Nitrate-Regulated Growth and Cytokinin Responses in Seminal Roots of Barley

Mariann E. Samuelson*, Lennart Eliasson, and Carl-Magnus Larsson

Department of Botany, Stockholm University, S-106 91 Stockholm, Sweden

ABSTRACT

The influence of nitrate availability on growth of seminal roots, and root cytokinin levels, was studied in barley (Hordeum vulgare L. cv Golf). Nitrate was continuously supplied to initially N-starved seedlings at relative addition rates (RA) of 0.03 to 0.21 per day (standard cultures) or at RA 0.09 per day in split root cultures with the nitrate additions distributed in ratios of 100:0 or 80:20 to the two subroots. Data were collected both during a phase of acclimation (first 10 days of N additions) and in the acclimated stage (>10 days after onset of N additions). Limitation of whole-plant growth was observed at RA <0.15 per day. The lateral root frequency increased with RA in plants of equal chronological age. However, the lateral root frequency was related to root size rather than to RA; roots of uneven age but having comparable total root lengths also had comparable lateral root frequencies. Growth of individual subroots in split root systems during acclimation was proportional to the fraction of the total N addition that was fed to the root. All subroots had comparable relative growth rates in acclimated plants, and their lateral root frequency correlated with total root length in the same manner as in standard cultures. Onset of N additions in a 80:20 split root culture resulted in doublings of zeatin riboside (ZR) levels in shoots and in the “80” root, whereas the response of the “20” root was small. No effect of perturbed nitrate availability on xylem translocation of ZR was observed. The ZR levels remained higher in the “80” root during acclimation but returned to the level of the “20” root after acclimation. Root cytokinin levels and xylem translocation in acclimated standard cultures were unaffected by RA in the lower range but increased at high RA. Arguments for involvement of cytokinins in the nitrate-regulated growth response are discussed.

The first growth response observed in plants subjected to N limitation is normally a retardation of leaf expansion, whereas dry matter accumulation and C gain may still proceed undisturbed for several days (4, 11, 18, 19). The subsequent retardation of dry matter accumulation is more severe in shoots than in roots, causing the R:T2 ratio to increase (19, 31). Also, root morphology responds to N availability. The frequency of lateral roots increases in the nitrate-fed root part in systems in which nitrate supply is restricted to only a portion of the seminal root axis (7) or to single root axes (8, 15). Rates of nitrate uptake (measured as influx and net uptake after readdition of nitrate) increase initially during starvation but subsequently decline (5). If plants are maintained under long-term nitrate-N limitation, Vmax for net nitrate uptake is reduced in proportion to the restriction in N supply (17, 21, 24). However, tissue N concentrations decline, and the loss in specific uptake capacity is, on a relative basis (unit N taken up per unit N in the plant tissue), thus largely compensated. Instead, relative Vmax for nitrate uptake in species such as duckweed (24) and barley (21) actually increases the lower the N availability becomes. Within the N-limited range, this increase in relative uptake capacity is largely explicable in terms of increased R:T. “Acclimation” of the N acquisition system to low environmental N availability is thus partly a growth response, for which the change in R:T is an important determinant of the plant’s fitness (17).

There are conflicting opinions regarding whether these growth responses need to be explained in terms of hormonal growth control (31). Lowered N availability may decrease the fraction of absorbed N that is translocated to the shoot, thereby indirectly increasing the sink strength of roots for other resources (25, 31). The stimulation of proliferation in the plus-N root in split root systems has been interpreted as a consequence of in situ utilization of N absorbed from the nutrient medium during initiation of lateral roots; thus, the increased meristematic and metabolic activity increases the sink strength for assimilates (8, 9). Although these views do not imply direct roles for plant hormones, it is well known that changes in N availability strongly affect hormone levels, with cytokinins as a prime example. Cytokinin concentrations of roots, shoots, and xylem sap decrease at low N supply (10, 12, 26, 29), and a role for cytokinins from roots in the control of R:T at nutrient limitation has been suggested (12–14). Exogenous application of cytokinins delay the growth retardation caused by N limitation (12, 13, 29). Cytokinins are also important moderators during lateral root initiation, whereas auxin is considered promotive (30). The current work was initiated to give an integrated picture of the changes in root growth and development and in cytokinin levels and translocation in barley caused by differences in nitrate-N availability. Rather than comparing plus-N and minus-N culture conditions, i.e. the extremes of N availability, these experiments concern properties of plants maintained over a wide range of growth-limiting relative rates of nitrate-N additions (RA; ref. 21). Experiments were also addressed to the effects of localized nitrate supply in split root cultures, again using plants growing under overall N limitation.

1 This work was supported by the Swedish Natural Science Research Council.
2 Abbreviations: R:T, root:total plant biomass ratio; IPA, isopentenyladenosine; RA, relative addition rate; RG, relative growth rate; ZR, zeatin riboside.
MATERIAL AND METHODS

Plant Material and Culturing

Seeds of spring barley (Hordeum vulgare L., cv Golf) were imbibed in distilled water for 6 h and then sown in vermiculite. After 3 d of germination in the dark, the remaining endosperm was removed, and the seedlings were gently rinsed. Four to eight seedlings were then placed together in 1-L containers (standard cultures). In the split root cultures, the root system was divided into equal halves (normally three + three seminal axes), and the seedlings were placed eight to 10 together in black plastic containers with two compartments of 1 L each. The plants were kept in place using Styrofoam holders, with the shoot base elevated approximately 0.5 cm above the surface of the nutrient solution. Crown roots contributed little to total root mass and, when they did, only toward the end of the culturing time at the highest nitrate supplies used. No special care had to be taken to divide crown roots between the split root compartments.

During the following days, the plants were grown in an N-free but otherwise complete and well-aerated nutrient solution (21). The cultures received nitrate from day 8 after sowing. Nitrate-N and a surplus of other ions (21) were added to sustain a chosen relative rate of increment in plant N according to:

\[ N_t = N_0 \times e^{R_t \cdot t} \]

where \( N_0 \) and \( N_t \) are the N contents at the start and end of the time interval \( t \), respectively. The value of \( N_0 \) was 0.6 mg N seedling\(^{-1}\) on day 8, which corresponds to a N concentration of approximately 27 mg N g\(^{-1}\) dry weight. Nitrate and other nutrients were added continuously via an infusion pump operating at constant speed, with the concentration of the infusion solution increased on a daily basis to maintain RA constant. The nutrient solution was changed completely once a week to avoid major changes in pH or accumulation of exudates. The standard cultures were maintained at RAs ranging from 0.03 to 0.21 d\(^{-1}\). The split root cultures were maintained at RA 0.09 d\(^{-1}\) with the nitrate addition divided between the subroots in ratios of 100:0 or 80:20. The plants were kept in a climate chamber at constant environmental conditions: temperature and humidity were 20°C and 70%, respectively. The PPFD was 180 \( \mu \)mol m\(^{-2}\) s\(^{-1}\) (enough to saturate growth up to RG 0.15 d\(^{-1}\)) in the spectral range 400 to 700 nm, using General Electric F 96 PG17-Cwx Power Groove fluorescent tubes as light sources.

Growth Measurements

Growth was measured as the increase in dry weight between two harvest occasions within the interval 18 to 25 d after sowing. Dry weights were determined after oven drying at 70°C for 48 h. The RG was calculated on an exponential basis, using the equation:

\[ RG = \frac{(\ln W_2 - \ln W_1)}{t} \]

where \( W_1 \) and \( W_2 \) are the weights at the beginning and end of the time interval \( t \), respectively. Root growth and proliferation in terms of lengths of seminal axes, lengths of laterals, and frequency of laterals were further quantified using a PC-XT-based digitizer (1).

Collection of Xylem Sap

Xylem exudate was sampled from cut surfaces 1 to 2 cm above the root-shoot transition, after subjecting the roots to pressure (0.5 to 1 MPa) in a Scholander-type pressure bomb. For the split root cultures, one subroot was excised 1 h before sampling so that the xylem sap subsequently collected emanated only from the remaining subroot. The sap (approximately 10 \( \mu \)L per plant) was collected with a glass micropipette and was stored at -80°C until analyzed.

Purification and Quantification of ZR and IPA

Plant material harvested on days 22 to 25 after sowing was ground in liquid N, and samples corresponding to 2 g fresh weight were boiled in 20 mL PBS (pH 7.4) for 5 min, with butyrylhydroxyloluluril added as an antioxidant. The extract was cooled and then centrifuged at 16,000g for 15 min. The material was then passed through polyvinylpolypyrrolidone columns and immunopurification columns containing monoclonal rabbit antibodies raised against ZR or IPA (2, 3). After elution with 8 mL methanol, the eluate was evaporated to dryness and the residue dissolved in 500 \( \mu \)L deionized water. Samples of 500 \( \mu \)L were injected into a Spherisorb ODS1 3 \( \mu \)m, 4 × 30-mm column attached to an ISCO model 2350 HPLC pump (ISCO Inc., Lincoln, NB) equipped with an ISCO model 2360 gradient programmer. Samples were eluted with 2% acetic acid and 50% acetonitril. Elution was performed using linear acetic acid:acetonitril gradients. For IPA, elution started with a 10:90 ratio which gradually changed to 15:85 (4 min), 25:75 (6 min), and 30:70 (13 min). For ZR, ratios were 1:99 (start), 3:97 (4 min), 10:90 (6 min), and 15:85 (9 min). The flow rate was 2 mL min\(^{-1}\) and the absorbance of the elute was recorded at 265 nm, using a UV detector (Spectro monitor 3100, Milton Roy, Riviera Beach, FL). Fractions of 2 mL corresponding to the retention times of ZR and IPA were collected and evaporated to dryness, and the residue was dissolved in 1 mL PBS (pH 7.4). The retention times for the two ribosides were clearly distinct from the retention times of their bases. Final quantification was done using an ELISA assay (2, 3).

Replicates

Data were obtained from at least three, normally five, different cultures. Because of some variation in absolute concentrations of cytokinins, data for ZR and IPA are from single experiments; however, there were no major qualitative differences between them.

RESULTS

General Development and Dry Matter Accumulation

Nitrate-N additions to the initially mildly N-starved seedlings started on day 8 after sowing. The following 10 d are
considered as an acclimation phase, characterized by adjustments of tissue N levels and overall RGs to the RA of nitrate-N addition. During the following days (the acclimated stage), growth in terms of whole-plant RG was essentially stable. Tillers and crown roots were absent at RAs up to 0.15 d$^{-1}$, whereas one or two tillers and few crown roots were observed at the end of the cultivation period at RA >0.15 d$^{-1}$. Shoot elongation took place in the acclimated stage, whereas booting and ear emergence were observed from day 35 onward, i.e., after the latest time of harvest in the present study.

The RG of standard cultures, measured in the acclimated stage as dry weight increments of whole plants, increased with nitrate availability up to RA 0.18 d$^{-1}$. Root RG was less affected by RA (Fig. 1). Thus, the R:T ratios were unstable in the acclimated plants, with the exception of medium RAs, for which root and shoot RGs were nearly equal. Below this range, R:T progressively increased, whereas it decreased at higher RAs. On day 25, R:T values ranging from 0.42 (RA 0.03 d$^{-1}$) to 0.31 (RA 0.21 d$^{-1}$) were measured. These growth data confirm those reported previously for barley (21) and are similar to those for another annual species, pea (17), maintained under RA limitation.

The whole-plant RG for the split root cultures maintained at RA 0.09 d$^{-1}$ was 0.076 ± 0.013 (average ±SD), and the R:T was 0.38 ± 0.08 on day 22 after sowing. These values conform to those obtained in the standard cultures (RG 0.079 ± 0.005 and R:T 0.38 ± 0.06 on day 22 after sowing). There was no effect on either of these parameters caused by uneven distribution of the total N addition to the individual subroots (E. Öhlén and C-M Larsson, unpublished data). However, the contribution of each subroot to total root mass increased with increased fraction of the total N addition that was fed to the root. An example of this, for the 80:20 root, is given in Figure 2. There was a gradual shift in the contribution of each subroot to total root mass during acclimation so that, in the acclimated stage, the weight proportion was approximately 65:35. Very few further changes were observed, indicating equal RGs of the subroots during acclimated growth. The growth response was reversible; reversal of the addition ratio resulted in a rapid reversal of the weight ratios (Fig. 2).

**Root Structure**

The total root length in standard culture plants harvested at the same chronological age (day 22 after sowing), increased drastically with RA, mainly dependent on a fivefold increase in the length of lateral roots over the RA interval from 0.03 to 0.18 d$^{-1}$ (Fig. 3). The data further indicate that a higher RA results in increased lateral root frequency (number of lateral roots m$^{-1}$ seminal axis), from 60 to 160 during the same RA interval (Fig. 3, inset). This effect was, however, mostly due to the fact that lateral roots were confined to only the upper region of the seminal axis at low RA. An additional measurement of lateral root frequency was, therefore, made on the basis of equal total root lengths (4.70 ± 0.13 m), i.e., plants were harvested at different chronological ages (days 32, 22, 18, and 16 for RA 0.03, 0.09, 0.12, and 0.18 d$^{-1}$, respectively). Compared in this way, the results did not reveal any tendency toward increased lateral root frequency at higher N availability (Fig. 3, inset).

Measurements of root lengths in the split root cultures (addition ratios 100:0 and 80:20) show that the difference in root mass of the subroots after acclimation is mainly explicable in terms of effects on lateral root growth, whereas effects on seminal axes were negligible. As expected from dry weight measurements (Fig. 2), these growth responses were compen-

---

**Figure 1.** RGs of whole plants, roots and shoots as a function of RA. RG was calculated from dry matter increment in the acclimated growth stage. Dashed line, RG = RA.

**Figure 2.** Weight proportions of subroots of plants growing at RA 0.09 d$^{-1}$, with the nitrate addition divided to the subroots at a ratio of 80:20, as a function of time. Also shown are data from a culture in which the nitrate ratio was reversed on day 11 from onset of N addition.
satory; thus, the sums of both lateral and total root lengths were similar in the 100:0 and 80:20 cultures. Also, the frequency of lateral roots increased with increased proportion of the total N addition that was fed each subroot (Fig. 4, inset). The data concerning lateral root frequency seem to fit into a general relationship between frequency and total root length which emerges when all root measurements are pooled (Fig. 5).

**Cytokinins**

The levels of endogenous ZR in the root system of standard cultures acclimated to growth-limiting RA were approximately 2 pmol g\(^{-1}\) fresh weight but increased substantially at surplus nitrate nutrition, i.e. RA 0.21 d\(^{-1}\) (Fig. 6A). The levels of endogenous IPA were approximately 6 pmol g\(^{-1}\) fresh weight and remained unaffected over the whole RA range studied (Fig. 6A). Shoot ZR and IPA levels were more variable, ranging between 2 and 5 pmol g\(^{-1}\) fresh weight for ZR and between 4 and 9 pmol g\(^{-1}\) fresh weight for IPA (Fig. 6B). Xylem sap contained ZR and IPA in similar concentrations. Translocation of cytokinins from roots to shoots (estimated as the product of transpiration and xylem sap cytokinin concentrations) was on a unit root weight basis on the order of 5 pmol g\(^{-1}\) fresh weight d\(^{-1}\). The translocation data resembled the data for root ZR in the sense that increased translocation was observed at high or supersaturating nitrate availability, whereas differences at growth-limiting RA were small (Fig. 6B).

The time course of changes in ZR levels during acclimation was studied in split root cultures with the nitrate distributed to the subroots at a 80:20 ratio (Fig. 7). Also, IPA levels were studied, but no changes were observed (data not shown). The subroots receiving 80% of the daily nitrate addition, as well

---

**Figure 3.** Length of seminal axes and lateral roots of 22-d-old plants grown at different RAs. Inset, Lateral root frequency as a function of RA in plants harvested at equal chronological age (22 d) and in plants with comparable total root length (4.70 ± 0.13) harvested on days 16, 18, 22, and 32 for RA 0.18, 0.12, 0.09 and 0.03 d\(^{-1}\), respectively.

**Figure 4.** Length of seminal axes, lateral roots, and total roots on day 22 for split root cultures maintained at RA 0.09 d\(^{-1}\) with the nitrate distributed to the subroots in ratios of 100:0 and 80:20. Inset, Lateral root frequency as a function of fraction of the total N addition fed to each subroot.

**Figure 5.** Relationship between lateral root frequency and total root length. Data are pooled for plants growing at different RAs and harvested at equal age or at unequal age but approximately equal total root length, and plants grown in split root cultures in which the total root length for each subroot has been multiplied with a factor of two.
as the shoot, doubled their ZR levels in 1 d, whereas no significant response was seen in the roots receiving 20% of the total nitrate dose. The ZR level in the “80” root remained above that of the “20” root for the whole acclimation period but eventually returned to levels close to the original and close to the “20” root. Also, the shoot ZR level gradually declined to close to the initial value. The time course of changes in translocation of cytokinins during acclimation was also studied, but because enough xylem sap for cytokinin analysis could not be sampled from the very young seedlings, cytokinin translocation as affected by changed nitrate availability was estimated in plants in which the addition ratio was reversed from 80:20 to 20:80 (cf. dry matter distribution in Fig. 2). There appeared to be no significant effect on ZR translocation following this treatment (Fig. 7, inset).

DISCUSSION

Growth Response

Whole-plant RG in acclimated cultures correlated to RA up to approximately 0.15 to 0.18 d⁻¹. Considered separately, the effect of RA was slightly more pronounced on shoot growth than on root growth (Fig. 1), in accordance with earlier results (21). The profound influence of external nitrate on development of the cereal root system reported previously (cf. “Introduction”) is, nevertheless, clearly evident in the present results. Thus, size (Fig. 3), lateral root frequency (Figs. 3 and 4), and biomass distribution within the root system (Figs. 2 and 4) respond strongly to nitrate availability. The first two consequences were also observed in Pisum cultured at an RA range of 0.03 to 0.28 d⁻¹, when the plants were harvested at equal chronological age (23).

The question arises whether these data should be interpreted as a genuine morphogenetic effect of nitrate or whether they should be regarded as consequences of different rates of general root development. The data indicate that increased nitrate availability accelerates root growth in terms of both length growth and branching. However, when a slowly growing root has reached the same total length as a more rapidly growing root, it has done so by reaching the same degree of branching but at a more advanced age. In split root cultures the biomass distribution within the root system is a consequence of an initial and transient response to nitrate availability (Fig. 4). During subsequent growth, the RG of individual subroots is fairly unaffected (Fig. 2). A general relationship between lateral root frequency and total root length can be suggested (Fig. 5), in which the data obtained for individual subroots in split root cultures also can be integrated. It does not seem, therefore, that nitrate availability qualitatively influences the growth of the barley root system, but it may accelerate or retard the rate of development.

Cytokinin Response

The first free cytokinin formed is probably IPA-5′-monophosphate derived from AMP and isopentenyl pyrophosphate (20). It is presumed that this compound is rapidly hydroxylated to yield the transzeatin cytokinins (20). The immunoaffinity chromatography and HPLC purification permitted routine analysis of ZR and IPA in the subsequent ELISA assay, and both compounds were consistently present in extracts from both roots and shoots and in xylem sap. This does not

Figure 6. Concentration and translocation of ZR and IPA as affected by RA. A, ZR and IPA levels in roots; B, ZR and IPA levels in shoots; C, translocation of ZR and IPA from roots to shoots per gram fresh weight root and day. Translocation was taken as the product of transpiration and xylem sap cytokinin concentration.

Figure 7. Changes in root and shoot ZR levels with time in a split root culture with the nitrate addition dosed in 80:20 ratios to the two subroots from day 0. Inset, Translocation of ZR when the addition ratio was reversed on day 11 after onset of nitrate addition; first data point shows levels before N availability was changed (cf. relative root size data in Fig. 2).
preclude the presence of other cytokinins in physiologically active amounts in the extracts, although major quantitative contributions by these most probably would have been visible in the HPLC chromatograms.

Of the two measured cytokinins, ZR shows rapid and consistent dose-related responses to nitrate application (cf. Fig. 7). The response is transient in both roots and shoots, indicating that ZR responds to perturbations in nitrate supply rather than to differences in long-term nitrate supply. Thus, on day 22 (acclimated stage), nitrate additions ranged from 0.9 to 26.5 mg N g⁻¹ root dry weight in the standard cultures growing at different RAs and from 0 to 9.6 mg N g⁻¹ root dry weight in the subroot receiving from 0 to 100% of the total N addition, without comparable differences in ZR levels. An increase in ZR was only observed at high or supersaturating RA, corroborating the data of Thorsteinsson and Eliasson (29) for RA cultured Lemna. It may be hypothesized that the ZR levels respond to a nitrogenous metabolite, the level of which is controlled by metabolic consumption during N-limited growth but that may accumulate when the N regimen is perturbed or when N availability is supersaturating for growth.

Although ZR (and IPA) was found in xylem sap, the data do not give clear indications to what extent shoot ZR originates from the root. There was no effect of a reversal of the addition ratio to the subroots of a split root system on translocation of ZR from the individual subroots (Fig. 7). The data concerning xylem translocation at low RAs indicate that the amount of ZR translocated to the shoot in 1 d is about equal to the momentary ZR content of the shoot (Figs. 6 and 7). Measurements of volume flow as transpiration or water absorption may to some extent underestimate actual xylem flux (28). However, cytokinin turnover in most plant tissues is known to be rapid (20, 22). These factors suggest that the increase in shoot ZR found after supply of N to the roots (Fig. 7) is not necessarily due to translocation of ZR from the roots. Another possibility is that nitrate (or a nitrogenous intermediate) translocated from the roots influence cytokinin metabolism in the shoot, thus leading to the observed changes in ZR levels in the shoot.

Are Cytokinins Essential for the Nitrate-Regulated Growth Response?

Wilson (31) summarized information concerning factors regulating R:T, e.g. macro- and micronutrient availability, light, temperature, ontogeny, and rationalized these data within the context of a “null hypothesis,” namely, that cause and effect relationships can be established without hormonal control. In doing this, relationships actually necessitating participation of plant hormones may be identified. It was found that responses of R:T to changed macronutrient availability could be explained by interactions between nutrient availability and translocation and concentrations in roots and shoots of, notably, reduced C and N. Any hormone response must thus be considered to be a consequence of the changed growth pattern or completely unrelated to the growth response.

The data presented here can be subject to similar scrutiny, following circumstantial and physiological arguments for a role for cytokinins in the nitrate-regulated growth response. The circumstantial arguments point to the correlation, in both magnitude and duration, between the nitrate-induced perturbations of root growth and ZR levels in the split root cultures (Figs. 2 and 7). However, in acclimated plants of both standard and split root cultures, ZR levels appeared to be more even. Based on this correlation, it is possible to argue that cytokinins may be involved in establishing the relative size of a sink, which is subsequently maintained during acclimated growth. On the other hand, in the classical view, a reduction in N translocation to the shoot following environmental N limitation will indirectly increase the relative sink strength of the root, indicating that a physiological role for cytokinins would not be necessary. This reasoning is, however, based on the assumption that N translocation is largely unidirectional (upward). Available data for N-sufficient cereals show that a large fraction of the recently assimilated N recovered in roots has cycled through the shoot. In addition, a substantial cycling of N through roots is observed (6, 16). Also, reduced soluble N generated during protein turnover is potentially accessible for translocation. Less is known about N cycling during N-limited growth; however, data for Pisum maintained under RA limitation indicate that translocation of N to the shoot exceeds N utilization in the shoot by a factor of three (P. Duarte and C.M. Larsson, unpublished data). Growth limitation by translocation of N to any organ, thus, is not a necessary consequence of environmental N limitation. A physiological role that can be suggested for cytokinins in this context is to regulate, directly or indirectly, unloading from the transport pools for further utilization in growth processes. A similar conclusion was reached in experiments in which cytokinins were fed to one part of a wheat root system (27).

In conclusion, the data presented here indicate that there may be both circumstantial and physiological arguments in favor of a role for cytokinins in the nitrate-induced growth responses. A more detailed knowledge of the physiology of N-limited growth is, however, required before such conclusions can be fully verified. Furthermore, it is necessary to know more precisely the direct causes for the nitrate-induced effect on cytokinin levels and whether roots affect shoot cytokinins directly via cytokinin translocation or indirectly via translocation of nitrate or nitrogenous intermediates. It appears that the concept of nutritional control used in this study can be useful in further studies of the involvement of plant hormones in nitrate-induced growth responses.

ACKNOWLEDGMENT

We are grateful to Dr. S. Adalsteinsson for lending us equipment for root measurements and for helpful assistance while using it.

LITERATURE CITED

3. Bollmark M, Kubat B, Eliasson L (1988) Variation in endoge-
GROWTH AND CYTOKININ RESPONSES TO NITRATE


7. Drew MC, Saker LR (1975) Nutrient supply and the growth of the seminal root system in barley. II. Localized, compensatory increases in lateral root growth and rates of nitrate uptake when nitrate supply is restricted to only part of the root system. J Exp Bot 26: 79–90


22. Nandi SK, de Klerk GJM, Parker CW, Palmi LMS (1990) Endogenous cytokinin levels and metabolism of zeatin riboside in genetic tumour tissues and non-tumour tissues of tobacco. Physiol Plant 78: 197–204


