How to Manipulate Axillary Bud Outgrowth in Tomato: Prospects for Practical Application?

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Abstract

Axillary bud outgrowth is controlled by apical dominance, which is regulated by the plant hormonal and plant nutritional status. In this study, we investigated perspectives for manipulation axillary bud growth by application of different N-forms to reduce manual work and thus production cost in greenhouse culture of tomato. Shoot growth was more affected than root growth when plants were supplied with NH_4^+ as a sole nitrogen, leading to a decreased shoot/root ratio. Also axillary bud outgrowth could be significantly reduced under NH_4^+ nutrition compared to NO_3^- nutrition. However, yield parameters such as number of flowers and trusses per plant, fruit per truss and total fruit yield were also decreased, and the incidence of blossom end rot in tomato fruit was increased by NH_4^+ applications. However, the fruit size was not affected by the supply of different N forms.

Key words: ammonium, axillary bud, *Lycopersicon esculentum*, nitrate

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Introduction

In greenhouse tomato production, several manual manipulation are performed very intensively and constantly, such as removing the axillary shoots, "truss pruning", removal of the oldest leaves, and keeping the main stem in optimal position. All these manual operations are necessary to maximise the quality and quantity of tomato fruits, but are also associated with high production costs. For tomato plants, a negative correlation between the weight of axillary shoots and yield has been demonstrated indicating that axillary shoots compete for nutrients and photosynthates with fruits. When de-shooting (removing of axillary shoots) was performed with low frequency, diameter and vigour of the stem were reduced, as well as the number of fruits per m^2 leading to a significantly lower yield (Navarrete and Jeannequin, 2000; Hartman, 1977). Thus, Navarrete and Jeannequin (2000) suggested an optimal de-shooting frequency between 7-14 days. However, the frequency of de-shooting also increases the mechanically induced stress (MIS), thus impairing yield (Biddington, 1986). Even simple shaking of tomato plants reduced the yield by 9% - 17% respectively when shaking was performed once or twice a day (Buitelaar, 1988). Also frequent mechanical measurements on leaves and fruits decreased height, leaf area and yield of sweet pepper plants (Kläring, 1999).

Lateral bud outgrowth is controlled by a process called apical dominance. In shoots, auxin derived from the shoot apex has been postulated to inhibit lateral bud growth either directly via entry into the lateral buds with subsequent repression of outgrowth or indirectly by involvement of i.e. activation of a second messenger, increased auxin-cytokinin ratio, secondary growth substances, nutrient diversion, auxin transport auto-inhibition, etc. (Bangerth *et al*., 2000; Li and Bangerth, 1999; Tamas, 1995; Cline, 1994; Bangerth, 1994; Cline, 1991; Martin, 1987). In many species, removal of the shoot apex resulted in the outgrowth of lateral branches (reviewed by Cline, 1991). Moreover, there is evidence that cytokinin is actively involved in the regulation of this process (Bangerth *et al*, 2000; Chatfield *et al*., 2000; Bangerth, 1994). Reduction of basipetal IAA transport leads to a strong increase in root derived cytokinin transport to the shoot. This increased delivery of cytokinin to the lateral buds promoted a strong increase in the production and export of IAA of lateral buds and their release from dormancy (Bangerth, 1994; Eklof *et al*., 1997; Kaminek *et al*., 1997).

It is well known that nitrogen (N) deficiency leads to a drastic decrease of cytokinin levels in the plants (Wagner and Beck, 1993; Kuiper *et al*., 1988). Moreover, there is evidence that the N form affects the endogenous levels of phytohormones such as cytokinin (Wang and Below, 1996; Gao *et al*., 1992; Smiciklas and Below, 1992). Our previous studies with tobacco (Walch-Liu *et al*., 2000) and tomato (Rahayu *et al*., 2001) revealed that NO_3^- supply compared to sole NH_4^+ nutrition increased the levels of zeatin+zeatin riboside (Z+ZR), the putative active forms of cytokinin, in the whole plant and at the same time particularly promoted shoot and leave growth. As a consequence of these enhanced cytokinin concentrations, the release of lateral bud growth may be stimulated more in NO_3^- than in NH_4^+ treated plants.

In this study, we focused on the effects of N form on axillary bud growth and yield quantity and quality of fruit in order to investigate options for minimization of cost-intensive manual removal of axillary shoots by manipulating plant growth and hormonal balances via fertilization management.

Material and methods

Plant cultivication

Seeds of tomato *(Lycopersicon esculentum* L. cv. Moneymaker*)* were placed on filter paper which was soaked with 10 mM $CaSO_4$ solution and kept in the dark at a temperature of 25°C. After germination, the seedlings were transferred to a hydroponic culture system (4 plants per 3 l pots) (Walch-Liu *et al*., 2000). After 4 weeks, each plant was transferred to 5 l pots, and after 8 weeks to 10 l pots until harvest time. Nitrogen was applied either as KNO_3 or $(NH_4)_2SO_4$ at a concentration of 2 mM N. For the NH_4^+ treatment, K_2SO_4 was added to compensate for K applied at the KNO_3 variant. Depletion of N in the nutrient solution lower than 50% was avoided by checking the N concentration in the culture medium at least once a day using a RQflex reflectometer (Merck, Darmstadt, Germany) and by re-supply of appropriate N amounts. The nutrient solution was replaced completely every 2 d and pH was adjusted to 6.8 - 7.2 by addition of $CaCO_3$. Plants were grown in a greenhouse with a temperature regime around 20-24^{o}C at day time and 18-16^{o}C at night time and additional light (300-400 µE m^{-2} s^{-1}) at a light/dark duration of 16/8 h.

The axillary shoot of the plants were removed by hand for all N treatments except the control plants every 3 days, after axillary shoot had reached about 2 cm length. Removal was performed for all axillary shoot including the ones located near the apex. The number of leaves at the main stem and the height of the main stem measured with a ruler was recorded at several defined times.

Yield parameters

After 14 weeks of growth, fresh weights of shoots, roots, axillary shoots of control plants and of single fruits were determined. The dry weight was obtained from oven-dried the fresh material at 70°C until the weight reached a constant value. Yield parameters, such as the number of flowers, trusses per plant, and number of fruits per truss were obtained by manual counting. The incidence of blossom-end rot was determined by visual observation after harvest of the fruits.

Analysis of Ca^{2+} concentration in the fruits

Fruits of the 1st – 4th fruit truss were separately analyzed, (3 fruits of different size per truss). Calcium concentration was determined according to Gericke and Kurmies (1952). About 250 mg dried fruit material was dry-ashed at 500°C for 5 hours and then dissolved in 1:30 diluted HCl solution. In the filtered solution Ca^{2+} concentration was measured by flame photometry (ELEX 6361, Eppendorf, Germany).

Analysis of organic acids in the fruits

Oven-dried fruit material was homogenised with 5% (v/v) H_3PO_4 (1ml acid per 10 mg dry weight). After centrifugation, the supernatant was diluted 10-fold with HPLC-elution buffer (18 mM KH_2PO_4; pH 2,1), and analysed by a reversed phase HPLC in the ion suppression mode. Separation was conducted on a 250 x 4 mm reversed phase column (GROM-SIL 120 ODS 5 ST, 5 µm particle size) equipped with a 20 x 4 mm Hypersil ODS guard column (Grom, Herrenberg, Germany). Sample solutions (20 µL) were injected onto the column and 18 mM KH_2PO_4 adjusted to pH 2.1 with H_3PO_4, was used for isocratic elution with a flow rate of 0.5 mL min^{-1} at 40°C and UV detection at 215 nm. Identification of organic acids was performed by comparing retention times and absorption spectra with those of known standards. The identity of citric, malic and oxalic acids was further confirmed by enzymatic tests (Boehringer, Mannheim, Germany) as described by Neumann and Römheld (1999).

Results

Effect of N forms on vegetative plant growth

Growth of tomato plants responded significantly to the supply of different N forms (Figs. 1A, C; 3). Shoot and root dry weight of NH_4^+ supplied de-shooted plants decreased by 38% or 21%, respectively, compared to NO_3^- supplied plants. Control plants without de-shooting behaved similar with a reduction of shoot dry weight by 43% at NH_4^+ supply compared to NO_3^- supplied plants (Fig. 1C). Although, root biomass was not influenced by the N form in control plants (Fig. 1C), the relation of shoot to root growth expressed as shoot/root dry weight ratio was significantly decreased at NH_4^+ supply compared to NO_3^- fed plants (Figs. 1B, D). Interestingly, the supply with different N forms had no significant effect on leaf number of the main stem, although NH_4^+-supplied plants had a significantly smaller height (Fig. 2) and smaller leaf area than NO_3^- supplied plants.

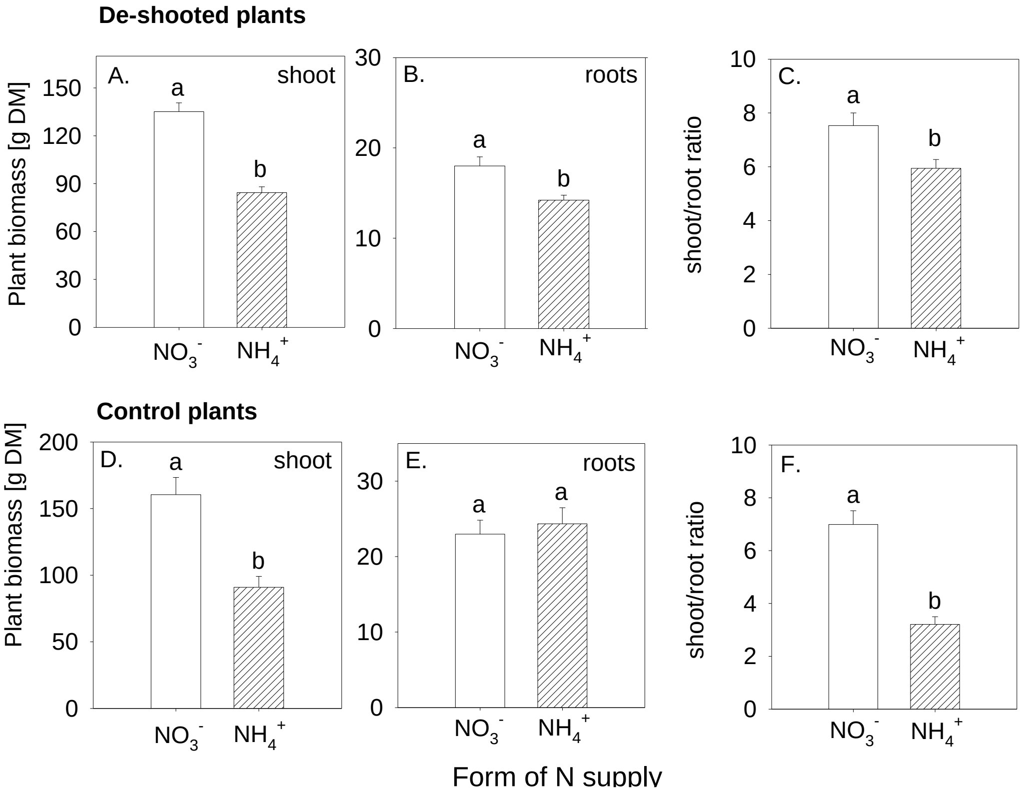

Fig. 1. Effect of different N forms on dry weight of shoots (A, D), roots (B, E) shoot/root ratio (C, F) of de-shooted tomato plants and of control plants.

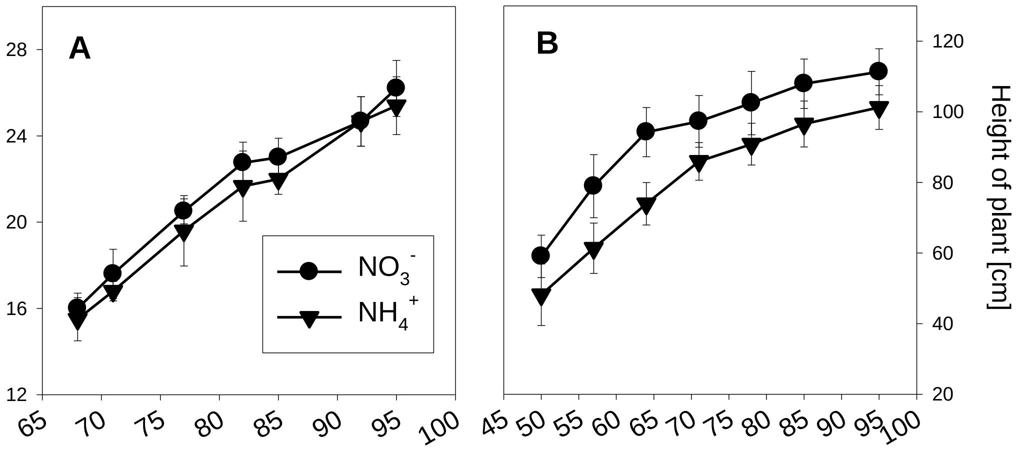

Fig. 2. Effect of different N forms on the number of leaves of the main stem (A) and height of tomato plants (B) at depending on plant age of de-shooted plants.

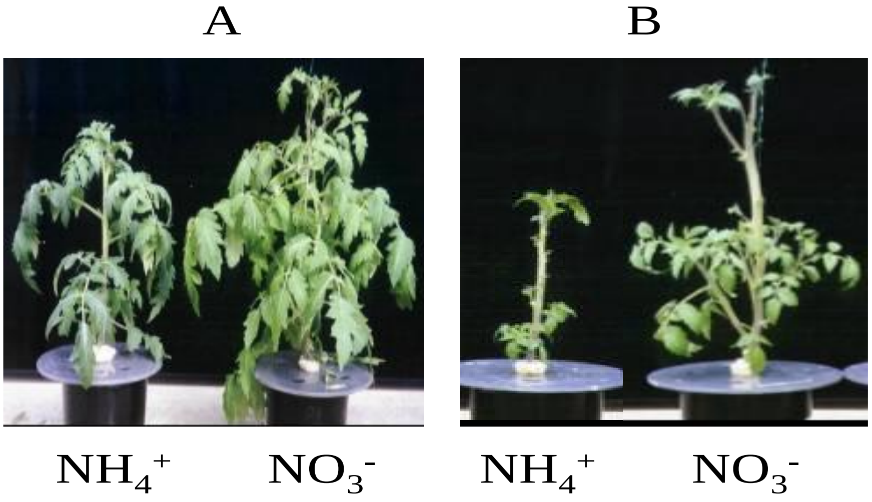

Fig. 3. Effects of different N forms on shoot growth (A) and axillary bud growth (leaves on main steam removed) (B). Plants were 8 weeks after sowing.

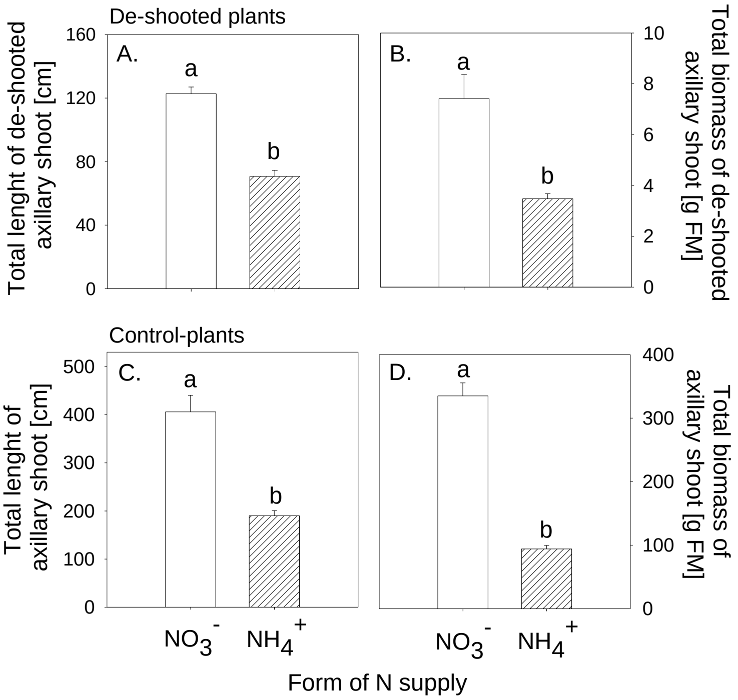

Fig. 4. Effects of different N forms on total length and weight of axillary shoot of de-shooted plants (A, B) and control plants (C, D).

Effect of N forms on axillary bud outgrowth

The supply of different N forms affected axillary bud outgrowth. In control plants (without removing the axillary shoot) developed less axillary shoot under NH_4^+ supply than NO_3^- treated plants (Figs. 3; 4A, B, C, D). Furthermore, compared to NO_3^- nutrition, NH_4^+ supply decreased significantly the length and weight axillary shoots by 33% and 53%, respectively of de-shooted plants (Figs. 4 A, B), and by 53% and 72%, respectively of control plants (Figs. 4 C, D).

Effects of N forms on yield

Fruit setting processes such as number of flowers, number of fruits, number of trusses, total fruit weight and single fruit weight were affected by the N form (Fig. 5). Compared to NO_3^- nutrition, NH_4^+ supply decreased the number of flowers, number of fruits and number of trusses by 40%, 21%, or 26%, respectively in de-shooted plants (Figs. 5 A, B, C), whereas mean fruit dry weight was not affected by the N form (Fig 5E). The final yield, particularly the total fruit weight was reduced by 48% in plants supplied with NH_4^+ instead of NO_3^- (Fig. 5D). Some fruits of NH_4^+-fed plants exhibited symptoms of blossom end rot, Ca^{2+} related disorder, which was not observed in fruits of NO_3^- supplied plants (Tab. 1). A similar trend was also obtained for control plants, which were not de-shooted (Fig. 5). While number of flower and trusses were decreased by NH_4^+ supply by 49% or 22%, respectively, (Figs. 5A, C), reduction of number of fruit was less pronounced (Figs. 5B). The total fruit yield and single fruit weight was not reduced by the NH_4^+ supply (Fig. 5D, E).

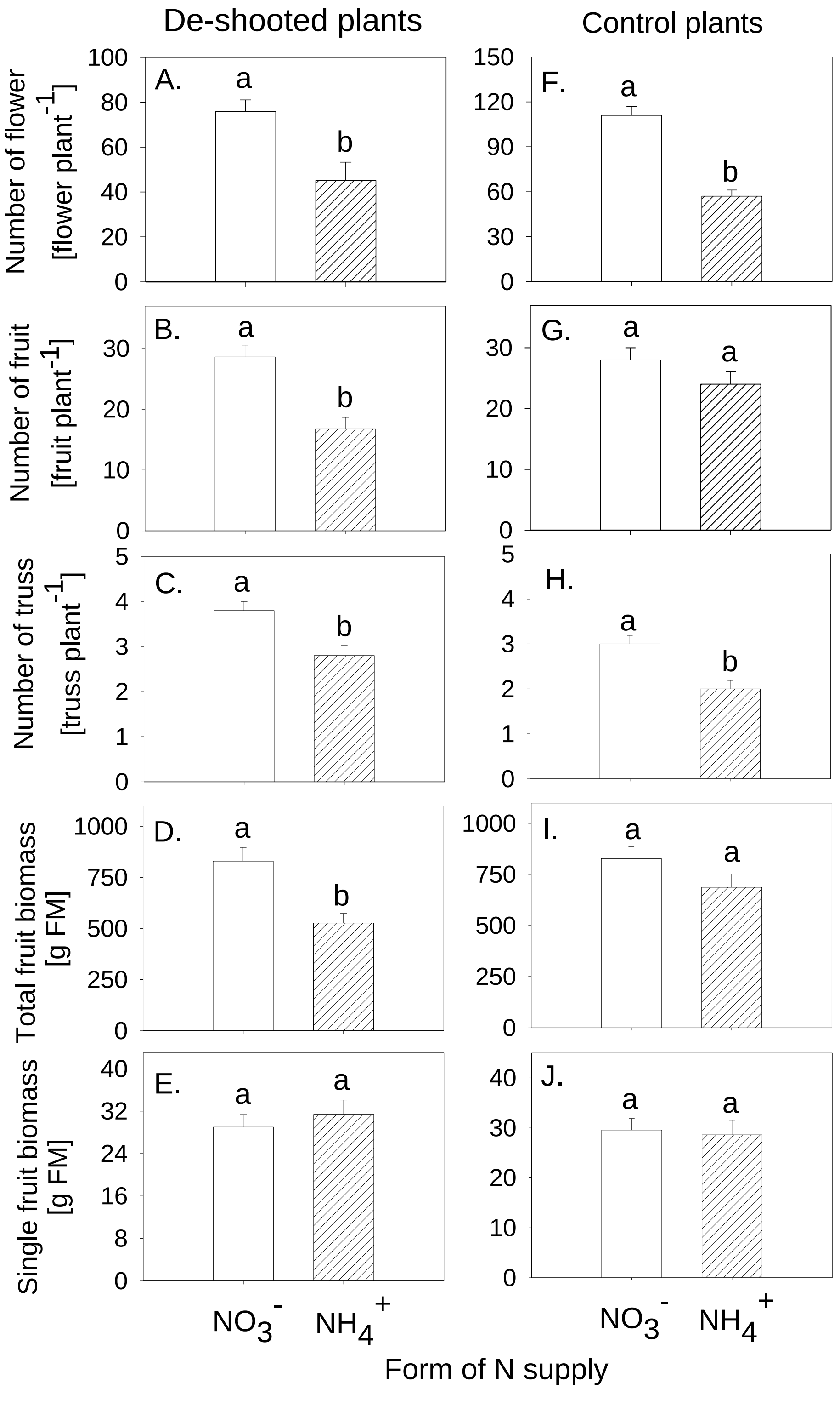

Fig. 5. Effect of different N forms on number of flower (A, F), number of fruit (B, G), number of truss (C, H), total fruit weight (D, I), and single fruit weight (E, J) of de-shooted and control plants.

Effect of N forms on Ca^{2+} and organic acid concentrations in fruits

Blossom end rot (BER), which was observed in fruits of NH_4^+ fed plants, is related to low Ca^{2+} concentrations caused by the limited ability of the plant to regulate Ca^{2+} distribution into growing regions and by the immobility of Ca^{2+} in the phloem and in non-vascular tissues (Bangerth, 1973). Therefore, Ca^{2+} concentrations were analysed in the fruits. However, the N from did not affect Ca^{2+} concentrations in fruits, although the fruits of NH_4^+ supplied plants showed incidence of BER (Table 1). Another cause for Ca^{2+} disorders could be its precipitation by chelators such as organic acids. However, NO_3^- supplied plants had even higher organic acid concentrations in the fruits than NH_4^+ fed plants (Tab.1).

Table 1. Effect of different N forms on incidence of blossom end rot (BER) of fruits, calcium concentration and organic acid concentration of fruits of de-shooted plants. Plants were grown in nutrient solution containing 2 mM nitrogen either solely supplied as NO_3^- or NH_4^+ and fruits were harvested 14 weeks after sowing.

Treatment	Ca conc. [mg (g DM)$^{-1}$]	Incidence of BER (%)	Organic acids [mg (g DW)$^{-1}$]					
			Malic	Shikimic	Citric	Fumaric	C-aconitic	aconitic
NO_3^-	1,40±0,05	0	20,5±1,1	46,8±1,4	22,7±1,2	17,8±2,4	6,4±1	13,7±1,5
NH_4^+	1,39±0,03	12,4±0,94	5,3±1,5	22,2±3,5	14,2±1,8	-	6,3±1,2	3,5±0,8

Discussion

NH_4^+ supply decreased plant growth

When plants were supplied with NH_4^+ compared to NO_3^-, shoot biomass was drastically reduced. (Figs. 1; 3A). These results are consistent with our previous studies with *Nicotiana tabacum* L. (Walch-Liu *et al.*, 2000), and also with other reports of *Beta vulgaris* L., *Lycopersicon esculentum* Mill., *Phaseolus vulgaris* L., and *Zea mays* L. (Raab and Terry, 1994; Cramer and Lewis, 1993; Magalhäes and Huber, 1989; Chaillou *et al.*, 1986). Reduced growth due to sole NH_4^+ supply was often related to decreased net photosynthesis caused by accumulation of NH_4^+ ions in the shoot (Claussen and Lenz, 1995; Goyal *et al*, 1982; Puritch and Baker, 1967), acidification of the external media (Gerendas *et al.*, 1997; Magalhäs and Wilcox, 1983), leading to NH_4^+ toxicity symptoms such as marginal necrosis and intervenal chlorosis on the leaves, wilting, stunted root growth or brownish roots (Goyal *et al.*, 1982; Maynard and Barker, 1969) or to a lack of osmolytes (i.e. nitrate, cations, and sucrose) involved in leaf expansion (Raab and Terry, 1995). However, previously we could demonstrate that these metabolic disorders related to NH_4^+ toxicity could be largely excluded by application of moderate NH_4^+ concentrations and by buffering the nutrient solution (Walch-Liu *et al.*, 2000). Ammonium-induced inhibition of leaf growth was associated with decreased zeatin+zeatin riboside (Z+ZR) concentrations in the xylem sap and in the plant tissue of tomato (Rahayu *et al.*, 2001) and of tobacco (Walch-Liu *et al.*, 2000). Cytokinins are involved in processes regulating leaf growth such as cell division and cell expansion (Francis and Sorrell, 2001; Ivanova and Rost, 1998).

Not only plant biomass, but also plant height was reduced by sole NH_4^+ supply, whereas number of leaves on the main stem was not affected, indicating that the N form had no affect on general plant developmental (Fig. 2). Similar results were shown for tomato, tobacco, and sugar beets (Rahayu *et al*, 2001; Walch-Liu *et al.*, 2000; Santamaria *et al.*, 1999; Raab and Terry, 1994).

NH_4^+ supply repressed lateral bud outgrowth

Ammonium nutrition significantly reduced axillary bud growth (Figs. 3, 4C, D) and also decrease the total length and weight of removed axillary shoot (Fig. 4 A, B). There is evidence that cytokinins are involved in the regulation of bud outgrowth. When cytokinins were applied to lateral buds, outgrowth was stimulated in intact plants (Mox, 1994). A study with *Arabidopsis* revealed that basally applied cytokinin was found to release outgrowth of lateral shoot from inhibition by endogenous or exogenous apical auxin (Chatfield *et al.*, 2000). Our previous studies with tomato and tobacco demonstrated that NH_4^+ supply obviously decreased cytokinin concentrations in the whole plant (Rahayu *et al.*, 2001; Walch-Liu *et al.*, 2000). Under these conditions low cytokinin levels may promote apical dominance leading to inhibition of lateral bud outgrowth.

However, the mechanisms controlling shoot branching and particularly the involvement of cytokinin are still obscure. Several models have been suggested to explain apical dominance involving an influence of auxin on cytokinin export from or biosynthesis by the roots (Bangerth, 1994; Eklof *et al.*, 1997; Kaminek *et al.*, 1997). According to this studies, reduction of basipetal IAA transport leads to a strong increase in root derived cytokinin transport to the shoot. This increased delivery of cytokinin to the lateral buds promoted a strong increase in the production and export of IAA of lateral buds and their release from dormancy. However, there are also evidence that the endogenous levels of these two hormones are not always correlated with the degree of shoot branching, in rms mutants of pea e.g. suggests that additional signals could be involved in regulating apical dominance (Beveridge *et al*., 1997a; Beveridge *et al*., 1997b; Beveridge *et al*, 1994).

NH_4^+ *supply reduced yield*

Ammonium nutrition decreased yield by reducing the number of flowers, the number of fruits and number of trusses (Figs. 5A, B, C, F, H) either due to increased flower and/or fruit abortion or due to low flower production. However, fruit size was not affected by the N form (Fig. 5E, J). Thus, the N form influenced flowering and fruit setting processes but not fruit growth.

Reduced yield of tomato plants receiving NH_4^+, instead of NO_3^- was also reported by other workers (Siddiqi *et al*., 2002; Sandoval-Villa *et al*., 2001; 1999). It was reported for legume that abscission processes leading to flower abortion may begin very soon after anthesis (Dybing *et al*., 1986; Brun and Betts, 1984; Huff and Dybing, 1980). This abortion may be caused by abscisic acid, a phytohormone which may be involved in promoting abscission of flowers and fruits (Taiz and Zeiger, 1998), since there is evidence that abscisic acid is increased by sole NH_4^+ nutrition (Peuke *et al*., 1994). Moreover, there is also evidence that cytokinin application to selected flower of soybean increased flower production and reduced the rates of flower abortion indicating that cytokinin levels play a significant role in determining total yield in soybean (Nagel *et al*., 2001; Reese *et al*., 1995; Mosjidis *et al*., 1993; Ghiasi *et al*., 1987; Dybing *et al*., 1986). Since in our previous studies with tomato (Rahayu *et al*., 2001) and tobacco (Walch-Liu *et al*., 2000) revealed that NH_4^+ supply decreased cytokinin levels and increased abscisic acid levels, it is likely that both flower initiation was decreased and flower abscission was increased.

Furthermore, there are reports that high cytokinin levels may increase fruit size during fruit development, because fruit size is a function of cell number rather than final cell size (Cowan *et al*., 2001) and control of final fruit size also depends on an early and high rate of cell division (Higashi *et al*, 1999; Bohner and Bangerth, 1988). However, in our study mean fruit size was not affected by lower cytokinin levels at NH_4^+ supply (Figs. 6E, 6.7E).

When de-shooting of plants was omitted, flower initiation was decreased by NH_4^+ supply similar to de-shooted plants. However the N form had no effect on number of fruit total yield and fruit size (Figs. 5G, I, J). Low frequency of de-shooting in tomato plants led to reduced vigour and diameter of the main stem, indicating a decreased vegetative growth compared to a de-shooting practiced every 7 days (Navarrete and Jeannequin, 2000; Navarrete *et al*., 1997). As a consequence of this decreased vegetative growth, yield and in particular number of fruits was reduced. Similarly, Hartmann (1977) reported that yield of tomato plants was decreased with increasing weight of axillary shoots remaining at the plant. It was suggested that a part of the assimilates produced by the main stem is diverted away from the fruits to the axillary shoots.

NH_4^+ supply reduced quality of fruit by increasing incident of blossom end rot (BER)

Fruits of NH_4^+-fed plants were affected at a higher percentage by the incidence of blossom end rot (BER) than fruits of NO_3^- fed plants. There are many reports confirming that application of NH_4^+ is associated with increased incidence of BER of tomato fruits (Siddiqi *et al*., 2002; Vielemeyer and Weissert, 1990; DeKock *et al*., 1982; Phil and

Lambeth, 1980). Moreover, when supplying both NO_3^- and NH_4^+, incidence of blossom end rot increased when the percentage of NH_4^+ reached 50% or more (Siddiqi *et al.*, 2002). Blossom end rot (BER) is associated with disintegration and increased ion permeability of cell membranes due to Ca^{2+} deficiency (Simon, 1978; Bangerth, 1973), as a consequence of the fundamental role of Ca^{2+} for membrane stability and cell integrity (Marschner, 1995). Blossom ends had always the lowest Ca^{2+} levels within the tomato fruit, and fruits affected by BER had lower Ca^{2+} levels than healthy fruits (Nonami *et al.*, 1995; Franco *et al.*, 1994; Adam and Ho, 1993; Ehret and Ho, 1986). However, in our study the N form did not affect Ca^{2+} concentrations in fruits (Tab. 1). These results are supported by our previous work with tobacco. Here we could not find a decrease of Mg^{2+} and Ca^{2+} concentration in expanding leaves of NH_4^+-fed plants compared to NO_3^- supply (Walch-Liu *et al.*, 2000). This is contradictions to many studies showing decreased cation concentrations (K^+, Ca^{2+}, Mg^{2+}) in plant supplied with NH_4^+ as nitrogen source (Siddiqi *et al.*, 2002; Jungk, 1977; Wilcox *et al.*, 1973; Barker *et al*, 1967). Furthermore, in our study the incidence of blossom end rot could not be related to Ca^{2+} levels (Tab. 1). Similar results are reported (Nonami *et al.*, 1995; Barker and Ready, 1994; Pill and Lambeth, 1980) that this missing relationship of BER and Ca^{2+} concentrations may be explained by a rather local Ca^{2+} deficiency, which is not reflected in Ca^{2+} concentrations of the whole fruit, since the Ca^{2+} concentration in the distal placental tissue of the fruit during the rapid expansion period is critical for the induction of BER in tomato, rather than the Ca^{2+} concentration of the whole fruit at maturity (Adams and Ho, 1992; Ehret and Ho, 1986).

For distribution of Ca^{2+} in fruits and thus incidence of BER, the development of xylem vessels and network of vascular bundles in the distal fruit tissue during fruit development may play an important role (Belda *et al.*, 1996; Belda and Ho, 1993). There is evidence that different levels of auxin or rates of auxin in transport together with other hormonal molecules such as cytokinins are important for vascular cell differentiation (Sachs, 2000; Aloni, 1993). Since in our previous studies NH_4^+ supply lead to decreased cytokinin levels compared to NO_3^- nutrition, (Rahayu, *et al.*, 2001; Walch-Liu *et al.*, 2000), it is likely that vascular cell differentiation and xylem formation in NH_4^+ fed plants is less pronounced than in NO_3^-supplied plants.

To explain the missing relationship of BER and Ca^{2+} concentrations it is also possibly that a part of Ca^{2+} was precipitated in the fruits by complexation with organic acids, e.g. citric acid. However, in our study NH_4^+-fed plants had less organic acids in the fruits than plants supplied with NO_3^- (Tab. 1), indicating that Ca^{2+} precipitation by organic acids might not be the cause for increased BER at NH_4^+ supply. Organic acid concentrations were also decreased in roots by NH_4^+ nutrition compared to NO_3^- supply (Marschner, 1995). The low levels of organic acid in NH_4^+ nutrition plants should be also considered, since the taste and aroma of tomato fruits are affected by organic acids concentration in the fruits (Stevens, 1986).

Conclusion

Our results showed that the specific application of different N forms can be used to manipulate the axillary bud outgrowth. Axillary shoot growth can be repressed by NH_4^+ nutrition, however, yield of NH_4^+-fed plants was reduced and fruits showed higher incidence of BER. Thus, an optimum yield should be gained by switching the supply of NH_4^+ to NO_3^- after initiation of the flowering process to decrease flower abscission and incident of BER or modify the NH_4^+/NO_3^- concerning the flowering process.

This type of using particular N-forms is valid or can be practised only under rather controlled growing conditions, e.g. if Nutrient Film Technique (NFT) is used. Further experiments should explore if altering NH_4^+/NO_3^- supply can reduce lateral shoot growth and possibly BER. With NFT control of the pH and N supply should be of no problem and could even be automated.

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