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Nitrate movement in xylem of lucerne plants

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Abstract

Nitrate content in lucerne stems and leaf blades immersed by cut ends in distilled water or in KNO_3 solution increased with the increase in KNO_3 concentration and with the duration of exposure under irradiance of 100 or 230 W m^{-2} PAR. The nitrate content increased from basal stem parts to apical stem parts and leaves. Nitrate was transported mainly with transpiration stream. Some flow variations occurred in stems causing time changes in nitrate content in different parts of stems.

Introduction

Besides atmospheric N_2 fixation, lucerne plants gain 50 % and more of nitrogen as nitrates (Smith and Sund 1965, Lee and Smith 1972a, Vance and Heichel 1981). The highest nitrate concentration occurs in stems, especially in their upper part (Plhák 1990), lower concentration is in leaves and roots. In all organs it decreases with age (Lee and Smith 1972a, Vance and Heichel 1981, Plhák 1990).

The nitrate transport with transpiration stream in detached lucerne shoots is the topic of the present paper.

Material and methods

Lucerne plants (*Medicago sativa* L. cv. Pálava) were grown in containers with soil in a growth chamber (irradiance of 100 W m^{-2}, light/dark temperature 25/18 °C and 60 % relative air humidity) or in the field. The plants in the phase of vegetative growth were used for experiments immediately after sampling or they were placed for 2 h with cut ends in distilled water in the dark to saturate with water. Then the plants were transferred to KNO_3 solutions of different concentrations and exposed under irradiance of 100 W m^{-2} PAR in the case of plants grown in growth chamber or under solar irradiance of 230 W m^{-2} PAR in the case of field grown plants.

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The nitrate content of stems or leaf blades was determined by two methods: (1) Nitrate was determined after its release from cut surface of stem or leaf blade segments into 20 ml extraction solution (0.04 M $Al_2(SO_4)_3$ + 0.02 M $CuSO_4$ 1:1). The elution of nitrate in extraction medium from tissue segments was linear with increasing number of cut surfaces of stem or leaf blade segments. No nitrate was released from the surface of undammaged stems. A suitable number of segments (50 stem segments of 2 mm length or 100 leaf blade segments - leaf blades cut transversally in two halves) were extracted during 2 h which was sufficient time interval for equilibrium to set up.
(2) Fresh plant tissue was ground in a mortar and homogenate was extracted with the above mentioned solution. The concentration of NO_3 was estimated by ion selective liquid electrode after Šenkýř and Petr (1989).

Transpiration rate was measured gravimetrically. All measurements were made in triplicate at least. The experimental results were statistically treated.

Results

Nitrate content in stems and leaf blades was remarkably higher (after 2 h exposure of shoots at irradiance of 100 W m^{-2}) when the shoots were immersed in KNO_3 solutions (concentration 0.4 % or higher) than in distilled water (Fig. 1a). Nitrate concentration increased in stems and in leaf blades with time of exposure (Fig. 1b).

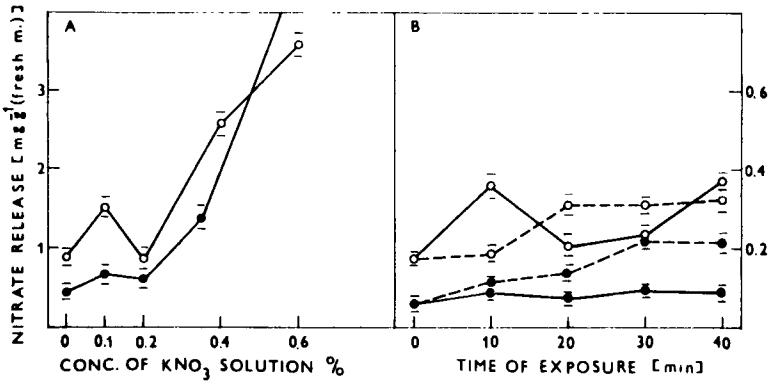

Fig. 1a. Release of nitrate from segments cut from the upper third of stems (*open symbols*) and from leaf blade segments (*closed symbols*). Lucerne shoots were placed with cut ends in increasing concentrations of KNO_3 solutions during 2 h exposure at 100 W m^{-2} irradiance, 25 °C temperature and 60 % relative air humidity.
Fig. 1b. Release of nitrate from segments cut from the upper third of stems (*open symbols*) and from leaf blade segments (*closed symbols*) of lucerne shoots placed with cut ends in distilled water (*full lines*) or in 0.4 % KNO_3 solution (*dashed line*) during the first 40 min of exposure.

Segments of all parts of stems from 0.4 % KNO_3 solution released about 3 times more nitrate than those from distilled water. The nitrate content increased usually from the basal to apical parts of stems (Fig. 2).

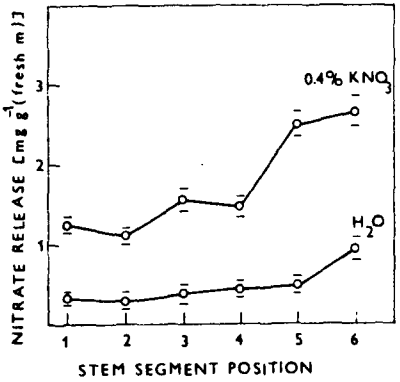

Fig. 2. Release of nitrate from stem segments cut in 5 cm vertical distance from basal (1) to apical (6) parts of stems. Before cutting segments, lucerne shoots were placed with cut ends into distilled water or 0.4 % KNO_3 solution and exposed 1 h at conditions mentioned in Fig. 1.

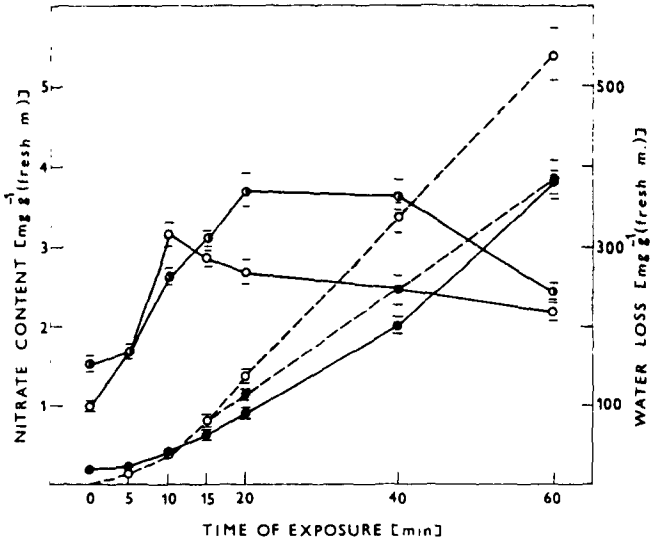

Fig. 3. Changes in nitrate content (*full lines*) in middle part (*open symbols*), in upper part (*half-closed symbols*) of stems and in leaf blades (*closed symbols*), and transpired water loss (*hatched lines*) by lucerne shoots placed in distilled water (*closed symbols*) or in 1 % KNO_3 solution (*open symbols*) during 60 min solar exposure (230 W m^{-2} PAR).

To compare quantitatively the nitrate transport with the amount of water transpired, total nitrate concentration measured by the tissue homogenate extraction

was measured simultaneously with transpiration rate in lucerne plants growing in the field during solar exposure.

A rapid increase in nitrate concentration in middle part of stems immediately after exposure was found in shoots immersed in 1 % KNO_3 solution. The increase continued up to 10 min and a decrease followed later. The rapid increase had a 5-min delay in upper part of stems, but continued for 40 min and the decrease followed later. The increase in nitrate concentration up to the end of exposure was detectable in leaf blades. The course of nitrate increase in leaves corresponded to the rate of water loss by transpiration (Fig. 3). Transpiration rate was lower when shoots were immersed in 1 % KNO_3 solution than in distilled water.

Discussion

Our preceding paper (Plhák 1990) as well as papers of other authors showed that lucerne plants growing in the field possess high nitrate concentration in stems and low in leaves. When nitrate fertilizers were applied, an increase in nitrate content especially in stems occurred (Smith and Sund 1965, Lee and Smith 1972b, Eskew *et al.*. 1973). Nitrate reductase is present mainly in leaf blades and only some 7 to 12 times lower amount in stems and roots, respectively (Eskew *et al.* 1973, Vance and Heichel 1981). According to Hall and Baker (1972) no nitrate is detectable in phloem tissue. The results obtained in present paper with cut shoots immersed in nitrate solutions show the entry of nitrate solution into lucerne shoots and its movement to middle and upper part of stems and to leaves essentially by transpiration stream. The velocity of nitrate movement was high enough that it was no doubt that nitrate moved through ascending xylem stream.

No differences between nitrate content in stems and leaf blades were observed when higher KNO_3 concentrations were used. In the case of 1 % KNO_3 solution the concentration in leaf blades reached after 1 h exposure 3 - 4 mg g^{-1} fresh matter. Such a high concentration was not detectable in leaf blades of field grown attached plants even if the same high nitrate concentration was present in upper part of stems (Plhák 1990). Plant integrity is therefore necessary for regulation of nitrate concentration in lucerne leaves.

The present results showed further that nitrate movement in xylem ascending flow was not as rapid as water transpiration stream. Some time delay of nitrate transport in stems was noticed. It caused nitrate accumulation followed by a decrease in different parts of stems which was estimated also in attached lucerne plants (Plhák 1992). This was observed also in some other plants when cation transport was studied and interpreted by temporal ion absorption on xylem walls (Isermann 1970).

References

Eskew, D.L., Schrader, L.E., Bingham, E.T.: Seasonal patterns of nitrate reductase activity and nitrate concentration of two alfalfa (*Medicago sativa* L.) cultivars. - Crop Sci. 13: 594-597, 1973.

Hall, S.M., Baker, D.A.: The chemical composition of *Ricinus* phloem exudate. - Planta **106**: 131-140, 1972.

Isermann, K.: Der Einfluss von Absorptionsvorgänge in Xylem auf die Calcium-Verteilung in der höheren Pflanzen. - Z. Pflanzenernähr. Bodenk. **126**: 191-203, 1970.

Lee, C.T., Smith, D.: Changes in the concentrations of nitrogenous fractions in alfalfa herbage with advance in maturity. - Agron. J. **64**: 326-327, 1972a.

Lee, C.T., Smith, D.: Influence of nitrogen fertilizer on stands, yields of herbage and nitrogenous fractions of field grown alfalfa. - Agron. J. **64**: 527-530, 1972b.

Plhák, F. [Nitrate content in lucerne in relation to growth and root activity.] - In: Produkčná Ekológia Plodín. Pp. 180-183. VŠZ, Nitra 1990. [In Czech.]

Plhák, F.: Comparison of diurnal changes in nitrate and potassium contents in lucerne shoots. - Biol. Plant. 34: 101-107, 1992.

Šenkýř, J., Petr, J.: [Nitrate ion selective electrode.]. - Chem. Listy (Praha) **73**: 1097, 1979. [In Czech.]

Smith, D., Sund, J.M.: Influence of stage of growth and soil nitrogen on nitrate content of herbage of alfalfa, red clower, trefoil, and bromegrass. - J. agr. Food Chem. **13**: 81-84, 1965.

Vance, C.P., Heichel, G.H.: Nitrate assimilation during vegetative regrowth of alfalfa. - Plant Physiol. **68**: 1052-1056, 1981.