RESEARCH ARTICLE

Utilization of $^{15}NO_3^-$ by nodulated soybean plants under conditions of root hypoxia

Luciana Nunes Menolli Lanza · Daniel Carlos Ferreira Lanza · Ladaslav Sodek

Abstract Waterlogging of soils is common in nature. The low availability of oxygen under these conditions leads to hypoxia of the root system impairing the development and productivity of the plant. The presence of nitrate under flooding conditions is regarded as being beneficial towards tolerance to this stress. However, it is not known how nodulated soybean plants, cultivated in the absence of nitrate and therefore not metabolically adapted to this compound, would respond to nitrate under root hypoxia in comparison with non-nodulated plants grown on nitrate. A study was conducted with ^{15}N labelled nitrate supplied on waterlogging for a period of 48 h using both nodulated and non-nodulated plants of different physiological ages. Enrichment of N was found in roots and leaves with incorporation of the isotope in amino acids, although to a much smaller degree under hypoxia than normoxia. This demonstrates that nitrate is taken up under hypoxic conditions and assimilated into amino acids, although to a much lesser extent than for normoxia. The similar response obtained with nodulated and non-nodulated plants indicates the rapid metabolic adaptation of nodulated plants to the presence of nitrate under hypoxia. Enrichment of N in nodules was very much weaker with a distinct enrichment pattern of amino acids (especially asparagine) suggesting that labelling arose from a tissue source external to the nodule rather than through assimilation in the nodule itself.

L. Nunes Menolli Lanza · L. Sodek (✉)
Departamento de Biologia Vegetal, Universidade Estadual de Campinas, C.P. 13083-862 Campinas, SP, Brazil
e-mail: lsodek@unicamp.br

L. Nunes Menolli Lanza
Laboratório de Botânica e Zoologia, Universidade Potiguar, C.P. 59056-000 Natal, RN, Brazil

D. C. Ferreira Lanza
Departamento de Bioquímica, Universidade Federal do Rio Grande do Norte, C.P. 59078-970 Natal, RN, Brazil

Introduction

The production of crops such as soybean can be adversely affected by waterlogging of the root system following even moderate rainfall, especially with compacted soils or soils with poor drainage properties. Waterlogging leads to a stress denominated hypoxia, a condition where the concentration of oxygen is insufficient for the roots to maintain their normal rate of respiration. Root hypoxia results from the extremely low rate of oxygen diffusion in water which is some 10^4 times lower than in air (Armstrong et al. 1994). Hypoxia caused by waterlogging is a stress that affects growth and survival of plants and provokes metabolic alterations especially of energy metabolism with a diversion of glycolysis to fermentation (Bailey-Serres and Voesenek 2008). Several studies have shown that the presence of nitrate (NO_3^-) under waterlogged conditions promotes greater tolerance of the plants (including soybean) to hypoxia (Malavolta 1954; Trought and Drew 1981; Allegre et al. 2004; Thomas and Sodek 2005; Horchani et al. 2010; Lanza et al. 2013). This beneficial effect has stimulated research into the uptake and metabolism of nitrate under hypoxia. There is evidence that hypoxia impairs both the uptake and assimilation of nitrate (Lee 1978; Trought and Drew 1981; Buwalda and Greenway 1989; Morard et al. 2004; Brandão and Sodek 2009; Oliveira et al. 2013a) although information on the metabolism of nitrate under such conditions is still inconclusive. Several studies indicate induction of the enzyme nitrate reductase under hypoxia (Garcia-Novo and Crawford 1973; Glaab and Kaiser 1993; Botrel et al. 1996; Morard et al. 2004; Horchani et al. 2010) but in other cases a decline in activity was observed (Brandão and Sodek 2009). Nevertheless, the reduction of nitrite, the

product of nitrate reductase, appears to be strongly impaired under hypoxia (Lee 1978; Botrel et al. 1996; Morard et al. 2004) leading to the accumulation of nitrite (Lee 1979; Libourel et al. 2006; Brandão and Sodek 2009; Horchani et al. 2010). Part of this nitrite can be reduced to nitric oxide, a process that may be important for NAD^+ regeneration (Igamberdiev and Hill 2004; Stoimenova et al. 2007; Oliveira et al. 2013b).

The study of uptake and metabolism of nitrate under hypoxia is quite complex in view of the presence of nitrate in the vacuole of root cells, a condition prevalent in plants cultivated with nitrate as N source. During hypoxia this endogenous nitrate is mobilized and metabolized (Sousa and Sodek 2002; Brandão and Sodek 2009) even when nitrate is present in the medium. A similar phenomenon occurs under normoxia when plants are transferred to a medium free of mineral N (Sprent et al. 1987; Lima and Sodek 2003; Oliveira et al. 2013a), a further indication that the capacity for nitrate uptake during hypoxia is limited. However, there is no information on the uptake and metabolism of nitrate after waterlogging of the root system of nodulated plants which, cultivated in the absence of mineral N, are naturally free of endogenous nitrate and therefore not metabolically adapted to its presence. The objective of the present study was, therefore, to verify whether the metabolic response to nitrate under root hypoxia of nodulated plants differs from that observed with non-nodulated plants grown on nitrate. The study was conducted with ^{15}N labelled nitrate supplied on waterlogging for a period of 48 h using plants of different physiological ages.

Materials and methods

Soybean (*Glycine max* L. Merr cv IAC-23) seeds were germinated in trays of vermiculite. Seedlings with fully expanded primary leaves (V1 stage - see Ritchie et al. 1982) were transferred to 1.4 L pots containing vermiculite, with 1 plant per pot. For nodulated plants the seedlings were inoculated with a liquid culture of *Bradyrhizobium elkani* (SEMIA 5019) (Norris and Date 1976) on transfer to the pots. The pots received Hoagland and Arnon's (1938) nutrient solution at one-third strength either free of N (0 mM NO_3^-) for nodulated plants or complete (5 mM NO_3^-) for non-nodulated plants. The experiments were set up when plants reached the desired developmental stage. For this purpose vermiculite was removed from the roots by careful washing with tap water and the plants transferred to a hydroponic system with one plant per pot containing 200 mL of nutrient solution. The following treatments were used: normoxia, with 5 mM $^{15}NO_3^-$ (11 % atom excess) and aeration; hypoxia, with 5 mM $^{15}NO_3^-$ without aeration and the solution surface covered with mineral oil. Each treatment consisted of 3 replicate plants. The pH of the nutrient solution was 6.5. Similar experiments were

carried out with plants at each developmental stage from V1 (primary leaf stage) to V7 (6th trifoliate leaf stage - for stage definitions, see Ritchie et al. 1982). After treatment for 48 h the roots, leaves (youngest fully expanded trifoliate) and nodules (where present) were collected. The first harvest of nodules was possible at the V4 (3rd trifoliate leaf) stage, since at earlier stages nodules were absent or too small.

The isotopic analysis of plant N was carried out on lyophilized material by the Stable Isotope Laboratory of the Centro de Energia Nuclear na Agricultura (CENA/USP), Piracicaba, SP following the procedures of Barrie and Prosser (1996). The ^{15}N enrichment of amino acids was determined after extraction, purification by ion-exchange and derivatization with MTBSTFA as described by Oliveira et al. (2013a) using Gas Chromatography/Mass Spectroscopy (GC/MS) according to a protocol adapted from Godber and Parsons (1998).

Results

Uptake of $^{15}NO_3^-$ by soybean plants

The data for ^{15}N enrichment after the 48 h of treatment shows that $^{15}NO_3^-$ was taken up under conditions of hypoxia with the distribution of isotope in roots, leaves and nodules of nodulated plants as well as roots and leaves of non-nodulated plants, at all the developmental stages investigated (Figs. 1 and 2).

In the roots of nodulated plants, the enrichment of ^{15}N was much less under hypoxia relative to normoxia (Fig. 1a). Under hypoxia the values were below 2 % whereas under normoxia above 3 %. Independent of treatment, there was a tendency for a decline in enrichment with plant age. In the leaves of nodulated plants the enrichment of ^{15}N was also lower for hypoxia relative to the normoxia controls at all stages of development (Fig. 1b). Under hypoxia the values of ^{15}N enrichment ranged from 0.2 and 1.6 %, while for normoxia they varied from 1.1 to 3.5 %. Despite the difference in values, the pattern was similar between hypoxia and normoxia with an increase recorded up to stage V3 followed by a decrease. In the case of nodules enrichment was extremely low for both hypoxia and normoxia, with values in the range 0.1 and 0.3 % (Fig. 1c).

The pattern of incorporation of ^{15}N in roots and leaves of an identical experiment conducted simultaneously with non-nodulated plants (Fig. 2) was quite similar to that observed for nodulated plants (cf. Fig. 1), as well as in terms of the absolute values recorded. For roots the enrichment was much lower under hypoxia with a tendency for a decline with plant age (Fig. 2a). For leaves ^{15}N enrichment was also lower under hypoxia for all ages.
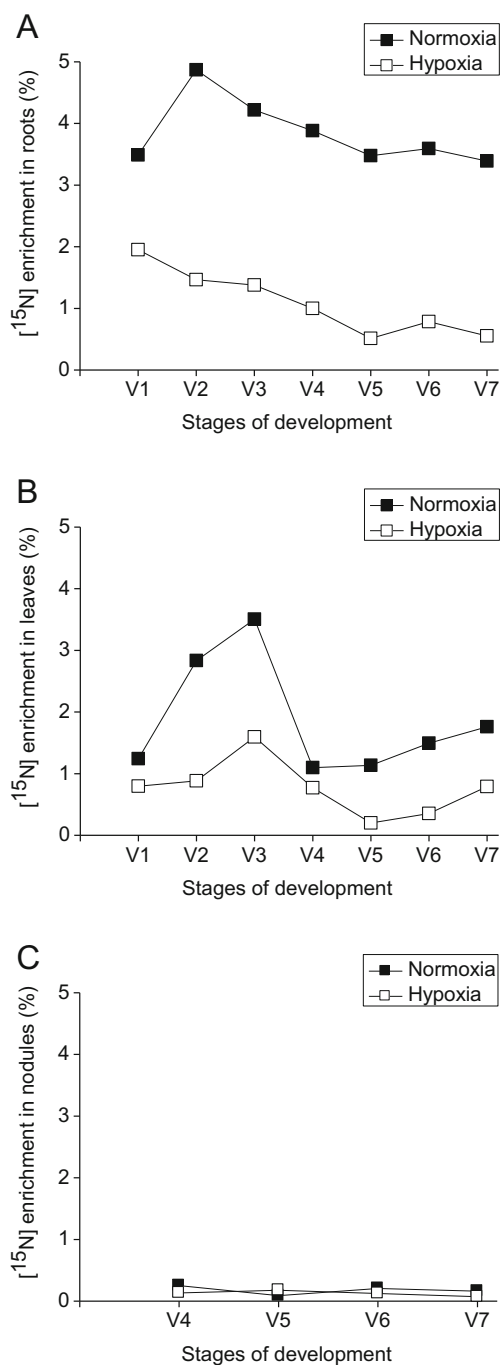
Fig. 1 Enrichment of N (%) of roots (**a**), leaves (fully expanded youngest trefoil) (**b**) and nodules (**c**) of nodulated soybean during different stages of plant development in normoxia (*black square*) and hypoxia (*white square*) 48 h after the application of $^{15}NO_3^-$ isotope. Stage of development V1 represents the primary or first pair of fully expanded leaves. Difference between hypoxia and normoxia (analysis of variance using a randomized block design): significant for (**a**) roots ($p<0.001$) and (**b**) leaves ($p<0.01$ %); not significant for (**c**) nodules

Fig. 2 Enrichment of N (%) of roots (**a**) and leaves (fully expanded youngest trefoil) (**b**) of non-nodulated soybean during different stages of plant development in normoxia (*black square*) and hypoxia (*white square*) 48 h after the application of $^{15}NO_3^-$ isotope. Stage of development V1 represents the primary or first pair of fully expanded leaves. Difference between hypoxia and normoxia (analysis of variance using a randomized block design): significant for (**a**) roots ($p<0.001$) and (**b**) leaves ($p<0.01$)

Incorporation of ^{15}N in amino acids

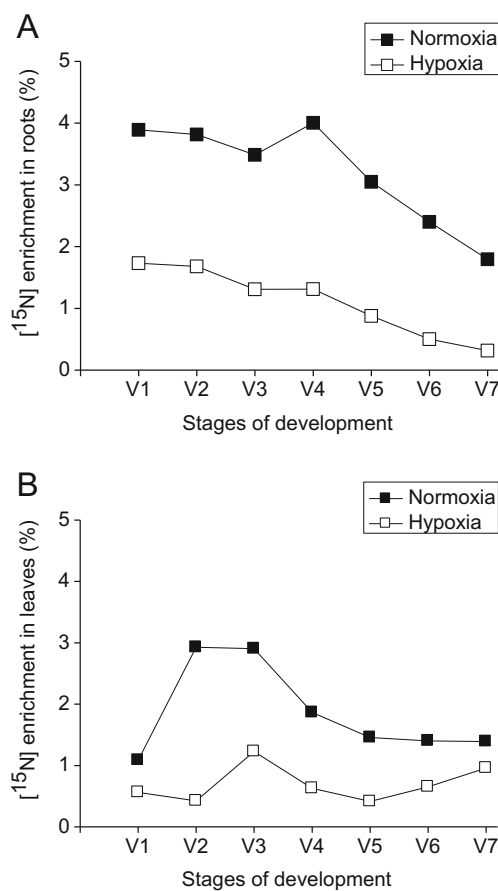Only data for the amino acids with greater enrichment of ^{15}N are presented, that is, Gln, Glu, Asn, Asp, Ala and Ser. These are also the amino acids closest to primary N assimilation. The data shown are the mean of all plant ages studied (V1 to V7) in view of the similarity of values between ages. In general, the incorporation of ^{15}N in these amino acids was observed in roots, leaves and nodules, both of nodulated (Fig. 3) and non-nodulated (Fig. 4) plants, although in the case of nodules the degree of incorporation was very weak and almost absent in the case of normoxia. For nodulated plants incorporation in all amino acids was greater under normoxia compared to hypoxia by a factor of about two, both for roots and leaves (Fig. 3). In leaves, Asn and Gln presented a greater degree of enrichment compared to other amino acids, but this is expected in view of the amides possessing 2 atoms of N which allows enrichment double that of amino acids with only 1 atom of N. In roots, the incorporation of ^{15}N in Asn was greater than Gln, both under normoxia and hypoxia, however in the leaf the opposite was found. In general, the data indicate that the flux of ^{15}N into amino acids was close to equilibrium, in view of the

enrichment values being generally similar for all reported amino acids (for this comparison values for amides should be divided by 2).

In the case of nodules, it is noteworthy that incorporation of ^{15}N in amino acids in the hypoxia treatment was weak, but even weaker or virtually absent for normoxia. An exception was Asn where incorporation was more substantial (around 4 %) both under normoxia and hypoxia, and also Gln where incorporation under normoxia was as high as under hypoxia (1.6 and 1.12 %, respectively; difference not significant).

In the case of non-nodulated plants substantial incorporation of ^{15}N into amino acids was found, both for leaves and roots (Fig. 4), however in general with values somewhat lower than those registered for nodulated plants. This difference may possibly result from dilution of the isotope by non-labelled endogenous nitrate present in non-nodulated plants. In the leaves incorporation of ^{15}N was lower under hypoxia except for Asn and Gln. Although Asn and Gln presented values for normoxia about double those found for hypoxia, the difference was not significant, due to the high variability between replicates. In the root, there was no significant difference between normoxia and hypoxia for all amino acids registered except for Ser, despite values being superior for normoxia in all cases. The incorporation of ^{15}N in the amides Asn and Gln stood out among the amino acids for normoxia in leaves (Fig. 4) similar to that found for nodulated plants (cf. Fig. 3), however by contrast to the data for nodulated plants it did not stand out in roots.

Discussion

The enrichment of the roots and leaves of soybean with ^{15}N after 48 h of hypoxia in the presence of ^{15}N-nitrate, both for nodulated and non-nodulated plants, allows two important conclusions. First, that nitrate is absorbed by roots under waterlogged (hypoxic) conditions and second that the isotope is transported to other parts of the plant. In the case of the non-nodulated plants this confirms a similar conclusion reached by Oliveira et al. (2013a). The fact that nodulated plants produced a similar response allows additional conclusions discussed below. Nevertheless, the enrichment of N under normoxia was much greater than for hypoxia indicating that nitrate uptake is much slower when plants are waterlogged. Therefore hypoxia impairs nitrate uptake but does not totally inhibit the process. This is in agreement with earlier studies with cereals (Lee 1978; Trought and Drew 1981; Buwalda and Greenway 1989) but contrasts data obtained with tomato where more, not less nitrate was taken up under hypoxia (Morard et al. 2004; Horchani et al. 2010).

The interpretation of the data obtained with nodulated plants must take into account that they were grown in the
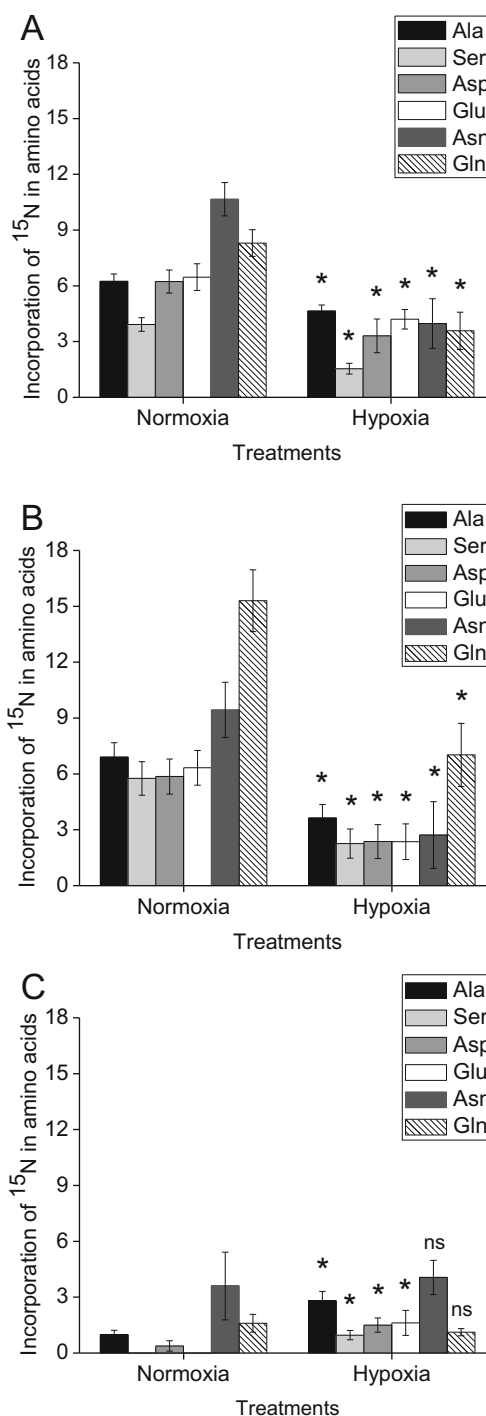
Fig. 3 Incorporation of ^{15}N in amino acids of nodulated soybean (% labelling of each amino acid). Data for (a) roots, (b) leaves (youngest fully-expanded) and (c) nodules after supplying plants with nutrient solution containing ^{15}NO$_3$ (11 % atom excess) for 48 h under normoxia and hypoxia. Data are the mean for all ages studied (V1 to V7). * = difference significant ($p<0.05$) by Student's t test between means for hypoxia and normoxia; ns = not significant

absence of nitrate and therefore totally dependent on N$_2$ fixation as a source of N. This contrasts with non-nodulated plants grown on nitrate and therefore dependent on nitrate
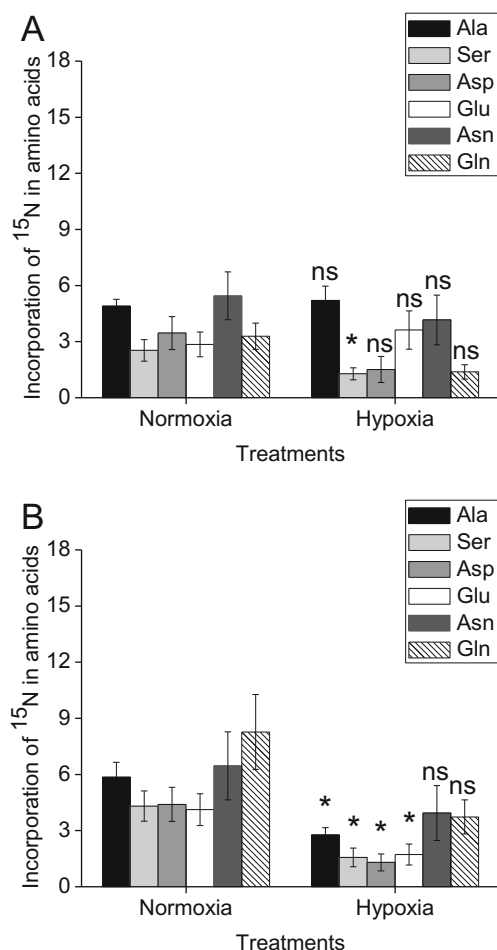
Fig. 4 Incorporation of ^{15}N in amino acids of non-nodulated soybean (% labelling of each amino acid). Data for (**a**) roots, (**b**) leaves (youngest fully-expanded) after supplying plants with nutrient solution containing $^{15}NO_3$ (11 % atom excess) for 48 h under normoxia and hypoxia. Data are the mean for all ages studied (V1 to V7). * = difference significant ($p<0.05$) by Student's t test between means for hypoxia and normoxia; ns = not significant

assimilation. The fact that the nodulated plants responded to hypoxia stress in a similar manner to the non-nodulated plants after the supply of nitrate-^{15}N indicates a rapid metabolic adaptation of the plant to the presence of nitrate even under hypoxic conditions. On waterlogging the process of N_2 fixation of the nodulated plants is suppressed within 1 h (Amarante and Sodek 2006) so the influence of this process would be minimal. The simultaneous supply of nitrate-^{15}N with the application of the hypoxic stress leads to its uptake, assimilation and transport to other parts of the plant since ^{15}N was found both in roots and shoots with incorporation in amino acids. This must involve the induction of NR activity in the roots, since the enzyme is absent in roots of nodulated soybean grown in the absence of nitrate, but can be induced within 24 h after addition of substrate (Antunes and Sodek, unpublished data). On the other hand, this capacity to take up and assimilate nitrate under hypoxia, although present, was strongly reduced in relation to normoxia, in view of the lower enrichment of roots and leaves under hypoxia. This phenomenon was observed both for nodulated and non-nodulated plants. The reduced capacity under hypoxia was of the same order of magnitude for nodulated and non-nodulated plants, consistent with our conclusion that nodulated plants adapt rapidly to the presence of nitrate even under hypoxia.

The fact that ^{15}N was incorporated efficiently in amino acids under hypoxia clearly demonstrates that the process of reduction and assimilation of nitrate does take place under hypoxia, not only in non-nodulated plants (see also Oliveira et al. 2013a) but also in nodulated plants. However, for roots and leaves of nodulated plants (and in the case of some amino acids of non-nodulated plants), the degree of enrichment was significantly less under hypoxia, indicating some limitation of the process of nitrate assimilation under these conditions. This limitation could be expected to be more severe for nodulated plants in view of the necessity to adapt their metabolism to the presence of nitrate. The enrichment of the amino acids was in general greater than the enrichment of total N, both for normoxia and hypoxia. This may be explained by the fact that amino acids are metabolically close to that of nitrate and will therefore receive the ^{15}N first. As may be expected, the immediate products and those closest to the assimilation of nitrate were the most enriched (Asn, Gln, Asp, Glu e Ala).

With regard to the nodules the data show that they too were enriched, though to a much lesser extent than in the roots or leaves, both under normoxia and hypoxia. The presence of labelled amino acids in the nodule is especially interesting, since the capacity of the nodule to reduce and assimilate nitrate is regarded as being very limited, although the subject is controversial (Sprent et al. 1987; Becana et al. 1989; Izmailov et al. 2003). Two aspects concerning the nodule data are noteworthy since they contrast the situation found for leaves and roots. First, the degree of enrichment of the amino acids was in general greater under hypoxia than normoxia. Second, Asn stood out among the amino acids in terms of enrichment level, both under normoxia and hypoxia. As such, the profile of ^{15}N incorporation in the amino acids analysed was different from that found in roots and leaves. It is possible, therefore, that the ^{15}N present in the amino acids was not assimilated in the nodule, but in a different location and some of these labelled amino acids translocated to the nodule. In this respect, it is pertinent to observe that in other studies it was suggested that at least part of the Asn of the nodule came from a different source and not from assimilation in the nodule itself (Atkins et al. 1988, 1990).

The only studies found in the literature that have used $^{15}NO_3$ under conditions of hypoxia were carried out by the group of Reggiani (Reggiani et al. 1995, 1997) with rice seedlings and more recently by our own group (Oliveira and Sodek 2013; Oliveira et al. 2013a) with root segments and intact plants of non-nodulated soybean. These investigations

demonstrated the incorporation of ^{15}N in several amino acids and Reggiani et al. (1997) concluded that there was no blockage of the nitrate assimilatory pathway under oxygen deficiency in rice. However, with regard to anoxia or hypoxia, rice is considered a case apart (Reggiani et al. 1993) in view of its capacity to germinate in anaerobic environments, being fully adapted to anoxic conditions. Although our own studies with soybean also demonstrate the operation of the nitrate assimilatory pathway during hypoxia, our conclusion is that the process is quite limited under these conditions. This conclusion is supported by other studies where nitrite was shown to accumulate under hypoxia (Lee 1979; Morard et al. 2004; Libourel et al. 2006; Brandão and Sodek 2009; Horchani et al. 2010). Some of the accumulated nitrite can be reduced to NO by mitochondrial activity where nitrite substitutes oxygen as terminal electron acceptor under oxygen deficiency (Stoimenova et al. 2007; Gupta et al. 2011; Oliveira et al. 2013b, c), thereby producing ATP and regenerating NAD^+ which may underlie the beneficial effect of nitrate on plant tolerance to hypoxia.

We conclude that in soybean plants under hypoxia provoked by waterlogging of the root system, nitrate is taken up by the roots and assimilated into amino acids, although both processes are limited in relation to normoxia. Nodulated plants, grown without nitrate, show a rapid metabolic adaptation to the presence of nitrate under hypoxic conditions in view of the response being very similar to that of non-nodulated plants.

References

Allegre A, Silvestre J, Morard P, Kallerhoff E, Pinelli E (2004) Nitrate reductase regulation in tomato roots by exogenous nitrate: a possible role in tolerance to long-term root anoxia. J Exp Bot 55:2625–2634

Amarante L, Sodek L (2006) Waterlogging effect on xylem sap glutamine of nodulated soybean. Biol Plant 50:405–410

Armstrong W, Brandle R, Jackson MB (1994) Mechanisms of flood tolerance in plants. Acta Bot Neerl 43:307–358

Atkins CA, Storer PJ, Pate JS (1988) Pathways of nitrogen assimilation in cowpea nodules studied using $^{15}N_2$ and allopurinol. Plant Physiol 86:204–207

Atkins CA, Dakora FD, Storer PJ (1990) Effect of oxygen pressure on synthesis and export of nitrogenous solutes by nodules of cowpea. Planta 182:565–571

Bailey-Serres J, Voesenek LACJ (2008) Flooding stress: acclimations and genetic diversity. Annu Rev Plant Biol 59:313–339

Barrie A, Prosser SJ (1996) Automated analysis of light-element stable isotopes by isotope ratio mass spectrometry. In: Boutto TW, Yamasaki S (eds) Mass spectrometry of soils. Marcel Dekker, New York, pp 01–46

Becana M, Minchin FR, Sprent JI (1989) Short-term inhibition of legume N_2 fixation by nitrate. I. Nitrate effects on nitrate reductase activity of bacteroids and nodule cytosol. Planta 180:40–45

Botrel A, Magne C, Kaiser WM (1996) Nitrate reduction, nitrite reduction and assimilation in barley roots in response to anoxia. Plant Physiol Biochem 34:645–652

Brandão AD, Sodek L (2009) Nitrate uptake and metabolism by roots of soybean plants under oxygen deficiency. Braz J Plant Physiol 21: 13–23

Buwalda F, Greenway H (1989) Nitrogen uptake and growth of wheat during O_2 deficiency in root media containing NO_3^- only, or NO_3^- plus NH_4^+. New Phytol 111:161–166

Garcia-Novo F, Crawford RMM (1973) Soil aeration, nitrate reduction and flooding tolerance in higher plants. New Phytol 72:1031–1039

Glaab J, Kaiser WM (1993) Rapid modulation of nitrate reductase in pea roots. Planta 191:173–179

Godber IM, Parsons R (1998) Translocation of amino acids from stem nodules of Sesbania rostrata demonstrated by GC-MS in planta ^{15}N isotope dilution. Plant Cell Environ 21:1089–1099

Gupta KJ, Igamberdiev AU, Manjunatha G, Segu S, Moran JF, Neelawarne B et al (2011) The emerging roles of nitric oxide (NO) in plant mitochondria. Plant Sci 181:520–526

Hoagland DR, Arnon DI (1938) The water culture method for growing plants without soil. Calif Agric Exp Station 347:1–39

Horchani F, Aschi-Smiti S, Brouquisse R (2010) Involvement of nitrate reduction in the tolerance of tomato (Solanum lycopersicum L.) to prolonged root hypoxia. Acta Physiol Plant 32:1113–1123

Igamberdiev AU, Hill RD (2004) Nitrate, NO and haemoglobin in plant adaptation to hypoxia: an alternative to classic fermentation pathways. J Exp Bot 55:2473–2482

Izmailov SF, Bruskova RK, Chechetka SA, Kirnos SV, Nikiforova TA, Satskaya MV (2003) Nitrate assimilation function of yellow lupine root nodules? Biol Bull 30:140–147

Lanza LNM, Rossi SC, Sodek L (2013) Nitrogen fertilization benefits soybean under flooding conditions Bragantia 72:2–9

Lee RB (1978) Inorganic nitrogen metabolism in barley roots under poorly aerated conditions. J Exp Bot 29:693–708

Lee RB (1979) The release of nitrite from barley roots in response to metabolic inhibitors, uncoupling agents, and anoxia. J Exp Bot 30: 119–133

Libourel IG, van Bodegom PM, Fricker MD, Ratcliffe RG (2006) Nitrite reduces cytoplasmic acidosis under anoxia. Plant Physiol 142:1710–1717

Lima JD, Sodek L (2003) N-stress alters aspartate and asparagine levels of xylem sap in soybean. Plant Sci 165:649–656

Malavolta E (1954) Studies on the nitrogenous nutrition of rice. Plant Physiol 29:98–99

Morard P, Silvestre J, Lacoste L, Caumes E, Lamaze T (2004) Nitrate uptake and nitrite release by tomato roots in response to anoxia. J Plant Physiol 161:855–866

Norris DO, Date RA (1976) Legume bacteriology. In: Shaw NH, Bryan WW (eds) Tropical pastures research; principles and methods. CAB, Brisbane, pp 134–174

Oliveira HC, Sodek L (2013) Effect of oxygen deficiency on nitrogen assimilation and amino acid metabolism in soybean root segments. Amino Acids 44:743–755

Oliveira HC, Freschi L, Sodek L (2013a) Nitrogen metabolism and translocation in soybean plants subjected to root oxygen deficiency. Plant Physiol Biochem 66:141–149

Oliveira HC, Salgado I, Sodek L (2013b) Involvement of nitrite in the nitrate-mediated modulation of fermentative metabolism and nitric oxide production of soybean roots during hypoxia. Planta 237:255–264

Oliveira HC, Salgado I, Sodek L (2013c) Nitrite decreases ethanol production by intact soybean roots submitted to oxygen deficiency.

A role for mitochondrial nitric oxide synthesis? Plant Signal Behav 8, e23578

Reggiani R, Mattana M, Aurisano N, Bertani A (1993) Utilization of stored nitrate during the anaerobic germination of rice seeds. Plant Cell Physiol 34:379–383

Reggiani R, Bertini F, Mattana M (1995) Incorporation of nitrate into amino acids during the anaerobic germination of rice. Amino Acids 9:385–390

Reggiani R, Bertini F, Mattana M (1997) Incorporation of nitrate nitrogen in rice seedlings transferred to anaerobic conditions. Amino Acids 13:183–188

Ritchie S, Hanway JJ, Thompson HE (1982) How a soybean plant develops. Ames, Iowa State University of Science and Technology, Coop. Ext. Serv. 20p. (Special Report, 53)

Sousa CAF, Sodek L (2002) Metabolic changes in soybean plants in response to waterlogging in the presence of nitrate. Physiol Mol Biol Plants 8:97–104

Sprent JI, Giannakis C, Wallace W (1987) Transport of nitrate and calcium into legume nodules. J Exp Bot 38:1121–1128

Stoimenova M, Igamberdiev AU, Gupta KJ, Hill RD (2007) Nitrite driven anaerobic ATP synthesis in barley and rice root mitochondria. Planta 226:465–474

Thomas AL, Sodek L (2005) Development of the nodulated soybean plant after flooding of the root system with different sources of nitrogen. Braz J Plant Physiol 17:291–297

Trought MCT, Drew MC (1981) Alleviation of injury to young wheat plants in anaerobic solution cultures in relation to the supply of nitrate and other inorganic nutrients. J Exp Bot 32:509–522