimate AOX engagement and estimated amounts of AOX protein by Western blotting at 25 and 35 °C before petal unfolding (data not shown). However, there was little difference in AOX engagement and amounts of AOX protein between these temperatures. Changes in growth temperature also caused only a small difference in AOX engagement in soybean cotyledons (Gonzalez-Meler *et al.* 1999). Moreover, Atkin, Zhang & Wiskich (2002) reported that the Q₁₀

of AOX is comparable to that of the cytochrome pathway under short-term changes in temperature in isolated mitochondria in soybean cotyledons. These observations support the hypothesis that AOX does not influence the magnitude of protein turnover cost at 35 °C. However, some previous studies have reported an increase in AOX engagement at low temperatures (McNulty & Cummins 1987; Stewart *et al.* 1990). To quantify precisely the respiratory costs under different growth temperatures, we should measure AOX engagement by means of the oxygen isotope technique (Ribas-Carbo, Robinson & Giles 2005).

We assumed here that RQ was always 1. The use of this constant value could result in some errors in cost estimation if the substrates were to vary during petal development or across temperatures. We found that total non-structural carbohydrate was abundant, on the order of mmol C g DW⁻¹ (data not shown). In the absence of active biosynthetic processes, therefore, RQ would be close to 1. On the other hand, active biosynthetic processes often increase RQ to more than 1 because of their high demand for reducing equivalents (Lambers *et al.* 1998). Thus, we would have underestimated the respiratory costs before petal unfolding. Differences in protein turnover cost among growth temperatures were observed mainly after petal unfolding. Consequently, we can conclude that RQ had little effect on the importance of protein turnover cost at 35 °C.

CONCLUSION

We evaluated the respiratory cost of each N-utilization process in *P. × hybrida* petals at three growth temperatures. Our results clearly revealed that, at high growth temperature, the high protein turnover cost accounted for the large portion of the increase in respiratory cost, and that at low temperature, the cost of nitrate uptake/reduction was high. Consequently, on the shoot basis, C investment in biomass was highest at 25 °C.

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REFERENCES

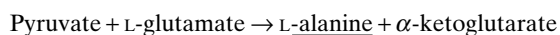
- Amthor J.S. (1989) *Respiration and Crop Productivity*. Springer-Verlag, New York, NY, USA.
- Amthor J.S. (1994) Respiration and carbon assimilate use. In *Physiology and Determination of Crop Yield* (eds K.J. Boote, J.M. Bennett, T.R. Sinclair & G.M. Paulsen), pp. 221–250. American Society of Agronomy, Madison, WI, USA.
- Amthor J.S. (2000) The McCree–de Wit–Penning de Vries–Thornley respiration paradigms: 30 years later. *Annals of Botany* **86**, 1–20.
- Atkin O.K., Zhang Q.S. & Wiskich J.T. (2002) Effect of temperature on rates of alternative and cytochrome pathway respiration and their relationship with the redox poise of the quinone pool. *Plant Physiology* **128**, 212–222.
- Atkin O.K., Scheurwater I. & Pons T.L. (2006) High thermal acclimation potential of both photosynthesis and respiration in two lowland *Plantago* species in contrast to an alpine congeneric. *Global Change Biology* **12**, 500–515.
- Barneix A.J., Cooper H.D., Stulen I. & Lambers H. (1988) Metabolism and translocation of nitrogen in two *Lolium perenne* populations with contrasting rates of mature leaf respiration and yield. *Physiologia Plantarum* **72**, 631–636.
- Boorer K.J. & Fischer W. (1997) Specificity and stoichiometry of the *Arabidopsis* H⁺/Amino Acid Transporter AAP5. *Journal of Biological Chemistry* **272**, 13040–13046.
- Bouma T.J., De Visser R., Jansen J.H.J.A., De Kock M.J., Van Leeuwen P.H. & Lambers H. (1994) Respiratory energy requirements and rate of protein turnover in vivo determined by the use of an inhibitor of protein synthesis and a probe to assess its effect. *Physiologia Plantarum* **92**, 585–594.
- Bunce J.A. (2004) A comparison of the effects of carbon dioxide concentration and temperature on respiration, translocation and nitrate reduction in darkened soybean leaves. *Annals of Botany* **93**, 665–669.
- Cannell M.G.R. & Thornley J.H.M. (2000) Modelling the components of plant respiration: some guiding principles. *Annals of Botany* **85**, 45–54.
- Dangl J.L., Dietrich R.A. & Thomas H. (2000) Senescence and programmed cell death. In *Biochemistry and Molecular Biology of Plants* (eds B.B. Buchanan, W. Gruissem & R.L. Jones), pp. 953–999. American Society of Plant Physiologists, Rockville, MD, USA.
- De Visser R., Spitters C.J.T. & Bouma T.J. (1992) Energy costs of protein turnover: theoretical calculation and experimental estimation from regression of respiration on protein concentration of full-grown leaves. In *Molecular, Biochemical and Physiological Aspects of Plant Respiration* (eds H. Lambers & L.H.W. van der Plas), pp. 493–508. SPB Academic Publishing, The Hague, the Netherlands.
- Ferguson D.L., Guikema J.A. & Paulsen G.M. (1990) Ubiquitin pool modulation and protein degradation in wheat roots during high temperature stress. *Plant Physiology* **92**, 740–746.
- Forbes P.J., Black K.E. & Hooker J.E. (1997) Temperature-induced alteration to root longevity in *Lolium perenne*. *Plant and Soil* **190**, 87–90.
- Gonzalez-Meler M.A., Ribas-Carbo M., Giles L. & Siedow J.N. (1999) The effect of growth and measurement temperature on the activity of the alternative respiratory pathway. *Plant Physiology* **120**, 765–772.
- Kikuzawa K. (1995) The basis for variation in leaf longevity of plants. *Plant Ecology* **121**, 89–100.
- Kronzucker H.J., Britto D.T., Davenport R.J. & Tester M. (2001) Ammonium toxicity and the real cost of transport. *Trends in Plant Science* **6**, 335–337.
- Kurimoto K., Day D.A., Lambers H. & Noguchi K. (2004) Effect of respiratory homeostasis on plant growth in cultivars of wheat and rice. *Plant, Cell & Environment* **27**, 853–862.
- Lambers H., Chapin F.S. & Pons T.L. (1998) *Plant Physiological Ecology*. Springer, New York, USA.
- Loveys B.R., Scheurwater I., Pons T.L., Fitter A.H. & Atkin O.K. (2002) Growth temperature influences the underlying components of relative growth rate: an investigation using inherently fast- and slow-growing plant species. *Plant, Cell & Environment* **25**, 975–987.
- Mae T., Makino A. & Ohira K. (1983) Changes in the amounts of

- ribulose biphosphate carboxylase synthesized and degraded during the life span of rice leaf (*Oryza sativa* L.). *Plant and Cell Physiology* **24**, 1079–1086.
- Marcelis L.F.M. & Baan Hofman-Eijer L.R. (1995) Growth and maintenance respiratory costs of cucumber fruits as affected by temperature, and ontogeny and size of the fruits. *Physiologia Plantarum* **93**, 484–492.
- McDermitt D.K. & Loomis R.S. (1981) Elemental composition of biomass and its relation to energy content, growth efficiency, and growth yield. *Annals of Botany* **48**, 275–290.
- McNulty A.M. & Cummins W.R. (1987) The relationship between respiration and temperature in leaves of the arctic plant *Saxifraga cernua*. *Plant, Cell & Environment* **10**, 319–325.
- Noguchi K., Go C.-S., Miyazawa S.-I., Terashima I., Ueda S. & Yoshinari T. (2001) Costs of protein turnover and carbohydrate export in leaves of sun and shade species. *Australian Journal of Plant Physiology* **28**, 37–47.
- Ono K., Terashima I. & Watanabe A. (1996) Interaction between nitrogen deficit of a plant and nitrogen content in the old leaves. *Plant and Cell Physiology* **37**, 1083–1089.
- Penning de Vries F.W.T. (1975) The costs of maintenance processes in plant cells. *Annals of Botany* **39**, 77–92.
- Penning de Vries F.W.T., Brunsting A.H.M. & van Laar H.H. (1974) Products, requirements and efficiency of biosynthesis: a quantitative approach. *Journal of Theoretical Biology* **45**, 339–377.
- Peterson G.L. (1977) A simplification of the protein assay method of Lowry *et al.* which is more generally applicable. *Analytical Biochemistry* **83**, 346–356.
- Ribas-Carbo M., Robinson S.A. & Giles L. (2005) The application of the oxygen-isotope technique to assess respiratory pathway partitioning. In *Plant Respiration: From Cell to Ecosystem* (eds H. Lambers & M. Ribas-Carbo), pp. 31–42. Springer-Verlag, Dordrecht, the Netherlands.
- Rosen H. (1957) A modified ninhydrin colorimetric analysis for amino acids. *Archives of Biochemistry and Biophysics* **67**, 10–15.
- Stewart C.R., Martin B.A., Reding, L. & Cerwicks S. (1990) Respiration and alternative oxidase in corn seedlings during germination at different temperatures. *Plant Physiology* **92**, 755–760.
- Van Iersel M.W. (2003) Short-term temperature change affects the carbon exchange characteristics and growth of four bedding plant species. *Journal of the American Society for Horticultural Science* **128**, 100–106.
- Van Iersel M.W. (2006) Respiratory Q10 of marigold (*Tagetes patula*) in response to long-term temperature differences and its relationship to growth and maintenance respiration. *Physiologia Plantarum* **128**, 289–301.
- Walker A.J. & Thornley J.H.M. (1977) The tomato fruit: import, growth, respiration and carbon metabolism at different fruit sizes and temperatures. *Annals of Botany* **41**, 977–985.
- Williams K., Percival F., Merino J. & Mooney H.A. (1987) Estimation of tissue construction cost from heat of combustion and organic nitrogen content. *Plant, Cell & Environment* **10**, 725–734.
- Zerihun A., McKenzie B.A. & Morton J.D. (1998) Photosynthate costs associated with the utilization of different nitrogen-forms: influence on the carbon balance of plants and shoot-root biomass partitioning. *New Phytologist* **138**, 1–11.
- Zhang P., Foerster H., Tissier C.P., Mueller L., Paley S., Karp P.D. & Rhee S.Y. (2005) MetaCyc and AraCyc metabolic pathway databases for plant research. *Plant Physiology* **138**, 27–37.

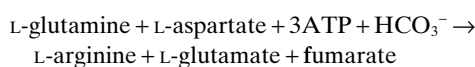
APPENDIX

Twenty pathways of amino acid synthesis for calculation of costs for amino acid and protein syntheses are shown. In the calculation of costs, substrates for amino acid synthesis in eqns 1–20 were derived from sucrose, in accordance with eqns 21–43 that follow. Each pathway and its name were based on the AraCyc metabolic map (<http://arabidopsis.org/tools/aracyc/index.jsp>). It was assumed that ATP incorporated into L-histidine in Reaction (9) was derived from *de novo* ATP synthesized in Reaction (33).

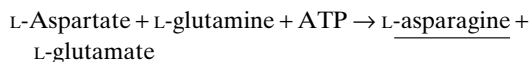
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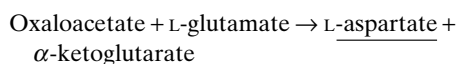
(2) Arginine biosynthesis II (acetyl cycle)



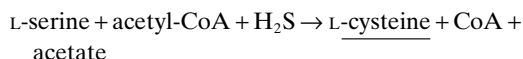
(3) Asparagine biosynthesis



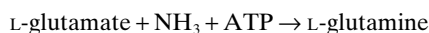
(4) Aspartate biosynthesis and degradation



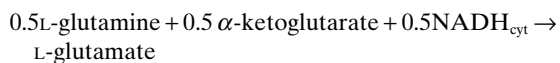
(5) Cysteine biosynthesis



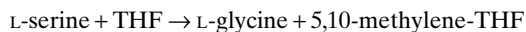
(6) Glutamine biosynthesis



(7) Glutamate biosynthesis II (NADH-glutamate synthase)



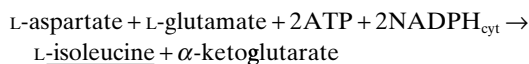
(8) Glycine biosynthesis



(9) Histidine biosynthesis



(10) Super-pathway of lysine, threonine, isoleucine and methionine biosynthesis



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- (11) Super-pathway of leucine, valine and isoleucine biosynthesis

$$\text{L-glutamate} + \text{acetyl-CoA} + 2\text{pyruvate} \rightarrow \text{L-leucine} + \alpha\text{-ketoglutarate} + \text{CoA} + 2\text{CO}_2$$
- (12) Super-pathway of lysine, threonine, isoleucine and methionine biosynthesis

$$\text{L-aspartate} + \text{L-glutamate} + \text{pyruvate} + \text{succinyl-CoA} + \text{ATP} + 2\text{NADPH}_{\text{cyt}} \rightarrow \text{L-lysine} + \alpha\text{-ketoglutarate} + \text{succinate} + \text{CoA} + \text{CO}_2$$
- (13) Super-pathway of lysine, threonine, isoleucine and methionine biosynthesis

$$\text{L-aspartate} + \text{L-cysteine} + 2\text{ATP} + 2\text{NADPH}_{\text{cyt}} + 5\text{-methyl-THF} \rightarrow \text{L-methionine} + \text{THF} + \text{pyruvate} + \text{NH}_3$$
- (14) Super-pathway of phenylalanine, tyrosine and tryptophan biosynthesis

$$\text{D-erythrose-4-phosphate} + \text{L-glutamate} + 2\text{PEP} + \text{ATP} + \text{NADPH}_{\text{cyt}} \rightarrow \text{L-phenylalanine} + \alpha\text{-ketoglutarate} + \text{CO}_2$$
- (15) Proline biosynthesis

$$\alpha\text{-ketoglutarate} + \text{L-ornithine} + \text{ATP} + 2\text{NADPH}_{\text{cyt}} \rightarrow \text{L-proline}$$
- (16) Serine biosynthesis

$$3\text{-phosphoglycerate} + \text{L-glutamate} \rightarrow \text{L-serine} + \alpha\text{-ketoglutarate} + \text{NADH}_{\text{cyt}}$$
- (17) Super-pathway of lysine, threonine, isoleucine and methionine biosynthesis

$$\text{L-aspartate} + 2\text{ATP} + 2\text{NADPH}_{\text{cyt}} \rightarrow \text{L-threonine}$$
- (18) Super-pathway of phenylalanine, tyrosine and tryptophan biosynthesis

$$\text{D-erythrose-4-phosphate} + \text{L-glutamine} + \text{L-serine} + 2\text{PEP} + \text{PRPP} + \text{ATP} + \text{NADPH}_{\text{cyt}} \rightarrow \text{L-tryptophan} + \text{L-glutamate} + \text{pyruvate} + \text{D-glyceraldehyde-3-phosphate} + \text{CO}_2$$
- (19) Super-pathway of phenylalanine, tyrosine and tryptophan biosynthesis

$$\text{D-erythrose-4-phosphate} + \text{L-glutamate} + 2\text{PEP} + \text{ATP} \rightarrow \text{L-tyrosine} + \alpha\text{-ketoglutarate} + \text{CO}_2$$
- (20) Super-pathway of leucine, valine, and isoleucine biosynthesis

$$2\text{pyruvate} + \text{L-glutamate} + \text{NADPH}_{\text{cyt}} \rightarrow \text{L-valine} + \alpha\text{-ketoglutarate} + \text{CO}_2$$
- (21) Sucrose degradation (by invertase) and Glycolysis II

$$0.25\text{sucrose} \rightarrow \text{pyruvate} + \text{ATP} + \text{NADH}_{\text{cyt}}$$
- (22) Arginine biosynthesis II (acetyl cycle)

$$2\text{L-glutamate} + \text{acetyl-CoA} + \text{ATP} + \text{NADPH}_{\text{cyt}} \rightarrow \text{L-ornithine} + \alpha\text{-ketoglutarate}_{\text{cyt}} + \text{CoA}$$
- (23) Sucrose degradation (by invertase)

$$\text{Sucrose} + 2\text{ATP} \rightarrow 2(\text{D-glucose-6-phosphate})$$
- (24) PRPP biosynthesis

$$0.5\text{sucrose} + 2\text{ATP} \rightarrow \text{PRPP} + \text{CO}_2 + 2\text{NADPH}_{\text{cyt}}$$
- (25) Super-pathway of oxidative and non-oxidative branches of pentose phosphate pathway

$$0.5\text{sucrose} + \text{ATP} \rightarrow \text{D-erythrose-4-phosphate} + 4\text{NADPH}_{\text{cyt}} + 2\text{CO}_2$$
- (26) Sucrose degradation (by invertase) and Glycolysis II

$$0.25\text{sucrose} \rightarrow 3\text{-phosphoglycerate} + \text{NADH}_{\text{cyt}}$$
- (27) Glycolysis II

$$0.25\text{sucrose} \rightarrow \text{PEP} + \text{NADH}_{\text{cyt}}$$
- (28) Super-pathway of glycolysis, pyruvate dehydrogenase, TCA and glyoxylate bypass

$$0.25\text{sucrose} + \text{CoA} \rightarrow \text{acetyl-CoA} + \text{ATP} + \text{NADH}_{\text{cyt}} + \text{NADH}_{\text{mit}} + \text{CO}_2$$
- (29) Super-pathway of glycolysis, pyruvate dehydrogenase, TCA and glyoxylate bypass

$$\text{Pyruvate} + \text{oxaloacetate} + \text{CoA} \rightarrow \text{succinyl-CoA} + 3\text{NADH}_{\text{mit}} + 3\text{CO}_2$$
- (30) Super-pathway of glycolysis, pyruvate dehydrogenase, TCA and glyoxylate bypass

$$0.5\text{sucrose} \rightarrow \alpha\text{-ketoglutarate} + 2\text{NADH}_{\text{mit}} + 2\text{NADH}_{\text{cyt}} + \text{ATP} + \text{CO}_2$$
- (31) Aerobic respiration and reaction 4.1.1.38

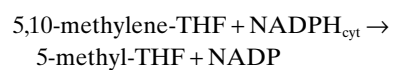
$$0.25\text{sucrose} + \text{CO}_2 \rightarrow \text{oxaloacetate} + \text{NADH}_{\text{cyt}}$$
- (32) Sulphate assimilation

$$\text{SO}_4^{2-} + 4\text{NADPH}_{\text{cyt}} + \text{ATP} \rightarrow \text{H}_2\text{S}$$
- (33) *De novo* biosynthesis of purine nucleotides I and formyl-THF biosynthesis

$$0.02\text{sucrose} + \text{AICAR} + \text{formate} + 2\text{ATP} + \text{aspartate} \rightarrow \text{fumarate} + {}_{\text{de}}\text{ATP} + 0.21\text{CO}_2$$
- (34) Carbon monoxide dehydrogenase pathway

$$\text{NADP}^+ + \text{formate} \rightarrow \text{NADPH}_{\text{cyt}} + \text{CO}_2$$

(35) formyl-THF biosynthesis



where: $_{\text{de}}\text{ATP}$ = ATP *de novo* synthesized

AICAR = 5-amino-4-imidazolecarboxamide
ribose

PRPP = phosphoribosylpyrophosphate

THF = tetrahydrofolate