Growth and photosynthesis of *Lycopersicon esculentum* (L.) plants as affected by nitrogen deficiency

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Abstract

Fully expanded leaves of tomato (*Lycopersicon esculentum*) growing with either complete or nitrogen-deficient nutrient solution were analysed for leaf water status, gas exchange and chlorophyll fluorescence during the vegetative and reproductive phases. N-deficiency did not affect leaf water relations but did decrease light saturated photosynthetic rate as well as stomatal conductance in the vegetative stage. A lower variable to maximum fluorescence ratio (F_v/F_m) was found in N-limited plants which also showed an increase in leaf starch content and in starch to sucrose ratio. The inhibition of photosynthesis and the alteration of photosynthates partitioning were responsible for the growth reduction in N-stressed plants. During the reproductive phase the limitation of photosynthesis may be due to a large accumulation of starch which determines both a decrease in the carbon demand from the sinks and a decrease in CO_2 conductance in the mesophyll.

Additional key words: carbohydrate partitioning, chlorophyll fluorescence, gas exchange, tomato, water status.

Introduction

The rapid photosynthetic rate in crop plants is reached by nitrogen fertilization which helped to the formation of leaves with high chlorophyll and RUBISCO content per unit area (*e.g.* Lawlor *et al.* 1989). However, for most crops, including tomato, fertilization is often excessive and causes pollution by nitrogen leaching.

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Abbreviations: Chl - chlorophyll; c_i - intercellular CO_2 concentration; E - transpiration rate; F_0 - ground fluorescence; F_m - maximum fluorescence; F_V/F_m, variable to maximum fluorescence ratio; g_w - stomatal conductance for water vapour; LAR - leaf assimilation rate; LMR - leaf mass ratio; NAR - net assimilation rate; P - pressure potential; P_N - net photosynthetic rate; q_{NP} - non-photochemical quenching; q_P - photochemical quenching; RGR - relative growth rate; RWC - relative water content; SLA - specific leaf area; VPD - vapour pressure deficit

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In the developed countries the awakening of public environmental consciousness has forced agriculture to revise the current cultivation techniques which are based on massive use of energy, water and chemicals and are responsible for serious pollution. One of the most important targets of the efforts that are being made has been to develop new cropping systems with low environmental impact. In greenhouse horticulture the employment of closed-loop soilless systems can provide an effective strategy to decrease the applications of fertilizers. Hydroponics may allow a precise splitting of mineral supply, thus improving fertilizer use efficiency; moreover, the recirculation of nutrient solution can substantially reduce the water contamination caused by nutrient leaching. In closed-loop hydroponics, however, plants may undergo cycles of low nutrient availability as the concentration of the recirculating solution may drop if a strict control system is lacking. Proper management of the mineral nutrition in soilless culture needs a precise knowledge of the crop requirements as well as the response to short periods of low nutrient supply.

The paper reports the data from work initiated to investigate the influence of N-deficiency on a tomato crop. On the plant's response to N stress a large body of papers were published, and our work is characterized by growing plants in water culture and using a low nitrate concentration in the nutrient solution (1 mM as against 10 mM in the control treatment) to expose the plants to N stress for a relatively short period (3 weeks). The research reported here was also designed to determine if tomato plants in two characteristic phenological phases, *i.e.* vegetative and reproductive, showed differential responses to N starvation.

Materials and methods

Plants and experimental conditions: Seedlings of *Lycopersicon esculentum* Mill. cv. Marmande were grown in pots with expanded clay and complete nutrient solution until the time of treatment. The composition of the nutrient solution was the following: 10 mM KNO_3, 1.5 mM KH_2PO_4, 2.5 mM $CaCl_2$, 1.0 mM $MgSO_4$, 0.05 mM FeEDTA (plus micronutrients in concentrations recommended by Hoagland). N-deficiency was applied during the vegetative stage (between 24 and 44 d from emergence) or during the fruiting stage (between 60 and 80 d from emergence) by growing plants in nutrient solution with N concentration of 1 mM (plus 9 mM KCl to achieve the same osmolality as the control solution). The nutrient solutions were renewed weekly and the pH was adjusted daily.

The experiments were conducted two times in the greenhouse under natural conditions in the spring season and repeated two times; minimum and maximum air temperatures were 17 and 28 °C, respectively, with daily global radiation ranging from 5.1 to 10.3 MJ m^{-2}.

The measurements of water relations, gas exchange, chlorophyll *a* fluorescence and pigment concentrations were performed on the fully expanded leaves at the third node numbered acropetally from the last fully expanded one.

Growth parameters: Leaf area and dry mass of stem, leaves and roots were determined and used to calculate relative growth rate (RGR), net assimilation rate (NAR), leaf area ratio (LAR) and specific leaf area (SLA) on the basis of the equations reported by Hunt (1978).

Measurements of leaf water relations: Relative water content (RWC) was measured gravimetrically on leaf discs. Leaf water potential (Ψ_w) was measured with a pressure chamber. Preliminary experiments showed a good correspondence between the values determined by the pressure chamber method and those obtained with a thermocouple psychrometer (data not shown). Osmotic potential (Ψ_s) was measured on expressed sap of frozen/thawed leaf tissues using a freezing-point osmometer. Pressure potential (P) was calculated as the difference between Ψ_w and Ψ_s.

Carbohydrate analysis: After being freeze-dried, leaf discs were extracted with 80 % ethanol. Saccharose was enzymatically assayed in the supernatant and starch was measured in the ethanol-insoluble pellet after digestion with amyloglucosidase as described by Ciompi *et al.* (1996).

Measurements of leaf gas exchange: Leaf gas exchange was measured in the laboratory using a temperature-controlled assimilation chamber connected with an *IRGA* in an open gas exchange system (*Heinz Walz*, Effeltrich, Germany). Temperature, humidity (VPD) and irradiance inside the leaf cuvette were maintained at values close to those recorded under greenhouse conditions between 12.00 and 13.00 h [22 - 26 °C, 1.1 - 2.2 kPa VPD), 1000 μmol(PAR) m^{-2} s^{-1}, supplied by a metal halide lamp (*OSRAM Power-Star HQI* 250 W) suspended above a water bath to remove infrared radiation]. The measurements were made at the ambient partial pressure of CO_2 (340 μmol mol^{-1}). Transpiration rate, E [mmol(H_2O) m^{-2} s^{-1}], net photosynthetic rate, P_N [μmol(CO_2) m^{-2} s^{-1}], conductance for water vapour, g_w [mmol(H_2O) m^{-2} s^{-1}], and intercellular CO_2 concentration, c_i, [μmol mol^{-1}] were calculated according to the equations reported by von Caemmerer and Farquhar (1981). Operational details are given elsewhere (*e.g.* Giannini *et al.* 1996).

Chlorophyll content and fluorescence: Measurements of leaf chlorophyll *a* fluorescence were carried out using a pulse amplitude modulation fluorometer *(PAM-2000, Heinz Walz*, Effeltrich, Germany). For details of the instrumentation see Guidi *et al.* (1997). The leaf was dark adapted for 30 min and ground fluorescence, F_0, was determined with a measuring modulated irradiance sufficiently low to not induce any variable fluorescence (< 1 μmol m^{-2} s^{-1}). The actinic and saturation irradiances were 150 and 15 000 μmol m^{-2} s^{-1}, respectively. Quenching coefficients were calculated as described by Schreiber *et al.* (1986).

Total chlorophyll was extracted from leaf discs with N,N-dimethylformamide for 24 h at 4 °C in the dark. The concentrations of chlorophyll were determined with a spectrophotometer using equations derived by Moran (1982).

Nitrogen content: The plant tissues were dried, weighed and ground, extracted with phosphate-sulphuric acid and then the total nitrogen was determined by Kjeldahl digestion and colorimetric analysis of NH_4^+.

Statistics: The paper reports data from representative runs as mean values of 3 - 10 replicates: each replicate was represented by one or two plants. Data were analysed by analysis of variance (*ANOVA*) and significantly different means were separated using the LSD-test.

Results

N-deficiency reduced leaf area and dry matter accumulation in both growth stages (Table 1). Root dry mass was not affected by N supply in the vegetative stage but it was reduced in fruiting plants. In both phases root-to-shoot ratio (on a dry mass basis) was higher in stressed plants than in the controls. Growth analysis showed that the decrease of RGR was due to a reduction in both NAR and LAR (Table 1). A slight decrease in SLA occurred as a consequence of N starvation, while no effects on LWR were observed.

Table 1. Growth analysis of hydroponically-grown tomato plants subjected to N-deficiency (-N) during the vegetative or the fruiting stage in comparison to controls (+N) grown with adequate N concentration of the nutrient solution. Means (n = 4) followed by the same letters are not significantly different at 5 % level according to *ANOVA* and LSD-test

	Vegetative phase		Reproductive phase	
	+N	-N	+N	-N
Stem and stalk dry mass	7.50a	4.10b	12.60a	8.70b
Leaf dry mass [g]	5.30a	2.60b	9.10a	5.00b
Root dry mass [g]	0.90a	0.70a	3.20a	2.40b
Fruit dry mass [g]			11.20a	7.00b
Root/shoot ratio	0.07a	0.10b	0.15a	0.18b
Total dry mass [g]	13.70a	7.40b	36.10a	23.10b
Total leaf area [cm^2]	2620.00a	1071.00b	3316.00a	1442.00b
RGR [% d^{-1}]	7.10a	4.00b	4.80a	2.60b
NAR [mg cm^{-2} d^{-1}]	0.66a	0.48b	0.37a	0.23b
LAR [cm^2 g^{-1}]	112.00a	88.00b	141.00a	126.00b
SLA [cm^2 g^{-1}]	274.00a	233.00b	429.00a	391.00b
LMR	0.50a	0.48a	0.32a	0.30a

N concentration of leaves, roots, stem and fruits was lower in stressed plants than in the controls in both growth stages (Table 2). N allocation was modified by N starvation; in fact, as compared to N-sufficient plants a larger amount of N was found in the roots of stressed plants, particularly during the reproductive stage when any differences in nitrogen content were recorded between stressed and control plants.

No consistent effects on leaf water content and potentials were found (Table 3).

In the vegetative stage N starvation decreased net photosynthetic rate (P_N), transpiration rate (E) and stomatal conductance (g_w) measured at saturating irradiance (Table 4). In stressed plants c_i was higher than in the controls because photosynthetic activity was affected to a larger extent than stomatal conductance. Quantum yield, compensating irradiance and dark respiration rate were not influenced by N nutrition. N-deficient plants also showed a lower saturating irradiance in comparison to the controls.

Table 2. N concentration and N content in different organs of the hydroponically-grown tomato plants subjected to N-deficiency (-N) during the vegetative or the fruiting stage in comparison to controls (+N) grown with adequate N concentration of the nutrient solution. Means (n = 4) followed by the same letters are not significantly different at 5 % level.

	Nitrogen concentration [% (d.m.)]				Nitrogen content [mg plant^{-1}]			
	vegetative phase		reproductive phase		vegetative phase		reproductive phase	
	+N	-N	+N	-N	+N	-N	+N	-N
Stem+stalk	1.4a	0.7b	1.0a	0.5b	100a	37b	131a	42b
Leaf	3.0a	1.7b	2.7a	1.2b	160a	44b	250a	60b
Root	1.9a	1.3b	2.0a	1.2b	16a	9b	63a	65a
Fruit			1.7a	0.9b			170a	56b

Table 3. Leaf water relations of hydroponically-grown tomato plants subjected to N deficiency (-N) during the vegetative or the fruiting stage in comparison to controls (+N) grown with adequate N concentration of the nutrient solution. Means (n = 4) followed by the same letters are not significantly different at 5 % level.

	Vegetative phase		Reproductive phase	
	+N	-N	+N	-N
RWC [%]	93.50a	94.00a	93.10a	91.00a
ψ_w [-MPa]	0.76a	0.67a	0.65a	0.74a
ψ_s [-MPa]	1.02a	0.94b	1.00a	1.08a
P [MPa]	0.26a	0.27a	0.35a	0.34a

In fruiting plants grown with reduced N supply P_N remained lower than in the controls (Table 4). E and g_w were not influenced by N-deficiency, which, however, increased the c_i/c_a ratio and the dark respiration rate and lowered the saturating irradiance and compensating irradiance.

Total chlorophyll (a+b) content per leaf area unit was considerably diminished in N-limited tomatoes in both growth stages (Table 5), but the Chl a/Chl b ratio was not affected.

During the vegetative stage the ratio between variable and maximum fluorescence (F_v/F_m) decreased significantly in stressed plants in comparison to the controls, which showed typical values of healthy plants (Björkman and Demmig 1987) (Table 5). The

alteration of the F_v/F_m ratio appeared attributable to the change in the ground fluorescence (F_0) which was increased by N-starvation. In fruiting plants the fluorescence parameters were not modified by N-deficiency with the exception of F_m which slightly decreased. The photochemical and non-photochemical quenching coefficients were not significantly affected by N-deficiency in either vegetative or reproductive phase.

N stress resulted in higher starch content of the leaves, particularly during the fruiting stage (Table 6). By contrast, saccharose content was similar in control and stressed plants in the vegetative stage, but it was increased by N-deprivation in fruiting plants.

Table 4. Leaf gas exchange parameters of hydroponically-grown tomato plants subjected to N-deficiency (-N) during the vegetative or the fruiting stage in comparison to controls (+N) grown with adequate N concentration of the nutrient solution. Means (n = 4) followed by the same letters are not significantly different at 5 % level.

	Vegetative phase		Reproductive phase	
	+N	-N	+N	-N
Net photosynthetic rate [μmol(CO_2) m^{-2} s^{-1}]	7.20a	3.60b	12.00a	7.30b
Transpiration rate [mmol(H_2O) m^{-2} s^{-1}]	2.00a	1.50b	2.90a	2.50a
Stomatal conductance [mmol(H_2O) m^{-2} s^{-1}]	149.00a	108.00b	205.00a	177.00a
c_i/c_a	0.75b	0.83a	0.71b	0.80a
Compensation irradiance [μmol m^{-2} s^{-1}]	14.00a	12.00a	21.00a	9.00b
Saturation irradiance [μmol m^{-2} s^{-1}]	1000.00a	700.00b	1200.00a	900.00b
Quantum yield [mmol(CO_2) mol^{-1}(photons)]	36.00a	38.00a	44.00a	42.00a
Dark respiration rate [μmol(CO_2) m^{-2} s^{-1}]	0.50a	0.40a	0.90a	0.40b

Table 5. Chlorophyll content and fluorescence in leaves of hydroponically-grown tomato plants subjected to N-deficiency (-N) during the vegetative or the fruiting stage in comparison to controls (+N) grown with adequate N concentration of the nutrient solution. The measurements of chlorophyll fluorescence were carried out on leaves dark adapted for 40 min. Means (n = 4) followed by the same letters are not significantly different at 5 % level.

	Vegetative phase		Reproductive phase	
	+N	-N	+N	-N
Total Chl content [mmol m^{-2}]	0.30a	0.20b	0.44a	0.26b
Chl *a*/*b* ratio	2.20a	2.30a	2.50a	3.00a
F_0	125.00b	142.00a	55.00a	55.00a
F_m	641.00a	615.00a	325.00a	292.00b
F_v/F_m	0.85a	0.77b	0.83a	0.81a
q_P	0.86a	0.81a	0.88a	0.91a
q_{NP}	0.26a	0.32a	0.27a	0.24a

Discussion

It is well known that N deficiency induces many morphological and physiological modifications in plants, resulting in strong inhibition of growth (Radin and Parker 1979, Clarkson and Hanson 1980, Radin and Boyer 1982). In our work, reducing N supply to tomato plants for a relatively short period substantially decreased leaf N concentration, leaf expansion, dry matter accumulation and increased carbon and nitrogen partitioning to the roots.

Table 6. Starch and saccharose contents in leaves of hydroponically-grown tomato plants subjected to N-deficiency (-N) during the vegetative or the fruiting stage in comparison to controls (+N) grown with adequate N concentration of the nutrient solution. Means (n = 4) followed by the same letters are not significantly different at 5 % level.

	Vegetative phase		Reproductive phase	
	+N	-N	+N	-N
Starch content [μg cm^{-2}]	56.53b	142.30a	56.06b	533.77a
Saccharose content [μg cm^{-2}]	112.28a	101.04b	54.02b	193.87a

Low pressure potential in the shoot has been reported as a factor responsible for growth reduction in N-limited plants (Radin and Boyer 1982, Radin 1983, Clarkson and Touraine 1994). In our work the maintenance of unchanged water status in N-stressed tomato plants indicates that under nutritional stress leaf elongation was not inhibited by reduced leaf N. These results agree with those reported by Chapin *et al.* (1988) on the same species.

Net photosynthetic rate declined markedly with N stress in tomato. In the vegetative stage this inhibition was associated with the decrease in g_w as also observed in N-deficient cotton plants (Radin and Ackerson 1981). On the contrary, stomatal components did not contribute to the decline in photosynthesis in fruiting plants. In the vegetative stage P_N varied with leaf N concentration and stomatal conductance. This suggests that the stomatal aperture was finely adjusted to the changing photosynthetic potential of the leaf although an increase in c_i/c_a was observed. Wong *et al.* (1979) proposed that stomatal aperture is related to the capacity of the mesophyll tissue to fix carbon. In their model, stomata act to regulate c_i when the capacity of the mesophyll tissue to fix CO_2 is altered. The data presented here for the vegetative phase in tomato are consistent with that model. The differences in the leaf conductance can be interpreted as a response to the differences in the capacity of the mesophyll tissue to fix CO_2 at low level of foliar N.

Some authors have suggested that the relationship between g_w, P_N and N content probably depends on the species (Thompson *et al.* 1992). In the case of tomato this differential response happened in two characteristics phases in its ontogeny.

The inhibition of leaf expansion growth and the reduction of g_w in the absence of any significant change in plant water status suggests the involvement of root signals (ABA, cytokinins) that indicate N-deficiency (Chapin *et al.* 1988).

The decline in chlorophyll content in N-deficient plants is widely reported in literature (*e.g.* Huber *et al.* 1989, Khamis *et al.* 1990, Ciompi *et al.* 1996). Although leaf chlorophyll content is not often correlated with carbon exchange rate, a statistically significant correlation (r = 0.97*) was observed in the present study. Strong correlation between photosynthetic capacity and chlorophyll content has also been found previously in maize subjected to nitrogen stress (Huber *et al.* 1989). The ratio Chl *a*/Chl *b* reflecting the thylakoid composition (Anderson *et al.* 1988) remained unchanged by N-deficiency as found in barley plants (Hák *et al.* 1993).

An increase in F_0 may occur if PS 2 reaction centres are damaged, or if the transfer of excitation energy from the antennae to the reaction centres is impeded or has a lower quantum yield. Also Kolber *et al.* (1988) reported in different species of marine unicellular algae that nitrogen limitation leads to substantial decreases in photosynthetic conversion of excitation energy. The decreasing value of F_v indicated the diminishing photosynthetic activity of the plant with N stress. It is also interesting to note that leaves of N-limited plants showed a lower F_v/F_m ratio as compared with leaves of controls. It would appear that N limitation may alter the photosynthetic machinery, both by increasing the F_0 and then probably diminishing the excitation energy transfer, and by decreasing the relative abundance of Chl. This may result in an increased sensitivity to photoinhibition (Rheil *et al.* 1986).

The observed decrease in P_N during the reproductive stage could be correlated with the high starch content as already observed in other species (Rufty *et al.* 1984, Huber *et al.* 1989, Paul and Stitt 1993). On the other hand, nutrient stress decreases sink activities, such as leaf expansion, more than it decreases photosynthesis (Radin and Boyer 1982). So the accumulation of starch may be induced by a decrease of the sink activity which leads to a reorientation of carbon allocation towards chloroplast storage forms, rather than towards a production of transport forms, *i.e.* saccharose (Foyer 1987).

During the reproductive stage, the decline in P_N was not related to a decrease in g_w, whereas c_i increased. The observed decrease of P_N could be correlated with the high starch content recorded in fruiting plants. The reduction in respiration compared with the control plant may be a sign of a slowing down of all synthetic activities. Nafziger and Koller (1976) have reported that the quantity of starch has an influence on the reduction of conductance of CO_2 by the mesophyll. In fruiting tomato plants, E was constant, together with g_w, and the increase in c_i/c_a ratio showed that substomatal CO_2 concentration remained stable under N starvation. The reduction of P_N because of the N-stress could then explained by a decrease of conductance of CO_2 by the mesophyll probably due to an accumulation of starch. On the other hand, fluorescence parameters were not affected by the stress, thus demonstrating that electron transport efficiency was not altered.

To conclude, the growth in N-stressed plants is lower because *1*) photosynthesis is reduced, and *2*) a significant proportion of the photosynthates is accumulated as starch in the roots, with a concurrent decrease in whole-plant leaf area and photosynthesis. A differential response was observed in vegetative and reproductive phases: in the former stage photosynthesis appears to be limited by stomatal and non-stomatal components, whereas in fruiting plants N-stress can induce a large

accumulation of starch which determines both a decrease of the carbon demand from the sinks and a decrease in CO_2 conductance in mesophyll.

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