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Effect of nicotianamine on iron re-mobilization in de-rooted tomato seedlings

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Summary. An exogenous supply of nicotianamine is essential for the redistribution of ^{59}iron via the symplast and the phloem to newly developing organs in de-rooted seedlings of the nicotianamine-less tomato mutant *chloronerva*. This observation supports the idea that nicotianamine could function as a translocator of iron within the symplast and the phloem.

Key words: *Lycopersicon esculentum* Mill. cv. Bonner Beste mutant *chloronerva* — De-rooted seedlings — Iron — Phloem transport — Remobilization

Introduction

In higher plants, long-distance transport of iron is almost entirely restricted to the transpiration stream from the root to the shoot (Tiffin 1966). There have also been, however, several reports of phloem transport of iron. Translocation of foliar-applied iron to other parts of the shoot and to the roots was observed in different species as early as 1960 by Doney et al. and later by Brown et al. (1965) and by Eddings and Brown (1967). Marschner et al. (1987) reported on a rather high basipetal iron translocation which is interpreted as phloem transport. According to Pate (1975), the participation of iron in the exchange between phloem and xylem and in the circulation of solutes in the whole plant must occur. Maas et al. (1988) measured an iron concentration of 7 μM in the phloem exudate of iron-deficient *Ricinus communis* plants and of 20 μM in iron-sufficient ones. These authors reported that iron travels in the phloem of *R. communis* as a complex with an estimated M_r of 2400. They concluded that the capacity of the phloem to re-distribute iron to the roots is sufficient to influence the development of iron-efficiency reactions according to strategy I of iron acquisition.

Investigations in our laboratory on the function(s) of the ubiquitous and probably essential plant constituent nicotianamine (NA) led to the conclusion that it might serve as an iron transporter within the cell, guiding iron(II) as an NAFe(II) complex to the sites of demand (Scholz et al. 1988). Experiments with the NA-less tomato mutant *chloronerva* provoked the assumption that NA could also affect the symplast and the phloem transport of iron. This idea was tested by an experiment on the re-mobilization of radioactive iron in de-rooted seedlings of the mutant as described below.

Materials and methods

Tomato seeds (*Lycopersicon esculentum* Mill. cv. Bonner Beste and its monogenic mutant *chloronerva*) were germinated in the dark on polyvinyl needles (Biolaston, VEB Biolastonwerk Friedrichroda, GDR), moistened with distilled water. After 4 days, when radicles had emerged, the water was replaced by nutrient solution of the following composition: $Ca(NO_3)_2$ 5 mM, KNO_3 5 mM, KH_2PO_4 1 mM, $MgSO_4$ 1 mM, H_3BO_3 46 μM, $MnCl_2$ 4.5 μM, $ZnSO_4$ 3.8 μM, $CuSO_4$ 0.3 μM, $(NH_4)_6Mo_7O_{24}$ 0.1 μM. Iron was added as FeEDDHA to a final concentration of 90 μM and labeled with 10 MBq ^{59}FeEDDHA. On day 6, the seedlings were exposed to light from fluorescent tubes Razno LB 40 white (USSR) and Narva lumoflor LS 40-1 (GDR) in a ratio of 1:1. The light regime was

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This paper is part 36 in the series *The 'normalizing factor' for the tomato mutant chloronerva*. For part 35 see Stephan and Grün (1989)

Abbreviations: EDDHA, ethylenediamine-*N,N'*-bis(*o*-hydroxyphenylacetic acid); *NA*, nicotianamine = (2*S*, 3'*S*,3''*S*)-*N*-[*N*-(3-amino-3-carboxypropyl)-3-amino-3-carboxypropyl]-azetidine-2-carboxylic acid

16-h light, 8-h dark; temperature 20° C (light), 18° C (dark); relative humidity about 70%. On day 8, when the primary leaves started to emerge, roots were cut and the cuttings inserted into a container (4.5 l) with aerated tap water for 4 h to remove adhering ^{59}FeEDDHA from the basal ends of the hypocotyls. Then three sets of nine plants each were set up and the water replaced by nutrient solution without added iron, the pH adjusted to 5.5 with KOH and treated as follows. The cotyledons of nine mutant cuttings were supplied by means of a smooth brush with an NA preparation from ripe seeds of *Phaseolus vulgaris* containing 84% NA by dry mass; the final NA concentration of this solution was 0.1 mM. Tween 20 (Atlas-Goldschmidt GmbH, Essen, FRG) was added as a wetting agent to a concentration of 0.05% (by vol.). The cotyledons of nine other mutant cuttings were treated with the NA-free solution (control) as were nine cuttings of the wild-type Bonner Beste for comparison. These treatments were performed five times per day. The nutrient solution was replaced every 3 days. On day 16 six plants from each set were selected for uniformity, dissected into organs and, after fresh mass determination, subjected to radioactive counting.

Results

Plant development

Without an exogenous supply of NA the mutant cuttings showed very little growth and development. Apart from a small primary leaf, only a tiny secondary leaf emerged. Both leaves were severely chlorotic with some small necrotic spots. Only a few adventitious roots were visible at the basal part of the hypocotyl but these did not exceed 11 mm in length. In contrast to this, the NA-treated mutant, as well as the wild-type cuttings, showed two well developed leaves of yellow-greenish to chlorotic appearance and numerous adventitious roots of up to 85 mm in length, although exhibiting distinct iron-deficiency symptoms such as increased branching of laterals and swelling of root tips. In most characteristics the mutant cuttings treated with NA very much resembled the appearance of the NA-containing wild-type cuttings. The differences in growth and development were also reflected by the fresh mass as recorded in Table 1 A.

Iron re-distribution

The amount of ^{59}iron transported to the shoot prior to cutting was significantly higher in the mutant than in the wild-type seedlings as was expected from earlier observations (Scholz et al. 1985a). After de-rooting and without an exogenous iron source this ^{59}Fe was re-distributed to the newly developed organs of the mutant supplied with NA in a very similar manner as in the NA-containing wild-type cutting. Comparison of the treated with the untreated mutant reveals that in the former about 10% of the total ^{59}Fe were retranslocated to the growing leaves and about 9% to the adventitious roots. The main source of this re-mobilized iron was the hypocotyl and the cotyledons (Table 1 B).

Very similar results were obtained in a second experiment (not shown) with older seedlings where the primary leaves were supplied with NA and the cotyledons rather than the hypocotyl were the main source of ^{59}Fe redistribution.

Discussion

These results are in accordance with other observations from our laboratory with respect to the

Table 1. Fresh mass (A) and distribution (B) of ^{59}Fe in organs from tomato cuttings

Parameter	Tissue	Mutant untreated	Mutant treated with NA	Wild-type untreated
(A) Fresh mass (mg)	Leaves	16.7 ± 4.6^{b}	40.8 ± 12.6^{b}	29.0 ± 7.5
	Cotyledons	79.5 ± 9.5	73.2 ± 9.9	62.3 ± 11.0
	Hypocotyl	86.5 ± 11.4	78.8 ± 13.0	68.3 ± 12.5
	Roots	Not present	44.5 ± 9.2	34.8 ± 13.0
	Total	182.7 ± 23.4^{c}	237.3 ± 39.0^{c}	211.3 ± 60.0
(B) Distribution of ^{59}Fe (% total)	Leaves	3.7 ± 0.5^{a}	14.0 ± 2.7^{a}	12.4 ± 3.4
	Cotyledons	64.7 ± 8.5	56.9 ± 8.5	65.8 ± 6.7
	Hypocotyl	31.6 ± 8.2^{c}	20.3 ± 7.2^{c}	15.2 ± 4.7
	Roots	Not present	8.7 ± 2.2	7.2 ± 2.1
	Total (cpm)	3132 ± 542^{b}	3192 ± 752	1914 ± 300^{b}

Seedlings were raised with ^{59}FeEDDHA and, after derooting, grown for 8 days in nutrient solution without added iron. In one set of mutants the cotyledons were supplied with 0.1 M nicotinamine (NA). Results are the means of six plants each ± standard deviation; ^{a} difference significant at 0.1%; ^{b} 1%; ^{c} 5% level fact that the mutant plants, in contrast to the wild-type ones, collapse at a low iron supply of about 1 μM FeEDTA but recover and retain the ability to develop a morphological and biochemical adaptive iron-deficiency response after addition of NA either to the leaves or to the nutrient solution (Scholz et al. 1985b; Stephan and Grün 1989). They are also in harmony with results of Becker et al. (1989) who demonstrated that the elongation growth of adventitious roots is NA-dependent.

It is clearly demonstrated that (a) phloem transport of iron from the shoot to the roots is also possible in tomato and (b) this transport is NA-dependent. Within the shoot the situation is not as obvious as between shoot and roots because participation of xylem transport in iron translocation from the hypocotyl or the cotyledons to the growing leaves cannot be completely excluded. It is, however, difficult to assume that a major part of the translocated ^{59}Fe was localized within the xylem at the time of root cutting. At least a significant portion of this iron must have been re-distributed via the symplast and the phloem from adjacent tissues or from the cotyledons. For the ^{59}Fe present in the adventitious roots, however, phloem transport seems to be the only way of import. That NA after application to the leaves of the mutant is transported to the roots has recently been demonstrated by Stephan, Scholz and Rudolph (unpublished results). Hence, co-transport of iron and NA seems to be plausible.

This experiment supports the view that NA is presumably essential for phloem loading and/or phloem transport of iron. In this context the question arises whether NA could be related to the iron(III) chelator in the phloem of *Ricinus communis* mentioned above (Mass et al. 1988). However, the relative molecular mass (M_r) of the NAFe(II) complex is about 360 as compared with 2400 for the iron(III) chelator of Maas et al. Moreover, at physiological pH, NA complexes ferrous rather than ferric ions (Beneš et al. 1983). If one follows the arguments of Mass et al. (1988), it could be concluded that NA might perhaps be the iron 'translocator' mentioned by these authors with "a high enough affinity to the ferrous iron" that cooperates with the iron(III) chelator in the process of phloem or symplast transport. The same authors also suggest a modulating effect of iron transported from the leaves to the roots via phloem on the iron-deficiency response mechanisms of the roots under low external iron supply. If a certain iron concentration in the root cells is attained, these inductive mechanisms are inhibited. This notion recalls our observation that in the NA-less mutant these mechanisms remain switched on even at rather high iron concentrations in the nutrient solution and high iron uptake rates, unless NA is supplied to the plants (Stephan and Grün 1989). In the light of the results presented above, this added NA restores the symplast and phloem transport of iron to the root cells. Thus, amongst other things, it may saturate the (in higher plants still hypothetical) repressor of the inductive iron-deficiency response mechanisms with iron(II) and inhibit the expression of the respective genes (Scholz et al. 1988).

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