Metabolic aspects of transpiration of leaves. The effect of the reduction of nitrates on the process of transpiration in *Lycopersicum esculentum* and *Chenopodium album*

J. BUCZEK

INTRODUCTION

In the former paper (Buczek, 1963) a relationship between transpiration and respiration in cut off leaves of the investigated plants has been established. It has been found that the differences in the amount of transpired water depended on previous infiltration of leaves either with nitrates or with ammonium salts. Namely, it appeared that in leaves infiltrated with nitrate ions the transpiration was distinctly increased, being either slightly inhibited or remaining unchanged in leaves infiltrated with ammonium ions. The results obtained suggest that the transpiration depends on metabolic process occurring in the tissues of leaves.

The above hypothesis was the starting point for the experiments described in the present paper, consisting in measurements of transpiration in tomato (*Lycopersicum esculentum*) and melweed (*Chenopodium album*) seedlings in presence of either nitrates or ammonium salts. The choice of the experimental plants was dictated by the fact that tomato leaves contain highly active nitrate reductase (Sanderson and Cocking 1964), while in *Chenopodium album* such an enzymatic system probably does not exist (Marthaler 1937). The purpose of the present paper was to investigate the role of certain processes of nitrogen metabolism in the process of transpiration.

MATERIAL AND METHODS

Tomato seedlings of the variety 'Best of all' and seedlings of *Chenopodium album*, sprouted on blotting paper, were placed in one-litre glass jars filled with nutrient solutions prepared with bi-distilled water and containing the following salts (g/l): $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ — 0,321; KCl — 0,311; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ — 0,264; KH_2PO_4 — 0,251; NH_4NO_3 — 0,214; Fe-citrate — 0,05. The basis solution was supplemented with the following microelements (mg/l): H_3BO_3 — 0,5; $\text{MnSO}_4 \cdot 5\text{H}_2\text{O}$ — 0,05; $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ — 0,05; $\text{H}_2\text{MoO}_4 \cdot 4\text{H}_2\text{O}$ — 0,05. The pH equal to 6,8 was, if necessary, adjusted to this value by means of 0,1 N NaOH or HCl. The plants were grown in a greenhouse. After two weeks they were either transferred to an identical fresh

medium (non-starved) or placed for one week in a medium without nitrogen (starved). In both cases the media were supplemented with the above mentioned amounts of microelements. When the seedlings in water cultures were three-weeks-old the roots of both starved (kept for two weeks in full medium and for one week in medium without nitrogen) and non-starved (kept for three weeks in full medium) seedlings were cut off in the root-neck region, and the rootless seedlings were placed in potometers filled with suitable media. The potometers were first weighed and then placed in a thermostate under constant thermic (25°C) and relative air humidity (ca. 70%) conditions. The intensity of electric light within the thermostat was 15000 Lx. After 24 hours the potometers were weighed again and the difference between the initial and final weights yielded the amount of water transpired by the seedlings. The results were computed for 100 cm^2 of leaf surface area.

The potometers were filled with the following media (g/l) given bidistilled water:

Medium without nitrogen, $-\text{N}$:	$\text{CaSO}_4 \cdot 2\text{H}_2\text{O} - 0.321$; $\text{KCl} - 0.311$; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O} - 0.246$; $\text{KH}_2\text{PO}_4 - 0.251$; $\text{Fe-citrate} - 0.05$.
Medium with nitrate ions, $+\text{NO}_3$;	$\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O} - 0.44$; $\text{KCl} - 0.259$; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O} - 0.246$; $\text{KH}_2\text{PO}_4 - 0.251$; $\text{KNO}_3 - 0.165$; $\text{Fe-citrate} - 0.05$,
Medium with ammonium ions, $+\text{NH}_4$;	$\text{CaSO}_4 \cdot 2\text{H}_2\text{O} - 0.321$; $\text{KCl} - 0.448$; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O} - 0.246$; $\text{NH}_4\text{H}_2\text{PO}_4 -$ 0.206 ; $(\text{NH}_4)_2\text{SO}_4 - 0.228$; $\text{Fe-citrate} -$ 0.05 .

The basic medium was supplemented with the above mentioned amounts of microelements. Possible changes in pH were adjusted to 6,8 by means of 0,1 N NaOH or HCl. This value remained constant throughout the 24-hour experiment.

In some potometers the media were supplemented with the following substances: 10^{-3}M L(+) cysteine (Loba-Chemie, Vien-Fischamed), $4 \times 10^{-6}\text{M}$ reduced forms of diphosphopyridine nucleotide — NADH (Reanal-Hungary), 10^{-3}M NaVO_3 (computed with respect to pure vanadium) (FOCH, Gliwice, Poland).

Within each combination 8 replications were carried out, each experiment being moreover, repeated at least twice. Statistical analysis was made by Student's t test for the confidence level = 0,95, $n_1 + n_2 - 2$ being the degree of freedom. The significance of differences was assumed to be statistically proved if the computed $t \geq 2,14$.

RESULTS

In the cut off tomato shoots placed in a medium containing nitrates transpiration was increased by about 30% (Table 1) as compared with the control (medium without nitrogen). This increased loss of water was observed both in previously starved and non-starved plants, being somewhat greater in the starved ones. No simi-

Table 1

Effect of NO_3^- and NH_4^- ions on transpiration in *Lycopersicum esculentum* and *Chenopodium album*

Plant	Medium	Starved			Non-starved		
		$\text{H}_2\text{O g per 100 cm}^2$	t	increase + inhibition — %	$\text{H}_2\text{O g per 100 cm}^2$	t	increase + inhibition — %
<i>Lycopersicum esculentum</i>	—N	4.09			2.11		
	+ NO_3^-	5.60	3.61	+36	2.80	3.99	+33
	+ NH_4^-	4.06	0.55	0	2.20	0.49	+5
<i>Chenopodium album</i>	—N	3.48			3.32		
	+ NO_3^-	3.48	0.0	0	3.31	0.02	—1
	+ NH_4^-	3.29	0.35	—5	3.64	0.47	+9

The experiment lasted for 24 hours.

$t \geq 2.14$

Table 2

Effect of NO_3^- and NH_4^- ions and cysteine on transpiration of *Lycopersicum esculentum* and *Chenopodium album*

Plant	Medium	Starved			Non-starved		
		$\text{H}_2\text{O g per 100 cm}^2$	t	increase + inhibition — %	$\text{H}_2\text{O g per 100 cm}^2$	t	increase + inhibition — %
<i>Lycopersicum esculentum</i>	—N						
	+cysteine 10^{-3}M	4.68			1.60		
	+ NO_3^-						
	+cysteine 10^{-3}M	6.45	5.48	+38	2.15	2.63	+34
	+ NH_4^-						
<i>Chenopodium album</i>	+cysteine 10^{-3}M	4.88	0.73	+4	1.58	0.23	—1
	—N						
	+cysteine 10^{-3}M	5.56			4.51		
	+ NO_3^-						
	+cysteine 10^{-3}M	5.46	0.31	—2	4.79	0.33	+6
<i>Chenopodium album</i>	+ NH_4^-						
	+cysteine 10^{-3}M	5.79	0.67	+4	4.21	0.38	—6

Table 3

Effect of NO_3^- — and NH_4^- —ions, cysteine and NADH on transpiration of *Lycopersicum esculentum* and *Chenopodium album*

Plant	Medium	Starved			Non-starved		
		H_2O g per 100 cm^2	t	increase + inhibition — %	H_2O g per 100 cm^2	t	increase + inhibition — %
<i>Lycopersicum esculentum</i>	i —N +cysteine 10^{-3}M +NADH $4 \times 10^{-6}\text{M}$	2.60			3.31		
	+ NO_3 +cysteine 10^{-3}M +NADH $4 \times 10^{-6}\text{M}$	4.32	12.43	+66	4.64	2.90	+40
	+ NH_4 +cysteine 10^{-3}M +NADH $4 \times 10^{-6}\text{M}$	2.82	0.74	+ 8	3.40	0.38	+ 6
<i>Chenopodium album</i>	—N +cysteine 10^{-3}M +NADH $4 \times 10^{-6}\text{M}$	3.90			4.50		
	+ NO_3 +cysteine 10^{-3}M +NADH $4 \times 10^{-6}\text{M}$	4.01	0.75	+ 5	4.82	0.42	+7
	NH_4 +cysteine 10^{-3}M +NADH $4 \times 10^{-6}\text{M}$	3.98	1.12	+ 2	3.78	1.48	—16

$t \geq 2,14$

lar relationship has been stated in shoots growing on medium containing ammonium salts. Ammonium salts produced no essential change in transpiration both in starved and non-starved plants.

On the other hand, in *Chenopodium album* (Table 1) neither previous starvation nor the kind of applied medium caused no essential change in the process of transpiration.

Another series of experiments (Table 2) was conducted under identical conditions, except that before the cut off shoots were placed in potometers the media had been supplemented with cysteine. In these experiments, as in the former ones, the medium with nitrates caused a pronounced increase in transpiration both in starved and non-starved tomato seedlings. In + NO_3 combination the addition of cysteine slightly increased the transpiration. Yet, a distinct increase of transpiration was observed in previously starved tomato seedlings if cysteine was given together with NADH (Table 3). In this combination the loss of transpired water was by 66% greater than in the control, and almost 20% greater than in the combinations: + NO_3 and + NO_3 supplemented with cysteine.

Both in starved and non-starved tomato shoots growing in potometers on media containing ammonium salts supplemented with cysteine (Table 2) or cysteine with NADH (Table 3), the transpiration did not differ from that in the control.

As in the former experiment, no essential differences in the intensity of transpiration were observed in *Chenopodium album* within all combinations (Tables 2 and 3). The differences observed were slight, nonessential and not proved statistically.

Table 4

Effect of NO_3^- and NH_4^+ ions on transpiration of *Lycopersicum esculentum* and *Chenopodium album*

Plants	Medium	Starved			Non-starved		
		H_2O g per 100 cm^2	t	increase + inhibition — %	H_2O g per 100 cm^2	t	increase + inhibition — %
<i>Lycopersicum esculentum</i>	—N +V 10^{-3}M	2.59			2.44		
	+ NO_3^- +V 10^{-3}M	2.56	0.11	—1	2.24	0.88	—8
	+ NH_4^+ +V 10^{-3}M	2.42	0.57	—7	2.30	0.39	—6
	—N +V 10^{-3}M				1.90		
<i>Chenopodium album</i>	+ NO_3^- +V 10^{-3}M				1.82	0.72	—4
	+ NH_4^+ +V 10^{-3}M				1.85	0.62	—2

Nutrient solution in potometers were not supplemented with molybdenum.

$t \geq 2.14$

Table 4 contains the results of experiments conducted on media supplemented with vanadium. Both starved and non-starved plants were grown in water cultures containing a normal dose of Mo ions. In the nutrient solution for the potometers, Mo ions were replaced by V ions given in the form of NaVO_3 .

The addition of vanadium ions has removed the positive effect of nitrates on the process of transpiration, that was observed in tomato shoots in former experiments. The results obtained for the combination + NO_3^- supplemented with vanadium did not differ from those obtained for control plants.

It may be affirmed in general that the addition of vanadium reduced the absolute amount of transpired water both in tomato and *Chenopodium album* shoots and, as it follows from Table 4, removed the positive effect of NO_3^- ions on the process of transpiration, nevertheless, it did not inhibit entirely the transpiration itself.

DISCUSSION

The above described experiments show that the loss of water in transpiring leaves may be actively controlled by some processes associated with metabolism. Thus, the reduction of nitrates, if it takes place in leaf tissues, may cause an increased loss of water in the process of transpiration.

From the above experiments it follows that the transpiration in tomato plants is increased in the presence of NO_3 ions, this relationship being not observed in *Chenopodium album*. Considering the fact that tomato leaves contain nitrate reductase of high activity (Sanderson and Cocking 1964) and that *Chenopodium album* probably does not (Marthaler 1937) it may be inferred that the increased transpiration is not so much related to the presence of NO_3 ions as to their reduction.

This hypothesis seems to be reasonable enough since it is well known that both nitrates and ammonium salts are equally used by tomato plants as a source of nitrogen, while *Chenopodium album* can assimilate only ammonium ions. Although this species is able to accumulate nitrates, nevertheless, it cannot reduce them (Marthaler 1937, Street and Sheat 1958). Consequently, the increased loss of water observed in tomato shoots growing on a medium supplemented with nitrates may be connected with the reduction of NO_3 ions. This conclusion has been confirmed by our experiments with cysteine and NADH. In presence of those substances added to the combination $+\text{NO}_3$ one observes that in tomato leaves of previously starved plants the transpiration distinctly increased. The combined effect of cysteine and NADH stated in our experiment will be clear if we consider the results of investigations of Sanderson and Cocking (1964) on the activity of nitrate reductase in tomato leaves. According to these authors, cysteine increases the activity of nitrate reductase, NADH being the true donor of hydrogen ions for this enzyme. In the light of the above facts, it may be supposed that addition of cysteine together with NADH brings about an increased intensity of nitrate reductase, and consequently, an increased transpiration.

This relationship could be distinctly seen in starved tomato plants. In non-starved plants this effect was not so pronounced. This is understandable, since in nonstarved tomato plants a certain amount of nitrates could be present both in plants growing on control medium as well as on medium containing ammonium ions. Consequently, the increased excretion of water in the process of transpiration observed in control plants could result from the reduction of NO_3 ions.

The experiments conducted on *Chenopodium album* show that transpiration in these plants does not depend on the form of nitrogen present in the medium. The addition of cysteine or NADH had no effect on the intensity of transpiration. This is easy to understand since, as we know from the above quoted authors, *Chenopodium album* has the ability to accumulate a certain amount of nitrate ions but is not capable of reducing. Therefore, the results of experiments conducted on *Chenopodium album* seem to confirm the hypothesis that the intensity of transpiration does not depend on the total metabolism of nitrogen but merely on the reduction of nitrates.

The hypothesis that the reduction of nitrates is responsible for the increased transpiration has been confirmed by our experiments conducted with vanadium. The addition of vanadium ions to the potometers containing NO_3 ions produced a decreased transpiration in tomato shoots. In these plants transpiration was equal to that stated in the control. This fact can be explained by two reasons: 1° according to Arnon and Stout (1939) vanadium cannot replace Mo ions in tomato plants, since these substances do not play the same role. 2° vanadium ions are considered to be a specific inhibitor of nitrate reductase. This last hypothesis has been confirmed by Spencer (1959) who proved that vanadium ions actually are a specific and efficient inhibitor of nitrate reductase in wheat embryos. Thus, the deficiency of Mo ions as well as the inhibitory properties of vanadium ions might eventually result in inhibition of the activity of nitrate reductase in tomato leaves, and this in turn, would exert a specific effect on transpiration.

In the light of the above facts, it seems that, while considering the process of transpiration, the contribution of some metabolic processes capable of regulating the loss of water in leaves should not be entirely neglected. It seems probable that in the active outflux of water from leaf cells into intracellular spaces, metabolic processes should be taken into account.

CONCLUSIONS

1. The uptake of nitrate ions by tomato shoots results in an increased transpiration.
2. Activators of nitrate reductase such as cysteine and NADH cause an increased transpiration in tomato plants taking up nitrate ions.
3. Nitrate ions and the above mentioned activators of nitrate reductase do not affect the intensity of transpiration in *Chenopodium album*.
4. Reduction of NO_3 ions in shoots (leaves) brings about an increased transpiration.

*Department of Plant Physiology,
University, Wrocław,
Kanonia 6/8.*

LITERATURE

- Arnon D. J. and P. R. Stout., 1939, Molybdenum as an essential element for higher plants, *Plant Physiol.* 14: 595—601.
- Buczek J., 1963, On the metabolic aspects of the transpiration of leaves, *Acta Soc. Bot. Pol.* 32: 511—530.
- Marthaler H., 1937, Die Stickstoffernährung der Ruderalpflanzen, *Jahrb. f. wiss. Bot.* 85: 76—106.
- Sanderson G. W. and E. C. Cocking., 1964, Enzymatic assimilation of nitrate in tomato plants I. Reduction of nitrate to nitrite, *Plant Physiol.* 39: 416—422.

- Spencer D., 1959, A DPNH-specific nitrate reductase from germinating wheat. *Australian J. Biol. Sci.* 12: 181—189.
- Street H. E. and D. E. G. Sheat, 1958, The absorption and availability of nitrate and ammonia, In *Handbuch der Pflanzenphysiologie* (W. Ruhland, ed.), Vol. VIII p. 150—165, Springer, Berlin.

Metaboliczny aspekt transpiracji liści. Wpływ redukcji azotanów na proces transpiracji u Lycopersicum esculentum i Chenopodium album

STRESZCZENIE

Badano wpływ jonów NO_3 względnie NH_4 oraz wpływ tych jonów z dodatkiem cysteiny, cysteiny + NADH, względnie z dodatkiem jonów wanadu, na intensywność transpiracji u odciętych od korzeni pędów pomidorów odmiany 'Best of all' i lebiody białej. Wyboru roślin testowych dokonano z uwagi na fakt, iż liście pomidorów posiadają bardzo aktywną reduktazę azotanową (Sanderson i Cocking 1964), natomiast lebioda biała prawdopodobnie nie posiada tego układu enzymatycznego (Marthaler 1937). Badania przeprowadzano w stałych warunkach temperatury i wilgotności względnej powietrza oraz oświetlenia.

Transpirację oznaczano wagowo przeliczając ilość wytranspirowanej wody na 100 cm^2 powierzchni liści. Stwierdzono, że: 1. Pobieranie przez pomidory jonów azotanowych wpływa na zwiększenie transpiracji. 2. Aktywatory reduktazy azotanowej takie jak cysteina i NADH wpływają na zwiększenie transpiracji u pomidorów pobierających jony azotanowe. 3. Te same czynniki (jony NO_3 , cysteina i NADH) nie wpływają na intensywność transpiracji lebiody białej. 4. Wysunięto przypuszczenie, że redukcja jonów NO_3 w liściach powoduje zwiększenie transpiracji.