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Comparison of diurnal changes in nitrate and potassium contents in lucerne shoots

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Abstract

During 195-min light exposure following 5 d in dark, nitrate content was studied in different organs of lucerne plants in early bud stage. Nitrate content varied considerably especially in stems. Rapid diurnal variations in nitrate content were found in lower and upper halves of stems, in petioles and in leaf blades. The results reflected discontinuous nitrate movement in lucerne shoots. The positive correlation between the diurnal course of the nitrate and potassium contents in different plant organs showed that the K^+ transport followed the NO_3^- transport. Similar diurnal changes were found also in Na^+ and Ca^{2+} contents. Discontinuous salt movements occurring in xylem sap flow were in contrary to continuous transpiration stream and could be a consequence of temporary adsorption or binding of salts in xylem vessels.

Introduction

Soil nitrate uptake and dinitrogen fixation are alternative sources of nitrogen for lucerne plants (Smith and Sund 1965, Lee and Smith 1972, Eskew *et al.* 1973). Soil nitrates taken mostly in the form of KNO_3 are transported up to the leaves, where they are metabolized by nitrate reductase (NR) and other enzymes into amino acids and proteins (Pate 1973). Malate synthesized in leaves is translocated as K-malate into roots, where it is metabolized in respiratory pathway. $KHCO_3$ produced in roots is exchanged for KNO_3 and so K^+ circulation occurs (Ben Zioni *et al.* 1971). It is possible to assume such transport and metabolic pathways of K^+ and NO_3^- in lucerne plants, where NR activity is high in leaves and low in roots and stems (Vance and Heichel 1981), and therefore NO_3^- is transported from roots to leaves.

In our preceding papers (Plhák 1984, 1987, 1988), remarkable changes in K^+ content were reported in separated lucerne overground organs indicating some unexpected discontinuous K^+ transport. It was not possible to recognize the cause of this K^+ movement from the results obtained.

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The main goal of the present paper was to estimate whether or not similar changes in nitrate content occur in lucerne shoots when K^+ is regarded as co-transported ion for NO_3^- and to try to explain this problem.

Material and methods

Four lucerne (*Medicago sativa* L.) strains - progenies of open pollinated clones 9 - cv. Hodonínka, 45 - cv. Košice, 60 - cv. Přerovská, 81 - cv. Flandria and cv. Vela - were used in experiments. The plants were grown in containers (56 × 36 × 8 cm) with soil in a greenhouse. One hundred and fifty plants of each cultivar were grown in the container. Samples for analyses were taken at early bud stage. In the first experiment with cv. Vela, the samples were taken after 195 min in light following 5 d in dark. In the second experiment, the sampling began at 5.00 followed at 6.00, then at 2-h intervals up to 18.00, and then at 5.00 next morning. During the night, *i.e.* from 18.00 to 5.00, the containers were placed in a dark room at 20 °C. Each sample of plant material consisted of 10 - 12 shoots. Plant samples were dried immediately at 70 °C in an oven with air circulation. Plant samples were then separated into leaf blades, petioles and upper and lower halves of stems. Solar irradiance was measured during sampling and average day irradiance was calculated (Plhák 1981).

Natrium and calcium contents were estimated by means of flame photometry. Total non-structural saccharide (TNS) content was estimated colorimetrically (Plhák 1981). Nitrate content in plant samples was estimated by liquid selective nitrate electrode (Šenkýř and Petr 1979). The NO_3^-, Na^+ and Ca^{2+} contents were expressed in % dry mass after TNS subtraction. In the first experiment, the NO_3^- content was expressed in mg g^{-1} fresh matter. Each analysis of separated plant organs represented mean of 12 shoots made in triplicate.

The statistical treatment of results included analysis of variance, *t*-test, and linear correlations.

Results

Experiment 1: Light exposure of cv. Vela plants following 5-d dark treatment induced rapid changes in nitrate content especially in stems, and less in leaf blades (Fig. 1). Intensive decrease in nitrate content occurred in upper third of stems during the first 15 min of light exposure, then an increase occurred during 90 min and followed by a decrease during further 90 min. The variations in the apical part of stems were opposite to the changes in the middle part of stems. In leaves no changes were noticed during the first 45 min light exposure and thereafter nitrate content increased slightly. The results obtained led us to study the diurnal changes in NO_3^- content in lucerne shoots.

Experiment 2: Using 1 to 2-h time sampling of lucerne shoots during the day, rapid variations in nitrate content were noticed in leaf blades, petioles, upper and lower

halves of stems of the four lucerne strains examined (Fig. 2 - 5). The variations were mostly highly significant (Table 1).

Table 1. Statistical evaluation of variability in nitrate content in different plant organs during 24-h time sampling by means of analysis of variance (a) and *t*-test (b).

(a)

Plant organ	F values for variability caused by time sampling		replication	
	clone 9	clone 45	clone 9	clone 45
leaf blades	112**	65**	4.2*	3.2
petioles	134**	47**	0.5	2.1
stems - upper half	92**	56**	2.2	2.8
stems - lower half	3.2*	23**	2.7	2.3

(b)

Differences between time of sampling	t_3 values for clone 9 leaf blades	petioles	stems upper half	stems lower half
5.00 - 6.00	3.2*	3.7*	8.4*	3.5*
6.00 - 8.00	7.4**	6.1**	5.9**	3.2*
8.00 - 10.00	1.1	5.3*	3.2*	2.1
10.00 - 12.00	8.2**	6.7**	1.8	1.8
12.00 - 14.00	4.2*	8.7**	3.4*	1.2
14.00 - 16.00	5.1*	10.2**	3.2*	0.9
16.00 - 18.00	6.5**	2.1	1.2	0.8
18.00 - 5.00	3.4*	3.3*	5.1*	3.3*

* significant at 0.05 level; ** significant at 0.01 level.

The changes in petioles and upper half of stems were positively correlated, and changes in leaf blades and lower halves of stems were negatively correlated in almost all strains. In other cases, the correlation was not significant (Table 2). The opposite changes in distant plant organs - in lower half of stem and in leaf blades - and similar changes in near organs - upper half of stem and leaf petioles - reflected discontinuous NO_3^- movement in shoots.

The same experimental plants were analyzed for K^+ content (Plhák 1987). As very similar K^+ variations during time sampling in separated lucerne overground organs were observed which reflected discontinuous K^+ movement in lucerne shoots, correlations between the changes in K^+ and NO_3^- contents were calculated. In most cases highly significant positive correlations in all strains were found (Table 3).

Besides K^+ and NO_3^-, diurnal changes in Na^+ and Ca^{2+} contents in the same shoots were studied. Variability of diurnal changes expressed as variation coefficient (V) of

all strains and all separated lucerne organs was in average highest in NO_3^- (V = 35), and lower in Na^+ (V = 15.9), in K^+ (V = 15.2) and Ca^{2+} (V = 10.7).

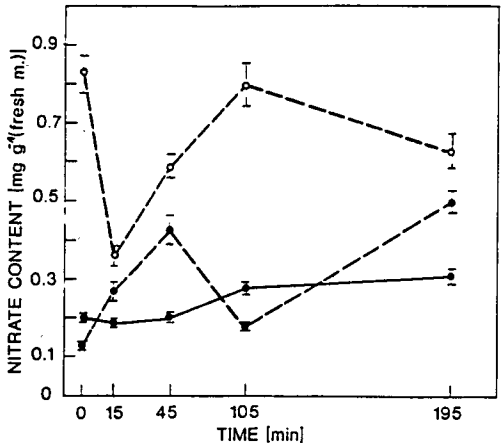

Fig. 1. Changes in nitrate content in leaf blades and stem of lucerne plants cv. Vela during 195 min. solar exposure (140 W m^{-2} PAR) after 5 d of darkening. *Full points, full line* - leaf blades, *empty points, dashed line* - stems, upper third, *full points, dashed line* - stems, middle part.

Table 2. Correlation between diurnal changes in NO_3^- content of different overground organs of lucerne strains of the clone 9 - derived from cv. Hodonínka, 45 - from cv. Košice, 60 - cv. Přerovská, and 81 - cv. Flandria.

Correlation between plant organs	Correlation coefficients (r) of strains examined				
	9	45	60	81	mean
Leaf blades *vs.* petioles	0.33	0.17	0.51	-0.39	0.16
Leaf blades *vs.* stems - upper half	0.43	-0.05	-0.21	0.42	0.15
Leaf blades *vs.* stems - lower half	-0.45	-0.40	-0.34	0.04	-0.29
Petioles *vs.* stems - upper half	0.61	0.79*	0.69	0.75*	0.71*
Petioles *vs.* stems - lower half	0.03	0.50	0.04	0.17	0.19
Stems - upper half *vs.* lower half	0.85**	-0.79*	0.30	0.22	0.15

* significant at 0.05 level; ** significant at 0.01 level.

Diurnal changes in K^+, Na^+ and Ca^{2+} contents in different overground organs were in some cases similar. Negative correlations occurred between changes in ion contents in leaf blades and lower halves of stems, and positive correlations between changes in the petioles and upper halves of stems (Table 4).

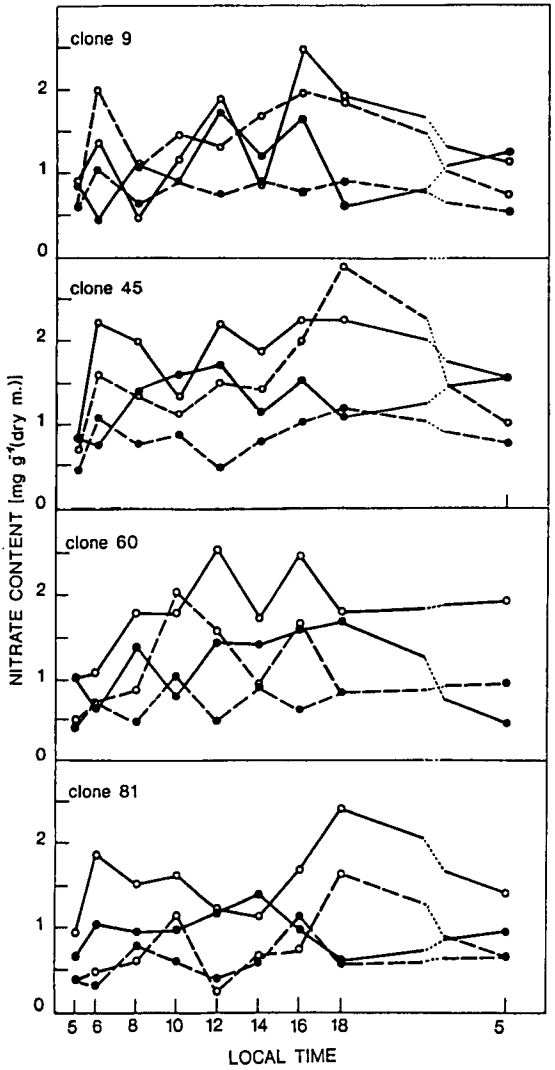

Fig. 2. Diurnal changes in nitrate content in overground organs of different lucerne strains. *Full points, full line* - leaf blades, *empty points, full line* - petioles; *empty points, dashed lines* - upper half of stems; *full points, dashed line* - lower half of stems.

Table 3. Correlation between diurnal changes in K^+ and NO_3^- contents in different overground organs of four lucerne strains.

Lucerne strains of the clone	Correlation coefficients (r) leaf blades	petioles	stems upper half	stems lower half
9	0.98**	0.84**	0.97**	0.56
45	0.84**	0.91**	0.91**	0.91**
60	0.73*	0.98**	0.96**	0.82**
81	0.68*	0.99**	0.95**	0.84**

* significant at 0.05 level, ** significant at 0.01 level

Table 4. Correlations between diurnal changes in K^+, Na^+ and Ca^{2+} contents of different aboveground organs expressed as average values of four lucerne strains of the clones 9, 45, 60 and 81.

Correlations between plant organs	Correlation coefficient (r) K^+	Na^+	Ca^{2+}
Leaf blades *vs.* petioles	-0.08	-0.80**	-0.39
Leaf blades *vs.* stems - upper half	-0.54	0.34	-0.06
Leaf blades *vs.*stems - lower half	-0.73*	-0.16	-0.23
Petioles *vs.*stems - upper half	0.77**	0.76**	0.71*
Petioles *vs.*stems - lower half	-0.04	0.45	0.79**
Stems - upper half *vs.*lower half	0.21	0	0.58

* significant at 0.05 level, ** significant at 0.01 level

Discussion

In our previous papers (Plhák 1984, 1987, 1988), remarkable diurnal changes in K^+ content in separated overground organs of lucerne were reported. The changes were opposite in distant organs and similar in near organs. They reflected the discontinuous K^+ movement in shoots and were judged as bidirectional long distance K^+ transport occurring perhaps in phloem.

Very similar NO_3^- and K^+ changes during time sampling established in present paper demonstrated that both these ions move in shoots together. As nitrates are detectable only in xylem sap and not in phloem sap (Hall and Baker 1972), the common path has to occur in xylem ascending way.

Discontinuous movement of Ca^{2+}, Mg^{2+}, Sr^{2+} in xylem sap flow of different plants was reported *e.g.* by Bell and Biddulph 1963 and Isermann 1970. Discontinuous movement of these cations was connected with cation adsorption on negatively charged places of xylem walls. Their following release and retranslocation to upper

part of plants was dependent on exchange reactions of accompanying cations (Bell and Biddulph 1963, Isermann 1970 and others).

Temporary KNO_3 immobility in xylem with transpiration stream was noticed also in our results when KNO_3 solutions were used as media for cut lucerne shoots (Plhák 1992). Temporary salt immobility in xylem can be one of regulation systems which can prevent abundant salt accumulation in leaves as a result of transpiration stream. The release of the salts accumulated in xylem can be connected besides exchange reactions perhaps also with their consumption in depletion places.

Nitrate reductase is located in lucerne mostly in leaves (Eskew *et al.* 1973, Vance and Heichel 1981). Both nitrate reductase, which reduces nitrate to ammonia, and photosynthetic processes, which produce carbon skeleton for amino acid synthesis are induced by light (Shawney and Naik 1982). In the darkness, depletion of metabolic reserves occurs and so the light exposure can promote metabolic processes connected with NO_3^- consumption and translocation. It was shown that these processes are influenced to some extent also by lucerne cultivars.

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