Response of Growth of Tomato to Phosphorus and Nitrogen Nutrition

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Abstract

A detailed growth analysis has been conducted to unravel the separate effects of nitrogen and phosphorus nutrition on growth of young tomato plants (*Lycopersicon esculentum* Mill. cv. Capita) and to study their interaction. We show that relative growth rate increased sharply with increasing plant P concentration before it levelled off, resulting in a broad plateau, while the response of relative growth rate (RGR, mg g^{-1} day^{-1}) to increasing plant N concentration was gradual and levelled off at high N concentrations, resulting in a small plateau. Possible causes of this different shaped response are discussed. Furthermore, we show that the importance of net assimilation rate (NAR, g m^{-2} day^{-1}) and leaf area ratio (LAR, m^2 kg^{-1}) in explaining the effects of N and P on growth may depend on the severity of the nutrient limitation. Finally we discuss the hypothesis that the regulation of the measured increase in dry-mass partitioning to the roots with decreasing N and P supply and the measured decrease in plant N concentration with decreasing P supply may be mediated by cytokinins. This paper summarises and discusses some of the data described in earlier papers (De Groot et al., 2001; 2002; 2003) on the regulation of growth by P and N nutrition.

INTRODUCTION

Besides sunlight and water, nitrogen and phosphorus can be major limiting factors for plant growth. The use of N and P fertilisers has increased dramatically over the last four decades. In greenhouses, crops like tomato are often grown on artificial substrates (e.g. rockwool). Plants are irrigated with water that contains a surplus of nutrients and this water may be recirculated. The uptake of nutrients by the crop may deplete some nutrients while other accumulate. Depletion as well as accumulation of nutrients may negatively affect crop growth, production and quality of products. The regular renewal of process water to counteract these negative effects is a burden for the environment. An accurate tuning of the supply of nutrients to the demand by the plant is necessary to minimise accumulation and prevent depletion of nutrients. Furthermore, this may be used to control the product quality. To achieve this accurate tuning, detailed knowledge of the regulation of growth by nutrients and their interaction with environmental factors is necessary. Furthermore, information on interactions between these nutrients is essential. A detailed growth analysis has been carried out to unravel the separate effects of N and P on growth of young tomato plants, and to study their mutual interaction. This paper summarises and discusses some of the data described in earlier papers (De Groot et al., 2001; 2002; 2003) on the regulation of growth by P and N nutrition.

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MATERIALS AND METHODS

Two experiments in which N or P supply was varied were conducted with tomato (*Lycopersicon esculentum* Mill. cv. Capita). At the beginning of the treatments (15 days after sowing (DAS) eight rates of N supply (N experiment) or seven rates of P supply (P experiment) were applied. P was supplied at a growth-saturating concentration of 1 mM $H_2PO_4^-$ (free access treatment) or was supplied daily to a P free nutrient solution (De Groot et al., 2001) at a relative addition rate ranging from 70 to 320 mg g^{-1} day^{-1}. The same treatments were applied for the N experiment, except that the relative addition rate ranged from 70 till 370 mg g^{-1} day^{-1} supplied to a N free nutrient solution (De Groot et al., 2002). The growth-saturating concentration in the N experiment was 12 mM NO_3^- (free access treatment). Plants were grown in 2.7 dm^3 containers with aerated nutrient solutions placed in a growth chamber with a photosynthetically active radiation (PAR) of 300 µmol m^{-2} s^{-1} for 16 hours a day (TL-D-HF, Philips, Eindhoven, The Netherlands) followed by 30 minutes of incandescent light (1 µmol m^{-2} s^{-1}). The relative humidity was 70% with a day/night temperature set to 23/23°C, which resulted in a day/night temperature of 24/23°C for the N experiment and 23/21°C for the P experiment. The composition of the macronutrients and trace elements are described in De Groot et al. (2001, 2002, 2003). This composition was based on experiments reported by Steiner (1984), who showed this composition to be optimal for the growth of tomato plants. Plants were harvested every fourth day after the start of the experiments (15 DAS). The data of 31 DAS are discussed in this paper.

For cytokinin analysis leaf material was extracted with 80% (v/v) ethanol. Ethanol was removed by evaporation under reduced pressure and the aqueous residues were taken up in water. Free and bound cytokinins were separated. The free cytokinins were purified by a combination of anionic exchange and reversed phase C18 column chromatography. Cytokinin quantification was performed by an enzyme-linked immunoassay (ELISA). A detailed description of the method can be found in De Groot et al. (2003).

The experiments were conducted twice, each time with three replicate plants per treatment per harvest, making a total of six replicate plants. Data were analysed with an ANOVA for the P experiment and with a REML (residual maximum likelihood) for the N experiment at a significance level of α=0.05. A more detailed description of the experiments can be found in De Groot et al. (2001) for the P experiment and in De Groot et al. (2002) for the N experiment.

RESULTS AND DISCUSSION

Growth Response to P and N Nutrition

The relation of growth or yield of a plant with the increasing concentration of a nutrient in the plant tissue resembles a saturation curve (Bates, 1971). Different nutrients may result in differently shaped curves (Burns et al., 1997), reflecting whether a nutrient has a more regulatory function or is a more structural component. In our experiments the growth response to plant N concentration indeed differed from the response to plant P concentration (Fig. 1). Relative growth rate (RGR, mg g^{-1} day^{-1}) increased sharply with increasing plant P concentration before it levelled off at high P concentrations, resulting in a broad plateau (Fig. 1). However, the response of growth to increasing plant N concentration was gradual and levelled off at high N concentrations, resulting in a small plateau (Fig. 1). Increasing the N supply rate from 320 mg g^{-1} day^{-1} to 370 mg g^{-1} day^{-1} to a constant rate of 12 mM did not increase the N concentration in the plant nor the relative growth rate of those plants (Fig. 1; De Groot et al., 2002). This shows that the two highest N supply rates and the free access treatment (12 mM nitrate) were high enough to gain maximal growth of these tomato plants. It is unlikely that the differently shaped curves of the response of growth to plant P and N concentration are caused by differences in accumulation of P and N since plotting RGR against the organic P and N concentration did not change the shape of the curves (De Groot et al., 2003). An explanation for these differences in response may be found in the roles of N and P in the machinery of the

plant's energy metabolism (De Groot et al., 2003).

Phosphorus and nitrogen play different roles in the energy metabolism of plants. A large portion of reduced N in a plant is associated with the machinery of the plant's energy metabolism, such as enzymes for photosynthesis and respiration, whereas a smaller portion is incorporated in structural cell components, such as structural proteins and nucleic acids. In contrast, P remains in its oxidised form and a relatively large part is incorporated in structural cell components, such as phospholipids and nucleic acids. A smaller portion of P is used as a component of the machinery of the plant's energy metabolism, where it is incorporated into phosphorylated intermediates of glycolysis and the Calvin cycle (Mengel and Kirkby, 1987). Therefore, from an increase in P supply almost all can be directly used for growth, while from an increase in N supply firstly the enzymes required to increase the rate of metabolism have to be synthesised (De Groot et al., 2003). This may offer an explanation for the steep increase and broad plateau of RGR with plant P concentration and the gradual increase and small plateau with plant N concentration as reported before.

Importance of NAR and LAR Depends on the Severity of the Nutrient Limitation

Plants can grow in a wide range of environments by adjusting their morphological and physiological characteristics to environmental conditions. The effects on growth of environmental factors like P and N limitation, can be determined by factoring RGR into the physiological component NAR (net assimilation rate, g m^{-2} day^{-1}) and the morphological component LAR (leaf area ratio, m^2 kg^{-1}) (Evans, 1972). In an analysis of literature, consisting of 75 observations, it was reported that on average the morphological component of relative growth rate (LAR) is more important than the physiological component (NAR) in explaining the effects of nutrient limitation on growth (Poorter and Nagel, 2000). However, in some experiments the opposite has been reported for several species including annual and perennial herbs, tomato and C_3 grass species (Corré, 1983; Guidi et al., 1998; Taub, 2002). We tested whether the severity of nutrient limitation can offer a possible explanation for the contradictive results reported in literature.

Our experiments showed that the slope of the relation between NAR and RGR was steepest at low RGRs (severe P and N limitation) and levelled off at higher RGRs (mild P and N limitation). The relation between LAR and RGR showed the opposite pattern (Fig. 2). This shows that at mild P and N limitation LAR was more important than NAR in explaining the change in RGR and that at severe P and N limitation NAR became more important. LAR is the product of SLA (specific leaf area, m^2 kg^{-1}) and LMR (dry-mass partitioning to the leaves) and reflects the morphological response of a plant, while NAR is largely the balance between photosynthesis and respiration and reflects the physiological response of a plant. The above implies that plants adjust to mild limitation by changing their morphology (leaf area, dry-mass partitioning) and to severe limitation by changing their physiology (photosynthesis, respiration) (De Groot et al., 2001; 2002).

Dry-Mass Partitioning

The decreases in N and P supply increased dry-mass partitioning to the roots (Fig. 3). This relation between dry-mass partitioning to the roots and leaf N concentration was linear and as long as the P supply treatments were not to severely limiting growth, the relation was the same for the N and P experiment. Only the three most limiting P supply treatments deviated from this relationship (Fig. 3). The relation between dry-mass partitioning to the roots and plant N concentration has been reported before to be strong and unaffected by growth irradiance (De Groot et al., 2002; De Pinheiro Henriques and Marcelis, 2000; Van der Werf and Nagel, 1996).

Plant N Concentration

Our experiments showed that plant N concentration decreased with decreasing P supply (De Groot et al., 2003). A possible explanation for the decrease in plant N concentration with P limitation may be found in the observed shift in dry-mass

partitioning from leaves, which have a high nitrogen concentration, to roots, which have a low nitrogen concentration. However, the N concentration of all plant organs decreased (De Groot et al., 2003), which means that there must have been an additional cause to explain the decrease in plant (and organ) N concentration with P limitation. N uptake may be decreased due to decreased energy availability in the roots (Rufty et al., 1993). However, root growth, as judged by the fraction of dry mass partitioned to the roots, increased (De Groot et al., 2003). Hence, decreased energy availability is unlikely to be a cause for the reduced N uptake with P limitation. We hypothesise that cytokinins play a role in the decrease in plant N concentration upon P limitation as well as in the regulation of dry-mass partitioning (Fig. 4).

A Hypothetical Model on a Role for Cytokinins

The regulation of dry-mass partitioning in response to nutrient limitation has been attributed to cytokinins (Beck, 1996; Kuiper et al., 1988; Van der Werf and Nagel, 1996). In leaves, high cytokinin levels promote cell division, while in roots high cytokinin levels inhibit cell division (Rayle et al., 1982). The reduced cell division in leaves upon decreased cytokinin levels may lead to a relative increase in root growth compared with shoot growth (Van der Werf and Nagel, 1996). As expected leaf cytokinin concentration decreased with decreasing nutrient supply and this was more pronounced for N than for P limitation (Table 1). In addition, the increase in dry-mass partitioning to the roots was more pronounced for N than for P limitation (Fig. 3; Ryser and Lambers, 1995). Dry-mass partitioning to the roots correlated well, for both the N and P experiment, with leaf cytokinin concentration (De Groot, 2002). Furthermore, addition of synthetic cytokinins to the nutrient solution can overcome the effects of a low mineral supply for dry-mass partitioning (Kuiper et al., 1988; Van der Werf and Nagel, 1996). This may suggest a direct relation between the decrease in cytokinin concentration upon nutrient limitation and the increase in dry-mass partitioning to the roots. However, further experiments in which the dry-mass partitioning is changed by wide ranges of nutrient supply with the simultaneous measurement of endogenous cytokinins are necessary to confirm this relation.

A low cytokinin concentration in the shoot inhibits nitrate reductase activity (Bueno et al., 1994; Lu et al., 1992), decreases net protein synthesis (Klämbt, 1977), and hence decreases N incorporation in the shoot (Simpson et al., 1982). It has been shown that increasing P limitation decreased leaf reduced-N concentration (De Groot et al., 2003), which is a measure for leaf protein concentration (Mengel and Kirkby, 1987). The decreased leaf protein level together with the decrease in leaf cytokinin concentration may offer an indication for the involvement of cytokinins in the response of plant N concentration to P limitation, via their effects on N metabolism. The suppression of P-starvation genes by supplying exogenous cytokinins to P-limited plants (Martin et al., 2000) is another indication of the involvement of cytokinins. It may be suggested that at P limitation, the decreased leaf cytokinin levels suppresses nitrate reductase activity and protein synthesis, and hence decreases plant N concentration (Fig. 4).

In conclusion, it is postulated that both the increase in dry-mass partitioning to the roots and the decrease in leaf reduced-N concentration with decreasing N and P supply may be the result of a decrease in cytokinin levels as summarised in our hypothetical model (Fig. 4). It should however be noted that this is a hypothesis that still has to be tested.

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Tables

Table 1. Total concentration (pmol g^{-1} dry mass, average of two pooled samples) of free zeatin riboside (ZR) equivalents and free isopentenyl adenine riboside (IPAR) equivalents in leaves. Within a column differences between means are indicated by different letters (n=2).

Nutrient treatment	N experiment	P experiment
Low (170 mg g^{-1} day^{-1})	95.0^{a}	141^{a}
High (320 mg g^{-1} day^{-1})	172^{ab}	217^{a}
Free Access	262^{b}	232^{a}
$LSD_{5\%}$	106.3	156.2

Figures

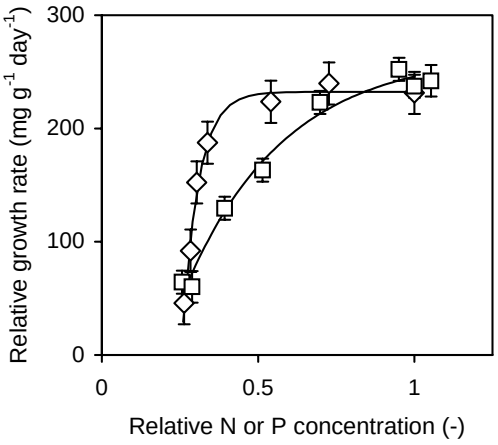

Fig. 1. Relative growth rate (RGR, mg g^{-1} day^{-1}) plotted against relative plant N or P concentration. The different plant N and P concentrations were reached by supplying the plants daily with nitrogen or phosphorus according to a supply rate of 70, 120, 170, 220, 270 and 320 mg g^{-1} day^{-1} for phosphorus. For nitrogen the supply rate of 370 mg g^{-1} day^{-1} was added to the treatments. A relative plant concentration of 1 represents the concentration of the free access treatment (12 mM nitrate, 1 mM phosphate). Diamonds: phosphorus, squares: nitrogen. Bars represent standard errors of mean, only shown when larger than symbol.

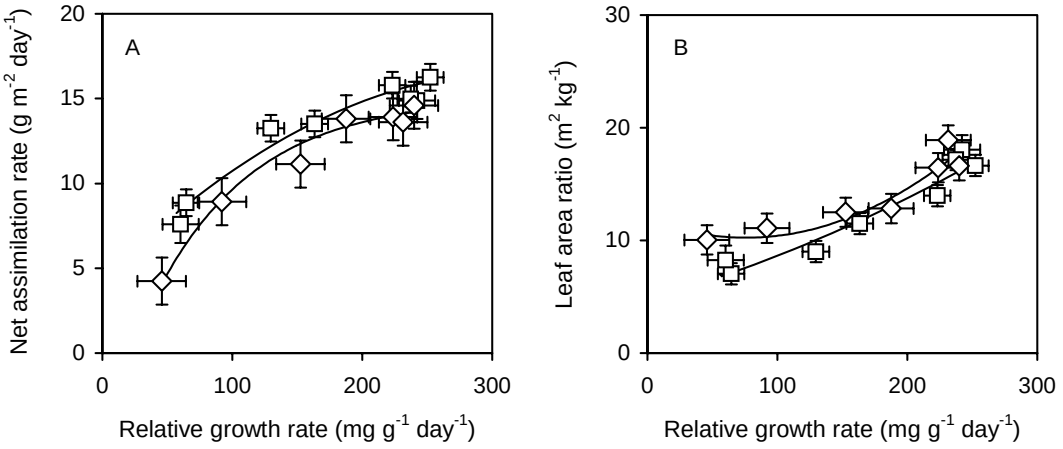

Fig. 2. Net assimilation rate (A) and leaf area ratio (B) plotted against relative growth rate. Diamonds: phosphorus, squares: nitrogen. Bars represent standard errors of mean, only shown when larger than symbol.

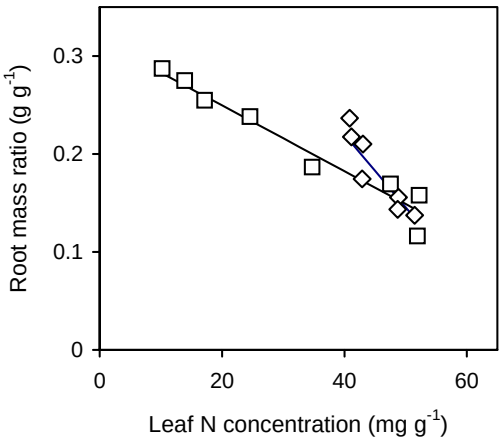

Fig. 3. Dry mass partitioning to the roots (root mass ratio) plotted against leaf N concentration. Squares: N experiment, diamonds: P experiment. Bars represent standard errors of mean, only shown when larger than symbol.

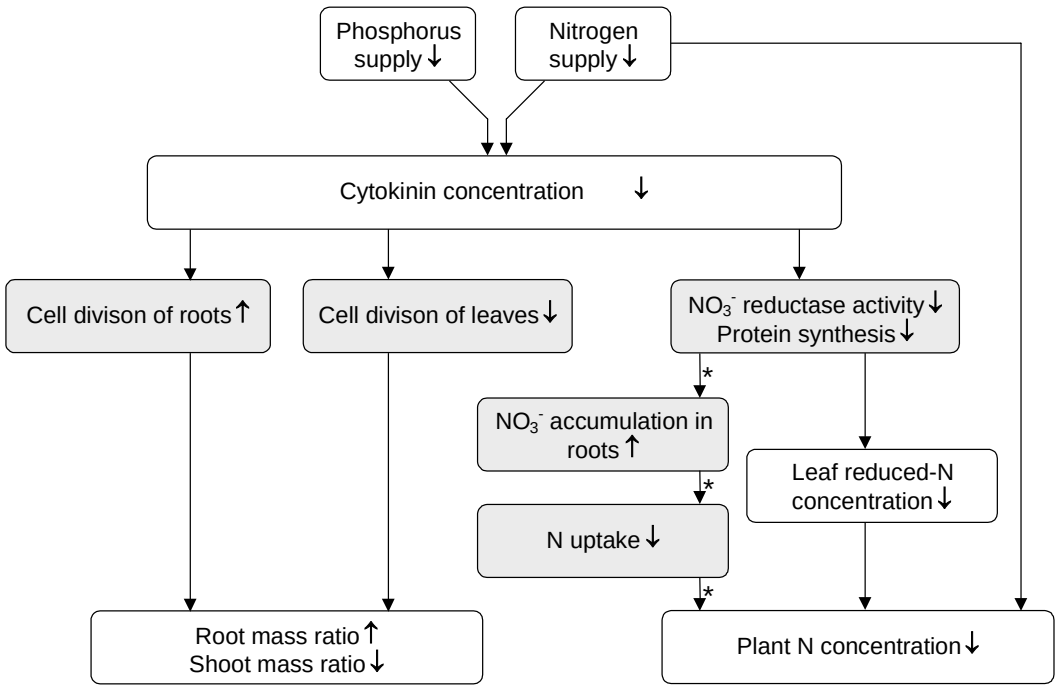

Fig. 4. Hypothetical model for regulation of dry-mass partitioning and plant N concentration mediated by cytokinins. This model is postulated based on results reported elsewhere (grey, see text for references), and results reported in De Groot et al. (2001, 2002, 2003) (white). In the text blocks: ↓ indicates a decrease, while ↑ indicates an increase. Arrows between text blocks indicate the sequence of effects. A * next to the arrows between text blocks indicates that the sequence of effects only applies to P limitation.