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Physiological disorders of the nicotianamine-auxotroph tomato mutant *chloronerva* at different levels of iron nutrition. I. Growth characteristics and physiological abnormalities related to iron and nicotianamine supply

U. W. STEPHAN* and Ž. PROCHÁZKA

**Central Institute for Genetics and Crop Plant Research, Academy of Sciences of the GDR, DDR-4325 Gatersleben, German Democratic Republic and Institute for Organic Chemistry and Biochemistry, Czechoslovak Academy of Sciences, 16610 Prague, Czechoslovakia*

SUMMARY

The mutant *chloronerva* of *Lycopersicon esculentum* Mill. cv. Bonner Beste suffers from retarded growth of shoots and roots and a diminished chlorophyll content of the youngest leaves. The roots exhibit thickened tips and root hair zones as symptoms of iron deficiency. These abnormalities were diminished to a certain degree by an increased iron supply.

Nicotianamine (NA) supply to the leaves leads to a shift in the mutant characteristics towards the wild-type (so-called phenotypical normalization). The relationships between expression of the genetic defect and iron nutrition and the degree of phenotypical normalization by exogenous NA are discussed.

Key-words: growth, iron supply, nicotianamine, *Lycopersicon esculentum* Mill.. mutant *chloronerva.*

INTRODUCTION

The mutant *chloronerva* (*Lycopersicon esculentum* Mill. cv. Bonner Beste (BB) mutant *chloronerva (chln)*) suffers from retarded growth of shoots and roots, chlorosis of the intercostal areas of young leaves, and an inability to form generative organs (Böhme & Scholz 1960; Scholz 1967).

The biochemical basis of these abnormalities is the lack of nicotianamine (NA), a non-protein amino acid, found in all multicellular plants hitherto examined (Noma *et al.* 1971; Rudolph & Scholz 1972; Rudolph *et al.* 1985). There is a close relationship between NA and the phytosiderophores excreted by graminaceous plants (Takagi 1976; Römheld & Marschner 1986; Marschner *et al.* 1987). Contrary to these phytosiderophores, which are ferric chelators, NA forms complexes with ferrous iron and some other divalent heavy metal ions (Beneš *et al.* 1983), and, moreover, no excretion by the roots was observed.

In addition to its visible growth impairments, *chln* is characterized by a disturbed iron metabolism. Its viability seems to break down with low iron concentrations but with

Abbreviations: BB: Bonner Beste, *chln: chloronerva*, EDTA: ethylenediaminetetra-acetate, NA: nicotianamine.

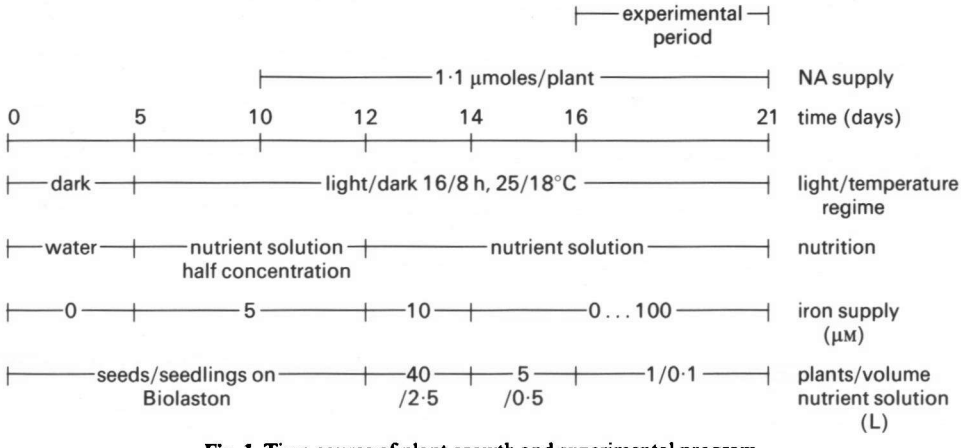

Fig. 1. Time course of plant growth and experimental program.

normal iron nutrition (10 μM FeEDTA) it takes up and translocates more than double the iron into the shoots than BB does (Scholz *et al.* 1985).

These abnormalities are partially or almost completely overcome by supplying the mutant with NA to the leaves or roots, dependent on the amount of NA supplied, or by grafting the mutant onto wild-type rootstocks (Böhme & Scholz 1960; Scholz 1983); denoted as phenotypical normalization.

The objectives of the investigations presented here are: (i) the relationship between iron supply within a wide concentration range and development of the mutant, and, (ii) the effects of NA supply at different iron concentrations in the medium on mutant development.

MATERIALS AND METHODS

Plant culture

Seeds were germinated on Biolaston (polyvinyl chloride needles, VEB Elastonwerk Friedrichroda, GDR) and the seedlings were grown in Hoagland's solution containing FeEDTA, as described earlier (Scholz *et al.* 1987). The light/dark regime was 16/8 h with temperatures of 25/18°C. The light intensity was 330 $\mu E\ m^{-2}\ s^{-1}$ and the relative humidity was approximately 65%. Fourteen-day-old seedlings were precultured for 2 days in groups of five plants in 500 ml nutrient solution containing different concentrations of FeEDTA (0, 2, 5, 10, 20, 50 and 100 μM; Fig. 1). All vessels for the culture with 0 and 2 μM Fe were washed with 3 M HCl and rinsed with distilled water before use. Our zero iron nutrition solution contained less than 0·1 μM Fe. This concentration is represented in the figures as 0·1 μM Fe.

Single plants, precultured with different iron concentrations for 2 days, were transferred into 100-ml tubes containing nutrient medium with the same iron concentrations as those applied during the preculture. The pH of these solutions was adjusted to 5·5 by the addition of KOH. During the experimental period (5 days) the loss of nutrient solution was compensated for daily with distilled water 4 h after the beginning of the light phase.

Plant growth analysis

At the end of the experimental period the main root length was measured. The adhering heavy metals were removed from the root surfaces by immersion in a solution containing

10^{-4} M $Ca(NO_3)_2$ and 10^{-4} M Na_2EDTA at 4°C for 30 min under stirring (Jooste & De Bruyn 1979, modified). This procedure was followed by estimation of the fresh weights of shoots and roots.

Chlorophyll content

The chlorophylls were extracted from the youngest leaflets on day 21 with 80% acetone (v/v) and the extinction at 646, 663 and 750 nm estimated. The calculations were performed according to Lichtenthaler & Wellburn (1983).

Phenotypical normalization of mutant plants

A 5×10^{-4} M NA solution was supplied to the leaves by a brush three times per day beginning at the time of leaflet emergence (10/11th day). Over a period of 10 days each plant received 2·2 ml solution containing 1·1 μmoles, theoretically corresponding to a mean tissue concentration of 400–1170 nmoles NA per gram fresh weight. This was dependent on the plant weight. Only one experiment of this kind was carried out because of a lack of substance.

Source of nicotianamine

L(–)nicotianamine was synthesized from azetidine-2-carboxylic acid according to a modified Kristensen & Larsen (1974) method as described by Procházka & Rudolph (1988).

RESULTS

Growth and development of plants

During the experimental period the root development of the wild-type reached a maximum at 2 μM Fe, whereas the mutant attained maximum growth at about 20 μM (Fig. 2c).

The diminished growth intensity of the mutant can also be deduced by comparison between fresh weights of shoots and roots. Under our experimental conditions *chln* exhibited approximately half the growth intensity compared with BB (Fig. 2a,b).

Visual symptoms of iron-deficiency in BB plants (chlorosis, retarded growth) could be observed at 0 and 2 μM Fe. Slight chlorosis appeared 24 h after the plants were placed into a low iron solution. In the course of the experimental period some zero-iron plants exhibited regreening of the youngest leaf parts. Iron-free cultivated *chln* plants did not show any development of the root. The leaves became fully chlorotic and small necrotic spots developed.

The chlorophyll content of the youngest leaves was lower in the mutant than in the wild-type. It increased up to 5 μM Fe in the medium and showed a tendency to level out in *chln* with a higher external iron supply (Fig. 3).

The formation of root hairs as a morphological indication of increased metabolic activity due to iron limitation was visible in the mutant up to an external iron concentration of 20 μM, whereas in the wild-type it was restricted to concentrations at and below 5 μM (Table 1). At the same time thickening of the root tips was observed in the mutant (contrary to the wild-type) with up to 10 μM Fe (Fig. 4).

Phenotypical normalization of the mutant by NA supply

In Fig. 2 the parameters of the phenotypically normalized mutant were recorded together with those of the wild-type and the untreated mutant. The properties of *chln* were shifted in the direction of BB by NA supply.

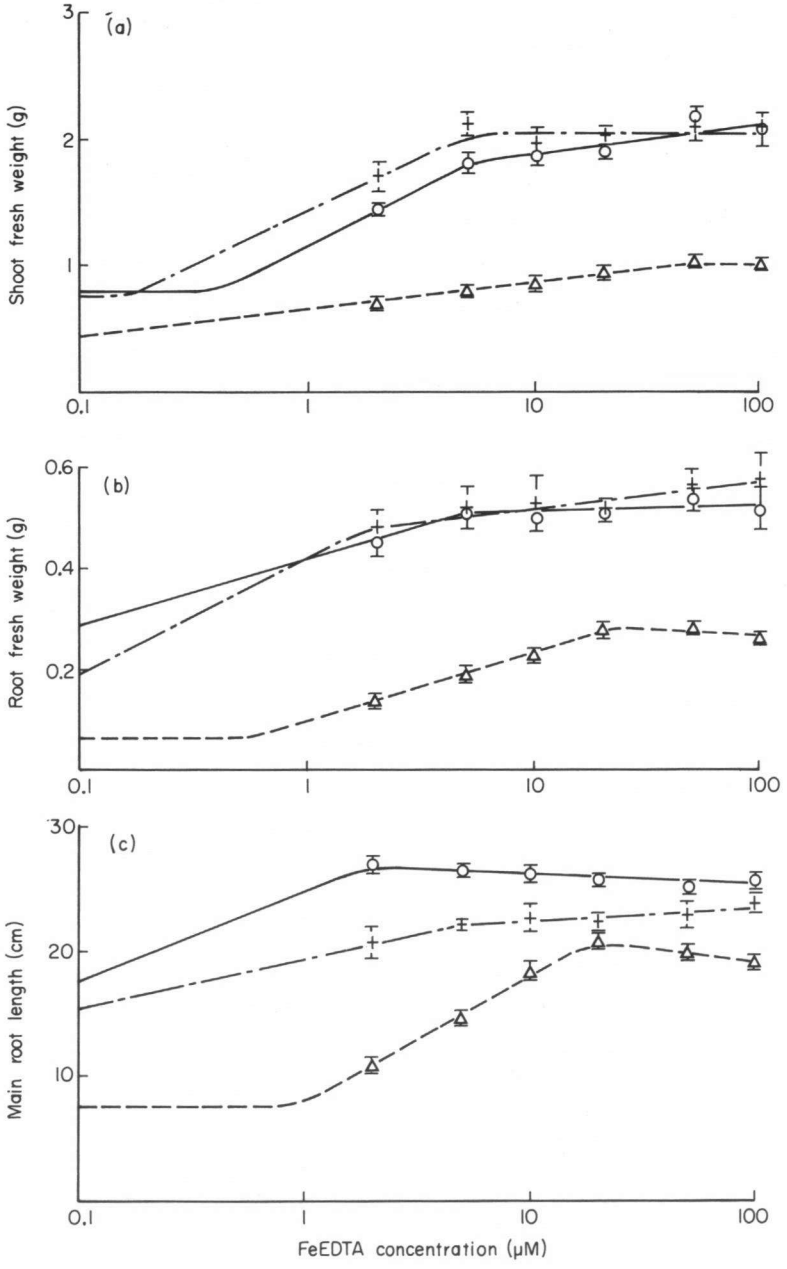

Fig. 2. Shoot fresh weight (a), root fresh weight (b), and main root length (c) at the end of the experiments (21 days). The points represent mean values of 24 plants from six experiments (wild-type, ○), 29 plants from seven experiments (mutant, △), and five plants from one experiment (mutant supplied with NA to the leaves, +), respectively. Bars indicate LSD, at $P=0{\cdot}05$.

The chlorophyll content of normalized mutant plants has not been estimated, but these plants were completely green without any signs of chlorosis, and appeared like wild-type plants.

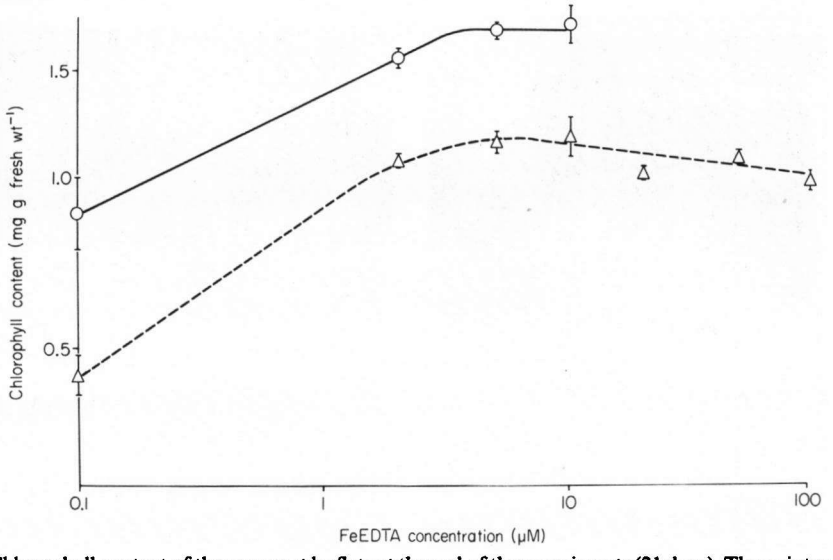

Fig. 3. Chlorophyll content of the youngest leaflets at the end of the experiments (21 days). The points represent mean values of five plants from one experiment (wild-type, ○) and 10 plants from two experiments (mutant, △), respectively. The contents at 20 and 100 μM Fe in *chln* are significantly lowered below that at 5 μM Fe at the 5% level (*t*-test). Bars indicate LSD at $P = 0{\cdot}05$.

Table 1. Root hair formation of wild-type and mutant plants at different FeEDTA concentrations. Nicotianamine (1·1 μmoles) was supplied to the leaves where indicated

Plant	Nicotianamine supply	Iron concentration (μM)						
		0	2	5	10	20	50	100
Wild-type	−	+	+	+	−	−	−	−
Mutant	−	−	+	+	+	+	−	−
Mutant	+	+	+	+	−	−	−	−

DISCUSSION

The concentration of 2 μM FeEDTA in the nutrient solution was sufficient for root extension growth of the wild-type, but suboptimal for the increase in fresh weight by shoots and roots due to the better development of secondary and tertiary roots which required approximately 5 μM (Fig. 2). The development of root hairs with up to 5 μM Fe (Table 1) can also be seen as an indicator of a persistent weak deficiency situation. Furthermore, since the chlorophyll content as a sensitive marker of the iron status was also increased up to 5 μM (Fig. 3), it is concluded that under our conditions this concentration is the lower threshold value for the full realization of the genetic potential of the wild-type. The sometimes observed regreening of the youngest leaf parts at zero iron culture is probably due to iron remobilization by rhizosphere acidification (Bienfait *et al.* 1985).

The mutant differed from the wild-type with respect to its growth characteristics in response to the iron supply. The drastic increase in primary root length with up to

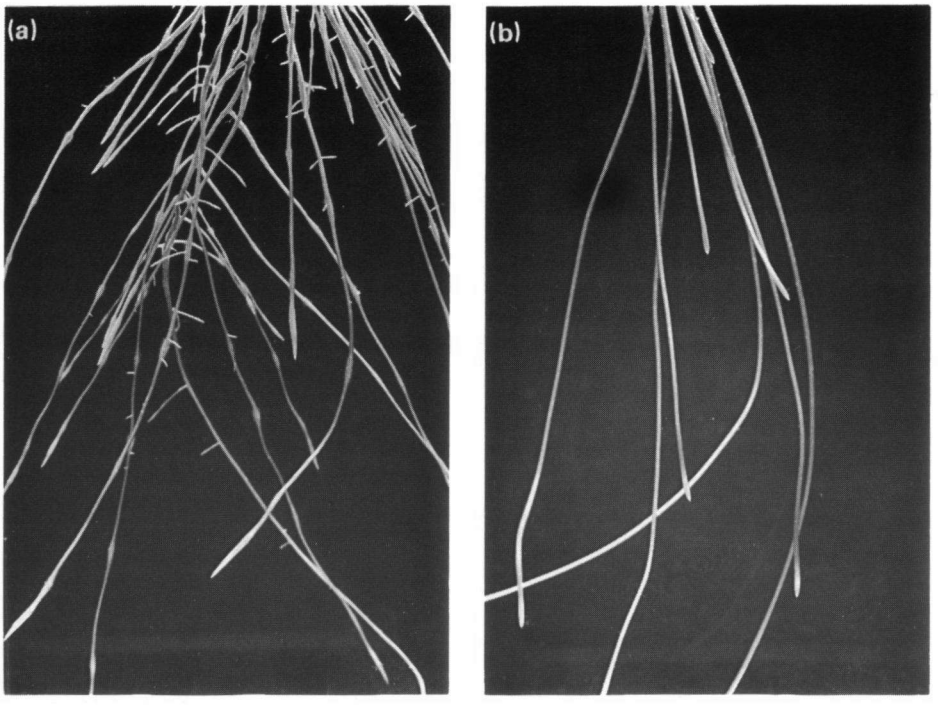

Fig. 4. Roots of 20-day-old tomato seedlings grown in nutrient solution with 10 μM FeEDTA. (a) Roots of the mutant *chloronerva* with thickened root tips and areas of root hairs as symptoms of apparent iron deficiency. (b) Part of the expanded system of thin lateral roots of the wild-type.

20 μM Fe indicated iron-deficiency below this concentration (Fig. 2c). On the other hand, even by increasing the iron supply the mutant was unable to respond by further increased growth and in no case was the level of the wild-type reached. The shoot and root fresh weights could not be increased by iron concentrations that exceeded 20 μM FeEDTA (Fig. 2a,b). The maximum chlorophyll content was reached at 5 μM Fe (Fig. 3). It seems that *chln* is unable to profit further from an increased iron supply. At the site(s) where development of iron-efficiency reactions are controlled, iron shortage must have been recognized (as the root hair formation indicates) up to 20 μM Fe (Table 1).

The stimulation by NA supply of main root longitudinal growth in the mutant has already been described by Scholz (1983) at 10 μM FeEDTA.

Phenotypical normalization is brought about by NA supply to the leaves. An increase in iron levels in the nutrition solution mimics the effect of NA, but even the highest iron concentration could not fully restore the wild-type appearance as NA does. The most straightforward explanation is that high FeEDTA levels improve the plant's iron status, but that in the absence of NA the distribution of iron between and within cells is unbalanced, thus resulting in suboptimal growth. Our results therefore support the proposal by Scholz *et al.* (1988) that the essential role for NA is as a cellular distributor of divalent metal ions.

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This is part 34 in the series 'The "normalizing factor" for the tomato mutant *chloronerva*'. For part 33 see Faust and Schreiber (1988).

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