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# Leaf area development of micropropagated potato plants: effects of leaf area of individual plants on logistic curve parameters and correlations among these parameters

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## Abstract

Leaf area increase of individual, *in vitro* produced potato plantlets was analysed over three growth phases: *in vitro* normalisation (3 weeks, 17 or 23 °C), transplant production (2 weeks, 18/12 or 26/20 °C) and tuber production (6 weeks, 18/12 or 26/20 °C). In each phase, initial leaf area (ILA) and final leaf area (FLA) of logistically growing plants were related to parameters describing logistic growth (leaf area = $A+C/(1+\exp^{(-B\times(t-M))})$; A: fitted minimum leaf area, B: fitted initial relative rate of increase, C: fitted maximum increment, M: fitted midpoint, MI: maximum rate of increase at M, MI = B×C/4).

Higher ILA was associated with higher FLA during normalisation and transplant production, but not consistently during tuber production. During normalisation, higher ILA led to higher A, C and MI-values, and at 23 °C also to higher B and earlier M. During transplant production, higher ILA was associated with higher C.

During normalisation, leaf area increase of plants with higher FLAs was characterised by higher A, C and MI-values, and at 23 °C also by earlier M. During transplant production, higher FLAs were associated with higher C and MI-values, and at 18/12 °C also with higher A. During tuber production, higher FLAs were associated with higher C, M and MI-values.

Correlations among parameters characterising logistic growth were of physiological origin, mathematical origin, or resulted from the actual shape of the logistic curve. A and B were positively correlated, likely because clearer S-shaped curves result in higher values of both. Both parameters usually were positively correlated with MI, likely because MI is a function of B, whereas A was correlated to B. Physiologically relevant correlations were found between M and C in the tuber production phase.

*Keywords:* growth analysis, *in vitro* plantlet, leaf area, logistic growth, seed production, *Solanum tuberosum* L., temperature.

## Introduction

Conventional seed potato (*Solanum tuberosum* L.) production systems have low rates of multiplication and high risks of infection with increasing numbers of field multiplication (Haverkort *et al.*, 1991). Micropropagation techniques, producing large numbers of disease free *in vitro* plantlets within a short period of time, may help to overcome these disadvantages (Jones, 1988; Struik & Wiersema, 1999). The fastest seed production system using *in vitro* plantlets constitutes four phases: *in vitro* multiplication (through nodal cuttings), normalisation (growing nodal cuttings to rooted plantlets), transplant production (acclimatisation of plantlets in a glasshouse) and tuber production (field) phases.

Different methods and conditions are used in different laboratories to grow plants in the various phases of seed production systems (Sipos *et al.*, 1988; Levy, 1988; Struik & Wiersema, 1999). These protocols, however, do not limit plant-to-plant variation and do not take into account the consequences that this variation may have in subsequent phases. Plant-to-plant variation may be caused by differences in size or other physical and physiological characteristics of the explants, variation in conditions during their growth and by the interactions between these characteristics and later events. Especially the behaviour immediately after transition is relevant and may be very variable: plants are damaged during the transition, they are often replanted deeper and they experience temporarily water loss. The same plants are also exposed to different growth conditions in the last three phases of the production scheme. Transplant shocks or boosts may, therefore, occur during transition of the plants from one phase to another (Sutter *et al.*, 1988; Grout, 1988; Sutter *et al.*, 1992; Tadesse *et al.*, 2001) and these will be reflected in leaf area growth and other developmental processes in the various phases.

Tadesse *et al.* (2000) indicated that leaf area of transplants at the end of the acclimatisation phase was positively influenced by leaf area of the same plantlet at the beginning of the phase. They also showed that temperature during the normalisation phase had a significant effect on leaf area growth during the transplant production phase that went beyond the effect of normalisation temperature on initial leaf area of the transplanted *in vitro* plantlet. The increase in leaf area with time usually follows a logistic growth in the normalisation, transplant production and tuber production phases (Tadesse *et al.*, 2001). It is yet unknown how the leaf area of an individual plant at the beginning of a phase affects the values of the different curve parameters of the logistic growth function of leaf area, nor is it known which parameters are important for achieving a high leaf area at the end of a phase.

This study therefore aims at:

- analysing plant-to-plant variation in leaf area growth in each of the three phases;
- correlating initial leaf area to different growth parameters;
- correlating final leaf areas to specific growth parameters in each phase;
- describing the effects of temperature in each of the three phases on these correlations;
- studying associations among different parameters.

## Materials and methods

Details on the experiment described in this paper have already been provided by Tadesse *et al.* (2001). Below we summarise the main aspects and provide further details relevant to this paper.

*Potato culture and treatments*
Potato (*Solanum tuberosum* L., cv. Gloria) plantlets were propagated *in vitro* by single-node cuttings on 10 ml medium containing MS salts (Murashige & Skoog, 1962) with vitamins, 25 g $l^{-1}$ sucrose, 8 g $l^{-1}$ agar and 0.0133 g $l^{-1}$ alar-64% (daminozide) in sealed 25 ×150 mm culture tubes at 17 or 23 °C and a photophase of 16 h for 21 days in the 'normalisation' phase. Rooted *in vitro* plantlets were then planted in transplanting trays with small cells filled with potting soil (75 plants $m^{-2}$) and grown in growth chambers with day/night temperatures of 18/12 or 26/20°C, a photophase of 14 h and a relative humidity of 80% for 14 days. This is the 'acclimatisation' or 'transplant production' phase. After acclimatisation, the plants were transplanted into 5-liter pots (16–6.4 plants $m^{-2}$; plants were spaced wider with time) filled with potting soil, and were grown in two glasshouses at a day/night temperature of 18/12 or 26/20°C, a photophase of 16 h and a relative humidity of 80% for 42 days in the 'tuber production' phase.

*Experimental design*
The experiment was carried out in a split-split plot design in 16 blocks where the tuber production temperature (TB) was randomised within the transplant production temperature (TP) and the latter was randomised within the normalisation temperature (N). The 128 plants in this experiment were part of a larger experiment with the same treatments, in total consisting of 992 plants. Out of the 128 plants, only those plants were further analysed in which leaf area increased logistically. Logistic curves best explained leaf area increment in all phases of growth (Tadesse *et al.*, 2001). Because of the experimental set-up, plant numbers per treatment were higher in earlier than in later phases. Three plants were omitted from the analysis because their fitted midpoints (M) were out of range.

*Measurements and statistical methods*
Leaf area was measured non-destructively before and after (trans)planting, and then every 3 days in the normalisation and transplant production phases and during the first week of the tuber production phase. From then on, measurements were taken weekly. Measurements of leaf area on the first and last day of every phase gave the 'initial leaf area' (ILA) and 'final leaf area' (FLA), respectively in all phases of growth.

Logistic curves describing leaf area increase (leaf area = $A+C/(1+\exp^{(-B*(t-M))})$) with time (t) were fitted in all phases for individual plants. The parameters of the logistic growth curve are: fitted minimum leaf area (A) at the moment $t \rightarrow -\infty$, fitted initial relative rate of increase (B), fitted midpoint (M) and fitted maximum increment (C). The maximum rate of increase in leaf area (MI) was calculated as B×C/4.

To study the relationship between the ILA or FLA and the curve parameters two approaches were used. In the first, curve parameter values for individual plants were related to ILA and FLA by linear regression separately for high or low temperatures in all three phases of growth. In the transplant and tuber production phases, this was done for plants from all temperature pre-treatments combined. Equations are provided whenever the relationship was significant. In the second approach, correlation coefficients were assessed to identify correlations between ILA or FLA and the curve parameters for individual treatments. In addition, correlation coefficients between curve parameters were assessed for individual treatments.

## Results

*Leaf areas of individual plants at the beginning and end of a phase*
In the normalisation phase, higher leaf areas of explants (ILA, initial leaf area) were associated with higher leaf areas of *in vitro* plantlets at the end of the period (FLA, final leaf area) under both temperatures (17 and 23 °C) of *in vitro* growth (Figure 1a).

Also after transplanting to soil, higher above-ground leaf areas were associated with higher leaf areas at the end of transplant production at both transplant production temperatures (18/12 and 26/20 °C), when data from both pre-treatments (17 and 23 °C during the normalisation period) were combined (Figure 1b). A positive association was also found within most individual pre-treatments (Figure 1b).

In the tuber production phase, higher initial leaf areas were associated with lower final leaf areas over all plants when temperature during tuber production was low (18/12 °C), but this negative association was only found within one pre-treatment (Figure 1c). Associations between ILA and FLA were not found at high (26/20 °C) temperature during tuber production (Figure 1c).

*Relationships between initial leaf area and parameters characterising its logistic increase*
*Normalisation phase.* Under both temperatures in the normalisation phase, a higher leaf area of the explant leaf led to higher fitted minimum leaf area (A) of the logistic curve describing the leaf area increase of the plantlet during the phase, to higher fitted maximum increments in leaf area (C) and higher maximum rates of increase (MI) (Figure 2). At 23 °C, a higher ILA also resulted in higher B-values and earlier midpoints (M) (Figure 2).

*Transplant production phase.* At the lower temperature during transplant production (and for data from both normalisation treatments combined), a higher ILA was associated with higher fitted minimum leaf area (A), maximum increment in leaf area (C) and the maximum rate of increase (MI) (Figure 3). At the higher temperature during transplant production a higher ILA was only associated with a lower B-value, a higher increment (C) and an earlier midpoint (M) (Figure 3).

These correlations were not always significant for both pre-treatments (Figure 3).

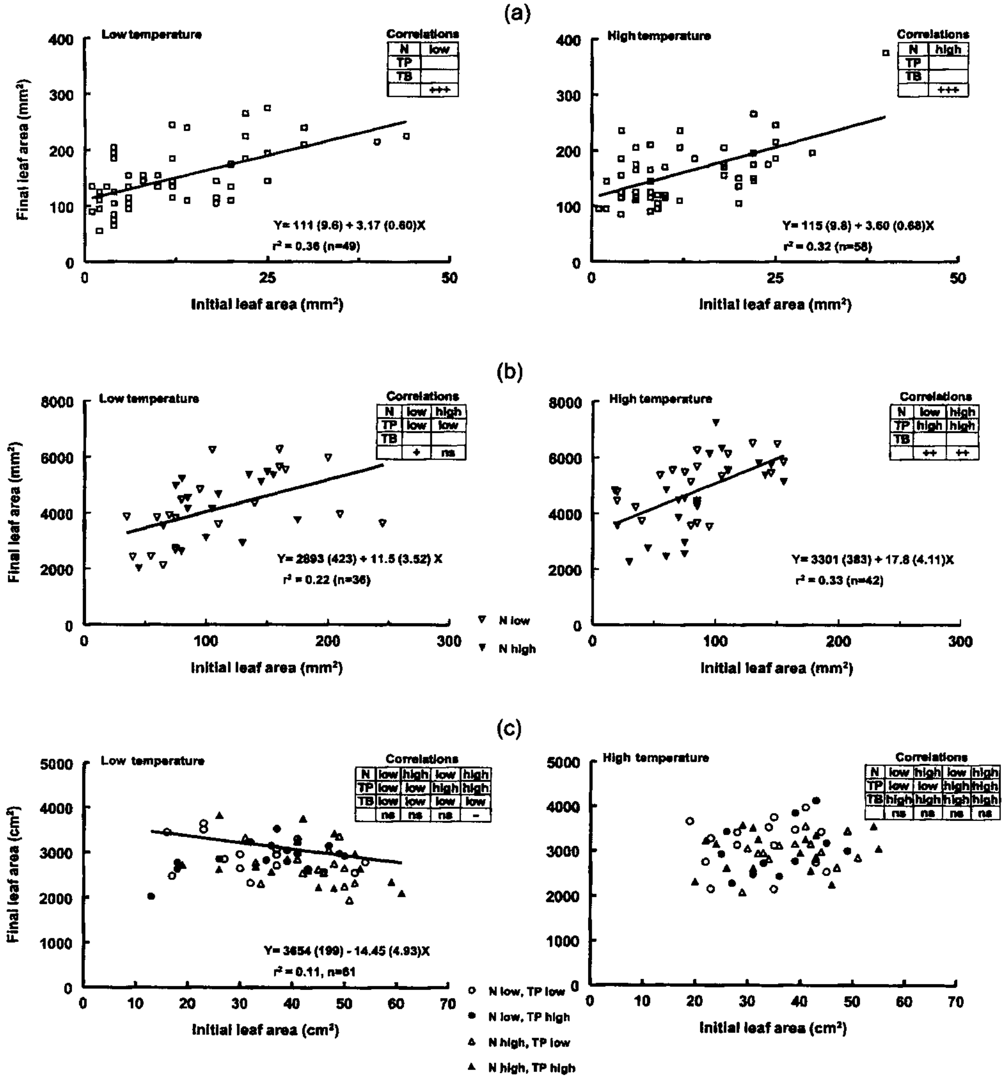


Figure 1. Initial leaf area plotted against final leaf area of *in vitro* propagated potato plantlets at high and low temperatures in the normalisation (a), transplant production (b) and tuber production (c) phases. +++, ++, + and – – –, – –, – indicate significant positive and negative correlation coefficients for the individual (pre-)treatments at $P < 0.001$, $0.001 \leq P < 0.01$, $0.01 \leq P < 0.05$, respectively. ns: not significant, $P \geq 0.05$. Low: 17 °C during normalisation, 18/12 °C during transplant or tuber production. High: 23 °C during normalisation, 26/20 °C during transplant or tuber production.

Only the positive associations between ILA and the fitted maximum increment (C) were consistently found. The positive association between ILA and the A-values at 18/12 °C and the negative association between ILA and the B-values at 26/20 °C were not significant for plants precultured at 23 °C (Figure 3). The positive associa-

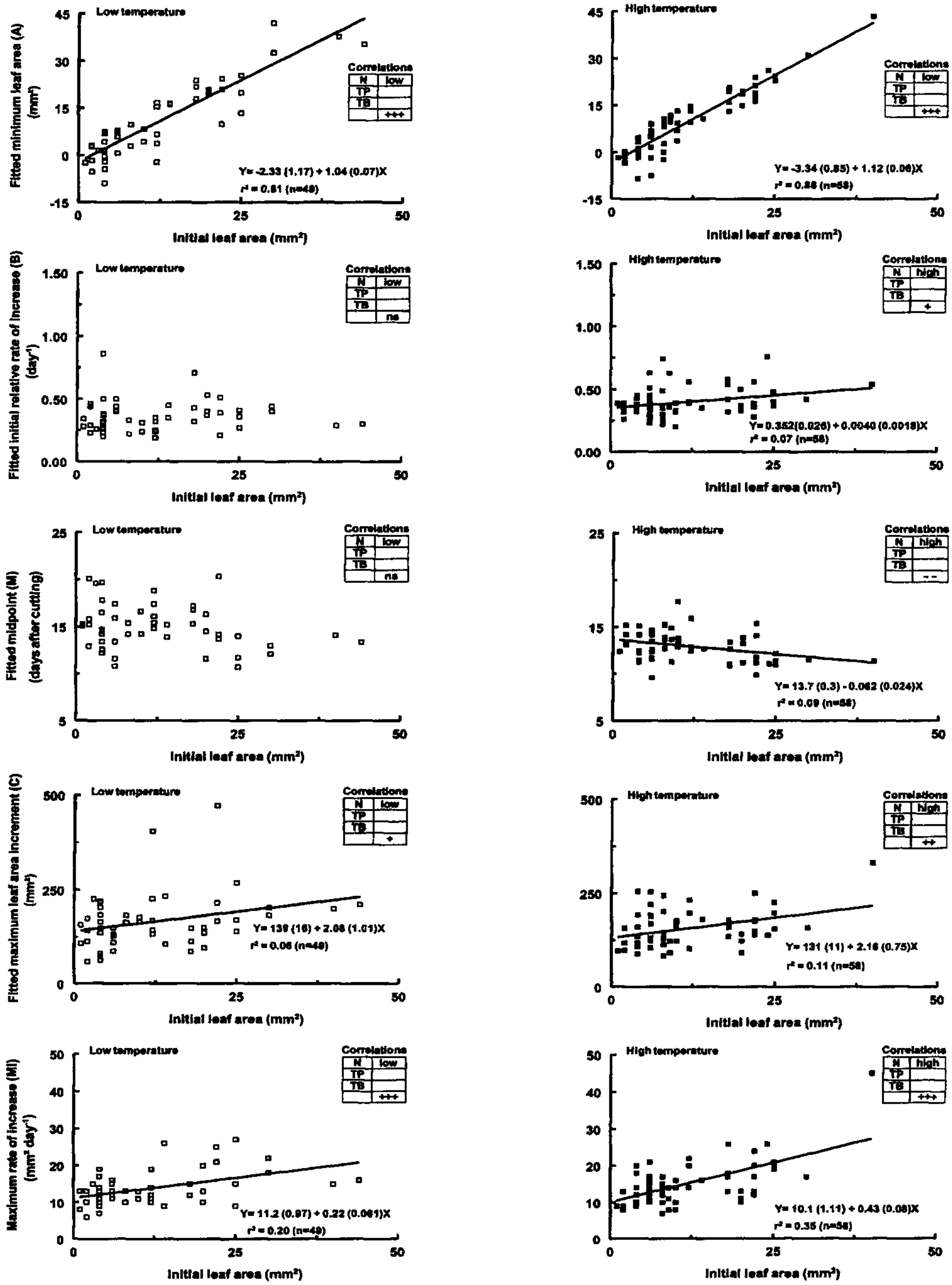


Figure 2. Initial leaf area (ILA) plotted against curve parameters defining logistic growth of *in vitro* propagated potato plantlets grown at low or high temperatures in the normalisation phase. For explanation of correlations and temperatures, see Figure 1.

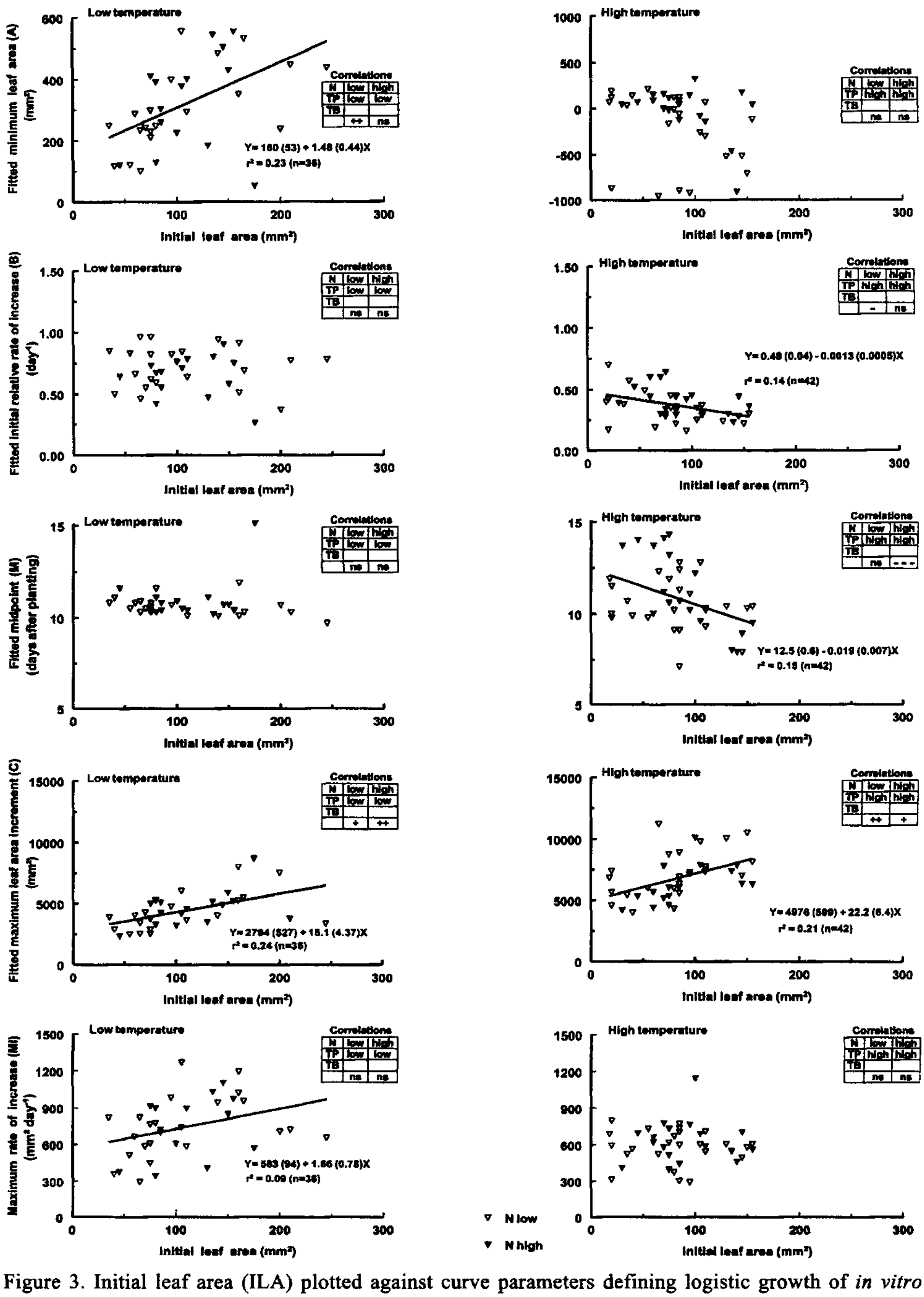


Figure 3. Initial leaf area (ILA) plotted against curve parameters defining logistic growth of *in vitro* propagated potato plantlets grown at low or high temperatures in the transplant production phase after they were precultured at two temperatures in the normalisation phase. For explanation of correlations and temperatures, see Figure 1.

tion between ILA and the maximum rate of increase (MI) at 18/12 °C even was not significant within both pre-treatments (Figure 3).

*Tuber production phase.* Over all pre-treatments, transplants with a higher ILA after transplanting to the tuber production phase, had higher A-values during tuber production (Figure 4). At the lower tuber production temperature, a higher ILA was also related to a higher B-value, later midpoint (M) and lower increment (C) (Figure 4).

Significant associations between ILA and fitted parameters over all pre-treatments, not always showed for each pre-treatment (Figure 4).

*Associations between logistic curve parameters and final leaf area*

*Normalisation phase.* At both 17 and 23 °C in the normalisation phase, higher fitted minimum leaf area (A), higher values for fitted maximum increment in leaf area (C) and a higher maximum rate of increase (MI) were associated with a higher final leaf area (FLA) at the end of the phase (Figure 5). At 23 °C also an earlier midpoint of the fitted logistic curve (M) was related to higher FLAs.

*Transplant production phase.* Associations – over both normalisation pre-treatments – between curve parameters and FLA in the transplant production phase were similar to those in the normalisation phase (Figure 6), except for the positive association between the A-value and the FLA, which was only found at the lower transplant production temperature.

Those associations being significant over pre-treatments, were also generally significant for both pre-treatments separately (Figure 6). The association between an earlier midpoint (M) and FLA at 26/20 °C was only significant for plants precultured at 23 °C.

*Tuber production phase.* When values for all pre-treatments were combined, lower initial rate of increase (B), later midpoints (M) and higher fitted increments (C) of the logistic curve and higher maximum rates of increase (MI), were all associated with higher FLAs at both temperatures during tuber production (Figure 7). At 26/20 °C also lower fitted minimum leaf areas (A) were associated with higher FLAs.

The positive association between the fitted increment (C) and the FLA was also found within all pre-treatments (Figure 7). The associations between the midpoint (M) and FLA were significant for 3 out of 4 pre-treatments at both temperatures (Figure 7). The positive association between MI and FLA was significant for all pre-treatments at the lower tuber production temperature, but only one pre-treatment at the higher tuber production temperature. The negative association between the B-value and FLA (Figure 7) was not significant within individual treatments at the low temperature, and only for plants of one pre-treatment at the high temperature. The latter was also found for the negative association between the A-value and the FLA at the high tuber production temperature.

*Correlations between parameters describing logistic growth*

The fitted minimum leaf area (A) and the initial relative rate of leaf area increase (B)

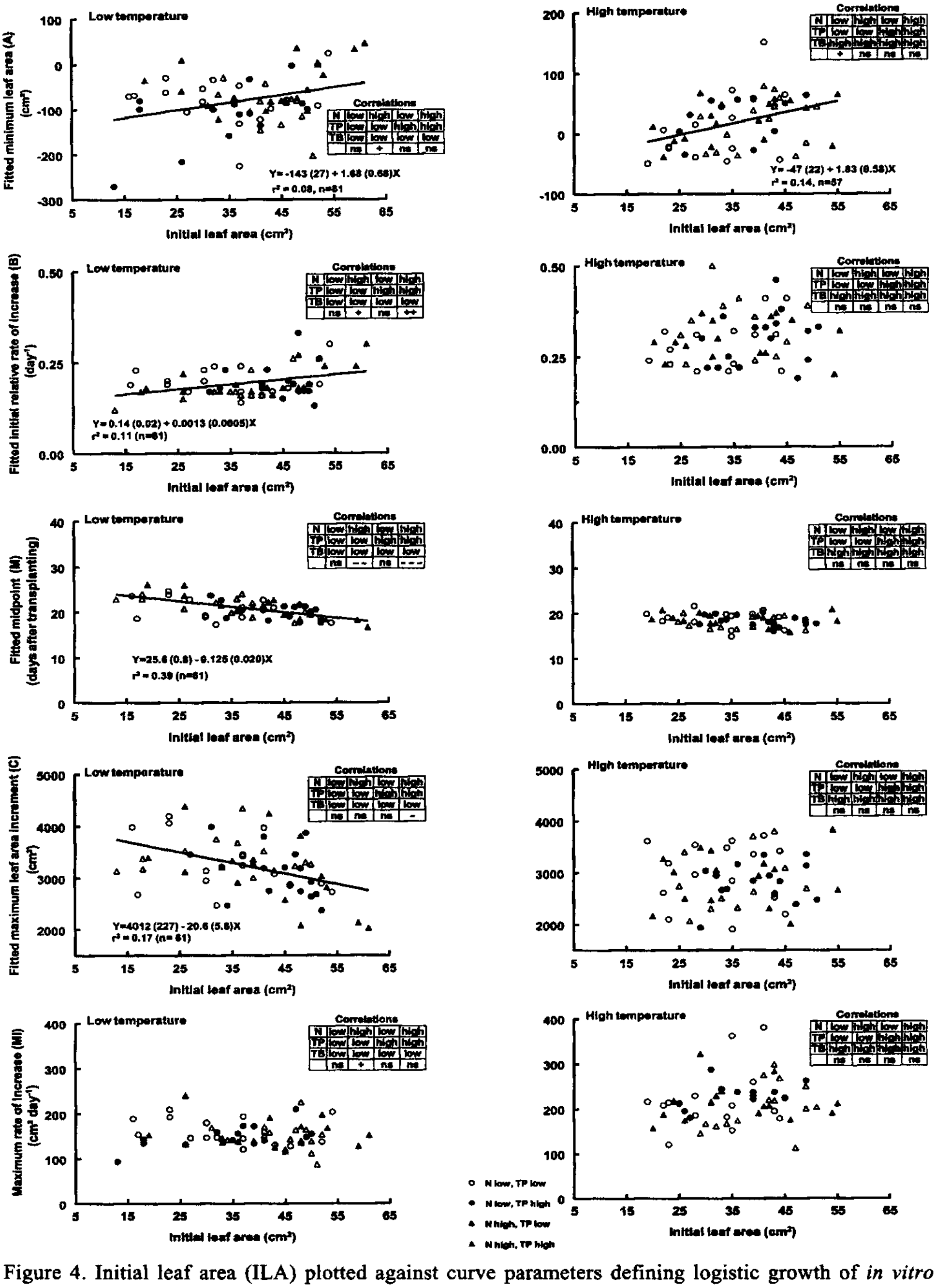


Figure 4. Initial leaf area (ILA) plotted against curve parameters defining logistic growth of *in vitro* propagated potato plantlets grown at low or high temperatures in the tuber production phase after they were precultured at two temperatures in the normalisation and transplant production phases. For explanation of correlations and temperatures, see Figure 1.

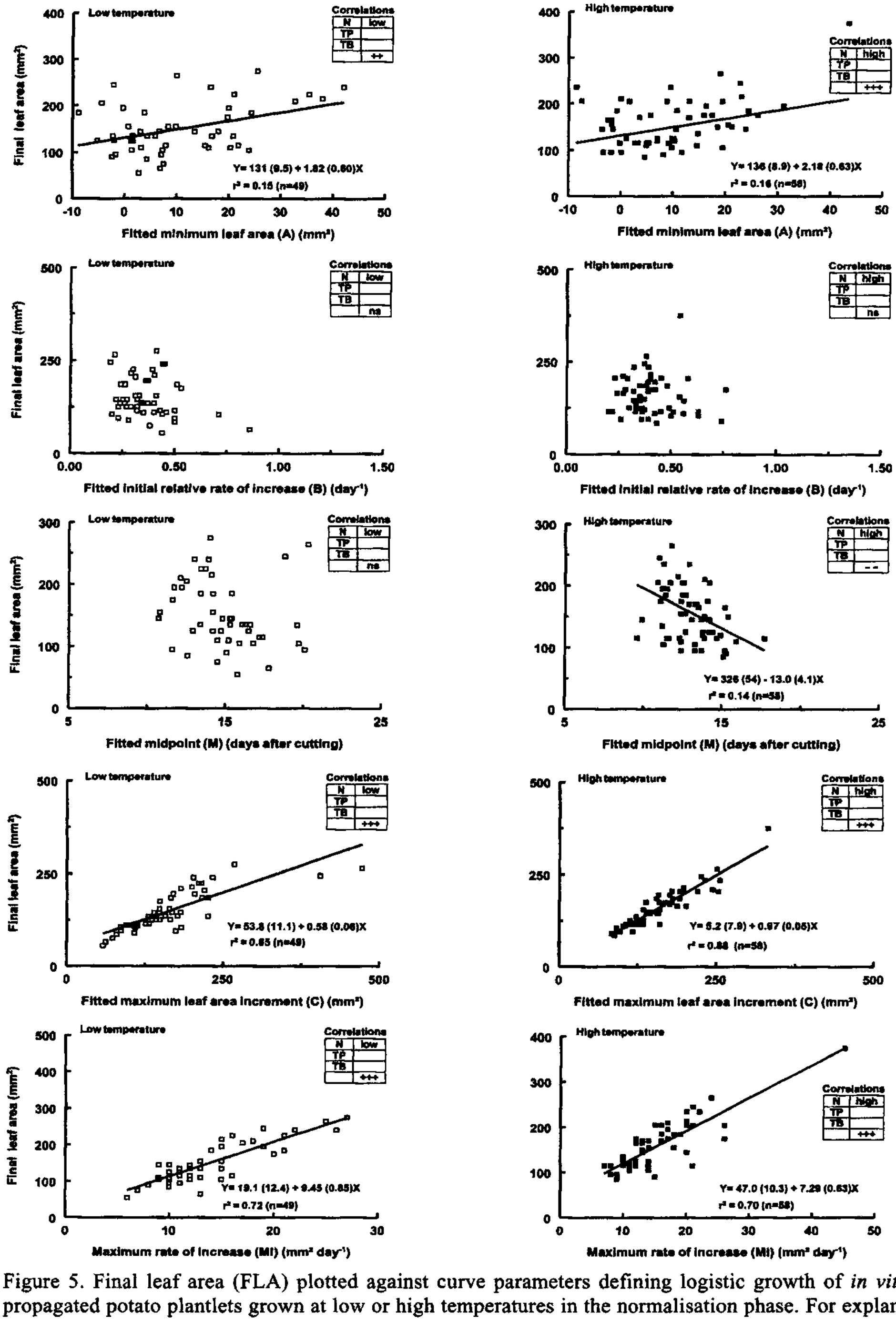


Figure 5. Final leaf area (FLA) plotted against curve parameters defining logistic growth of *in vitro* propagated potato plantlets grown at low or high temperatures in the normalisation phase. For explanation of correlations and temperatures, see Figure 1.

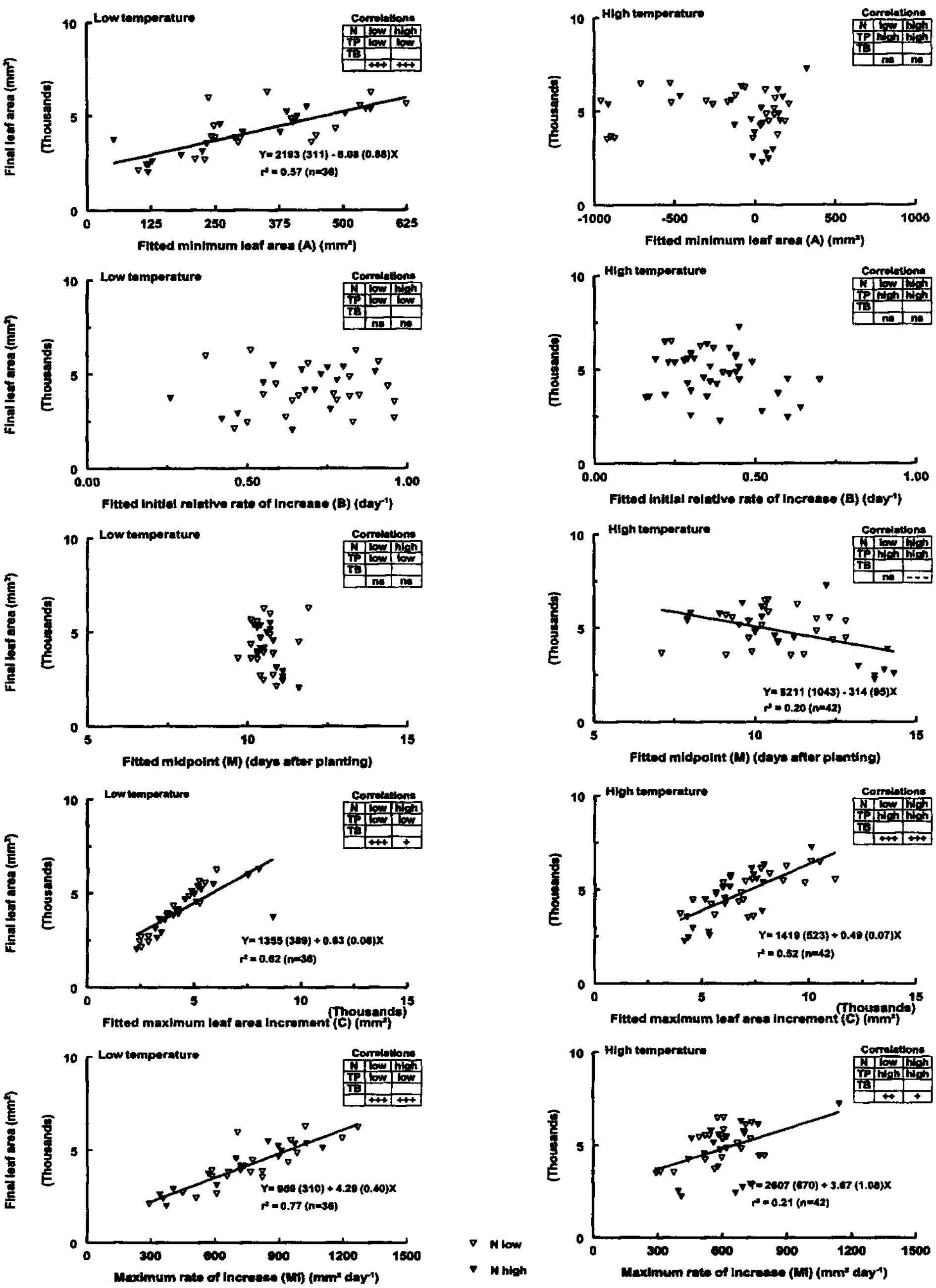


Figure 6. Final leaf area (FLA) plotted against curve parameters defining logistic growth of *in vitro* propagated potato plantlets grown at low or high temperatures in the transplant production phase after they were precultured at two temperatures in the normalisation phase. For explanation of correlations and temperatures, see Figure 1.

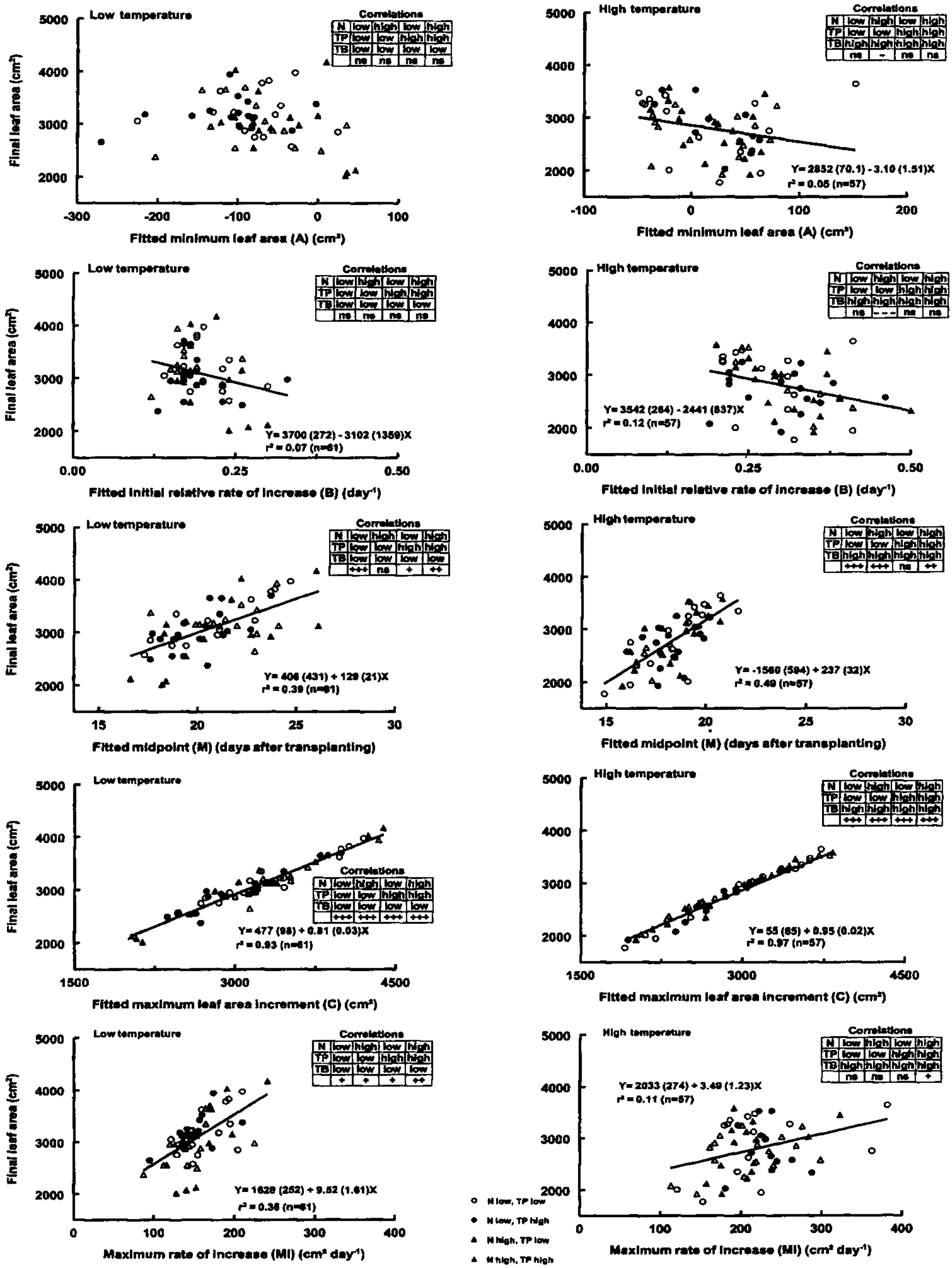


Figure 7. Final leaf area (FLA) plotted against curve parameters defining logistic growth of *in vitro* propagated potato plantlets grown at low or high temperatures in the tuber production phase after they were precultured at two temperatures in the normalisation and transplant production phases. For explanation of correlations and temperatures, see Figure 1.

were significantly, positively correlated in the three phases of growth in all 14 possible sets of plants (Table 1).

The fitted minimum leaf area (A) was significantly associated with the fitted mid-point (M) in 7 out of 14 sets of plants (Table 1). Six out of the seven significant correlations and six out of the remaining seven non-significant correlations between A and M showed a negative relation. The significant correlations were mainly found under high temperature.

The fitted minimum leaf area (A) was usually not significantly correlated with the fitted increment (C) (Table 1). There were, however, a significant positive and a significant negative correlation at low and at high temperatures, respectively, in the transplant production phase, and a single significant negative correlation at high temperature in the tuber production phase. All non-significant correlations in the transplant production and tuber production phases were negative (not shown).

The fitted minimum leaf area (A) was well and positively correlated with the maximum rate of increase in leaf area (MI) in 12 out of 14 sets of plants (Table 1).

The initial relative rate of increase in leaf area (B) was significantly correlated with the fitted mid-point (M) in the three phases of growth, in 11 out of 14 sets of plants. Of these correlations, 8 were negative (Table 1). A higher initial relative rate of increase in leaf area (B) resulted in a lower fitted mid-point (M) at higher temperature (23 °C) in the normalisation phase and at lower temperature (18/12 °C) in the transplant production phase. In the tuber production phase plants precultured at high transplant production temperature showed a negative correlation, while those precultured at low transplant production temperature had a positive correlation in three out of four sets of plants.

The correlation of the initial relative rate of increase (B) with the fitted increment (C) was only significant in 5 out of the 14 cases (Table 1), but was consistently negative in all cases including the non-significant correlations (Table 1).

The initial relative rate of leaf area increase (B) was consistently significantly correlated with the maximum rate of increase in leaf area (MI), in 12 out of the 14 cases. All correlations (including the two non-significant) were positive (Table 1).

The fitted mid-point (M) of the logistic curve had a significant, positive correlation with the fitted increment (C) in the transplant production and tuber production phases in 8 out of the 12 possible cases (Table1).

The fitted mid-point (M) was significantly negatively correlated with the maximum rate of increase in leaf area (MI) at high temperature in the normalisation phase (Table 1). There was no significant correlation in the transplant production phase. Only one (positive) correlation was significant in the tuber production phase.

The fitted increment (C) was only positively correlated to the maximum rate of increase in leaf area (MI) in the normalisation phase, at low temperature in the transplant production phase after a low normalisation temperature and at one temperature combination (high normalisation-high transplant production-low tuber production temperature) in the tuber production phase (Table 1).

Table 1. Linear correlations between parameters[a] describing logistic growth of *in vitro* produced potato plantlets in three phases of growth, as affected by different temperature treatments.

| | Normalisation phase | | Transplant production phase | | | | Tuber production phase | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Temperature[b] (°C) during | | | | | | | | | | | | | | |
| Normalisation | low | high | low | high | low | high | low | high | low | high | low | high | low | high |
| Transplant production | | | low | low | high | high | low | low | high | high | low | low | high | high |
| Tuber production | | | | | | | low | low | low | low | high | high | high | high |
| n | 49 | 58 | 21 | 14 | 24 | 18 | 16 | 14 | 16 | 15 | 15 | 12 | 16 | 15 |
| *A with:* | | | | | | | | | | | | | | |
| B | + | +++ | + | ++ | +++ | ++ | +++ | +++ | +++ | +++ | +++ | ++ | +++ | +++ |
| M | ns | – | ns | – | ns | + | ns | ns | – | ns | ns | – – | – | – |
| C | ns | ns | + | ns | – | ns | ns | ns | ns | ns | ns | – – | ns | ns |
| MI | ++ | +++ | +++ | +++ | +++ | + | ++ | +++ | ++ | ns | ++ | + | +++ | ns |
| *B with:* | | | | | | | | | | | | | | |
| M | ns | – – | – – | – – | ns | ns | ++ | – – | – – | – – | + | +++ | – – – | – – |
| C | – – – | ns | ns | ns | – – – | – | ns | ns | ns | – | ns | – – | ns | ns |
| MI | ns | +++ | + | ++ | +++ | + | + | +++ | ++ | ns | +++ | ++ | +++ | + |
| *M with:* | | | | | | | | | | | | | | |
| C | ns | ns | ns | + | + | ns | +++ | ns | +++ | +++ | +++ | ++ | ns | ++ |
| MI | ns | – – – | ns | ns | ns | ns | ns | ns | ns | ns | ns | + | ns | ns |
| *C with:* | | | | | | | | | | | | | | |
| MI | +++ | +++ | ++ | ns | ns | ns | ns | ns | ns | ++ | ns | ns | ns | ns |

[a] A: fitted minimum leaf area; B: fitted initial relative rate of increase; M: fitted midpoint; C: fitted maximum leaf area increment; MI: maximum rate of increase in leaf area at M.

[b] low: 17 °C during normalisation, 18/12 °C during transplant or tuber production, high: 23 °C during normalisation, 26/20 °C during transplant or tuber production.

+++, ++, + and – – –, – –, – indicate significant positive and negative correlation coefficients at $P < 0.001$, $0.001 \leq P < 0.01$, $0.01 \leq P < 0.05$, respectively. ns: not significant, $P \geq 0.05$.

## Discussion

*Relationship between ILA and FLA*

A higher initial leaf area (ILA) was clearly associated with a higher final leaf area (FLA) in the normalisation and transplant production phases (Figure 1). This proves that the status of a plantlet at the beginning of these phases has an effect on the status of the plantlet at the end of the same phase. In line with this, Seabrook & Douglass (1994) reported that removal of the subtending leaf of explants resulted in *in vitro* plantlets with smaller leaf area, and Tadesse *et al.* (2000) reported that *in vitro* produced plants with higher above-ground leaf areas at the beginning of the transplant production phase ended up with higher leaf areas at the end of the phase. The associations found are consistent with the general view for crop plants that, as long as a young, self supportive, vegetative plant is grown without inter-plant competition and free of stress, later leaf area is related to early leaf area (Goudriaan & Van Laar, 1994), and show that this also can occur under conditions where growth is limited as is the case under conditions where the leaf area increase is logistic. The results imply that the use of nodal cuttings with larger explant leaves to start normalisation, may lead to transplants with larger leaf areas at the moment of transplanting into the field.

In the tuber production phase, associations between ILA and FLA were not found at high temperature, probably because high temperature enhanced leaf senescence (cf. Struik & Ewing, 1995) and increased variation among plants. At the low temperature during tuber production, plants with higher ILA ended up with lower FLA when all data were combined, probably because the most advanced plants had the highest ILA at the beginning of the phase and were the first to senesce or were earlier to partition a relatively large part of their dry matter to tubers, thus limiting haulm development. However, the negative correlation at low temperature during tuber production was only found to be significant within one pre-treatment (i.e. plants pretreated at high temperatures during normalisation and transplant production phases), indicating that also the pre-treatments themselves affected the overall relationship (Figure 1c).

*Relationship between ILA and logistic curve parameters*

Associations between the ILA of individual plants at the onset of the normalisation phase and the parameters characterising their logistic increase in leaf area indicated that a higher ILA led to higher fitted minimum leaf area (A) and a higher fitted increment (C) at both temperatures. The latter likely resulted from a more steep increase of the logistic curve, and was associated with a higher maximum rate of increase (MI). A higher ILA therefore leads to faster growth. This, plus the higher fitted minimum leaf area, explains the positive association found between ILA and FLA in the normalisation phase. The positive associations between ILA and the initial relative rate of increase (B) and the negative ones between ILA and the curve mid-point (M) at 23°C indicate that at the high temperature during normalisation larger explants also had higher initial relative increase rates and a more advanced plant development.

At low temperature during transplant production, associations between ILA and curve parameters (over all data) were similar to those in the normalisation phase: a higher ILA was associated with higher fitted minimum leaf area (A), the fitted increment in leaf area (C) and the maximum rate of leaf area increase (MI). These findings are consistent with the idea that plants with a higher ILA started at a higher level and increased faster. However, only the correlations between ILA and C were significant for all pre-treatments. Also at high temperature during transplant production, only the positive correlations between ILA and C were significant for all pre-treatments. The positive association between ILA and FLA in the transplant production phase therefore is caused by a stronger leaf area increase in plants with a higher ILA. The lack of consistent relations between ILA and A likely results from the fact that when the increase of leaf area with time is more gradual, the S-shape becomes less pronounced and the A and B-values will become lower. These values indeed were found to be correlated (Table 1). A-values even may drop to low negative values (Figure 3). A more gradual increase could be found for instance when leaf area starts to increase very clearly soon after the onset the phase, as is also the case when temperatures during transplant production are higher (cf. Tadesse *et al.*, 2001).

In the tuber production phase, none of the associations found between ILA and curve parameters (Figure 4), also was found consistently within all pre-treatments. Different responses for plants from different pre-treatments may therefore have directed the regression over all data (Figure 4). The lower number of plants available in each pre-treatment also made it more difficult to establish the significance of a correlation, but often correlations within pre-treatments also were extremely weak (not shown). Clearest were the negative associations between ILA and M, suggesting a more advanced growth for the transplants with the largest leaf areas. The association between ILA and the curve parameters usually not being significant at high temperature in the tuber production phase – and for many pre-treatments at the low temperature –, however, is consistent with the absence of a clear association between ILA and the FLA in this phase.

*Relationship between logistic curve parameters and FLA*

At the end of the *in vitro* phase, higher fitted minimum leaf area (A), higher fitted increment (C) and a higher maximum rate of leaf area increase (MI) were associated with a higher FLA (Figure 5). One of the probable reasons for a higher fitted minimum leaf area (A) is a higher ILA and usually a higher ILA most likely results in a higher FLA before plants senesce. Besides, a higher C value indicates that the logistic curve is more steep with a higher maximum rate of leaf area increase (MI) and, thus, will give rise to a higher FLA. An earlier mid-point was associated with a higher FLA at 23°C in the normalisation phase, indicating that also growth of plants achieving a higher final leaf area was more advanced at that temperature.

Associations between curve parameters and FLA in the transplant production phase were very similar to those in the normalisation phase. Higher FLAs were correlated with higher fitted increments in leaf area (C) and higher maximum rates of increase (MI), and at low temperature also with higher A-values.

At both temperatures during the tuber production phase, higher fitted increments

(C), later midpoints (M) and higher maximum rates of increase (MI) were all associated with higher FLAs. Later mid-points suggest that leaf area development over time was slower and thus plant senescence was probably delayed.

*Relationships among parameters characterising the logistic leaf area increase*
Relationships among parameters characterising the logistic leaf area increase of individual plants in different phases may exist for different reasons. Firstly, there can be mathematical relations between parameters. For instance, the maximum rate of increase (MI) is a function of B and C (MI = B × C/4). Secondly, effects on different parameters could have the same physiological basis. For instance, larger explant leaves may lead to higher fitted minimum leaf areas (A) in the normalisation phase (Figure 2) and also to faster growth, thus increasing C or MI (Figure 2; Seabrook & Douglass, 1994). Finally, relationships among parameters may result from the actual way the logistic curve is best fitting through the data values. Slight changes in data may cause effects on curve fitting that could result in simultaneous changes in values of different parameters. For instance, when the S- shape becomes a bit less sharp not only the B-value, but also the fitted A value will decrease.

Out of the relations tested, three groups seem to be of interest: (1) relations among A, B, and MI, (2) relations between C and MI and (3) relations between M and A, B or C.

*Relationships among A, B and MI.* The fitted minimum leaf area (A) and the initial relative rate of leaf area increase (B) were so well and positively correlated in all possible cases (Table 1) that it is tempting to suggest that a higher fitted minimum leaf area is likely to occur when initial leaf area (ILA) is high and that this is associated with more substrate for growth to realise a higher initial relative rate of leaf area increase (B). However, Figures 3 and 4 showed that high ILAs in the transplant and tuber production phases were not consistently related to a higher A or B. It is therefore likely that in these phases the very clear positive associations between A and B mainly resulted from the simultaneous effect that a change in the course of the logistic curve has on both parameters, and much less from a higher initial leaf area as a shared physiological cause. This is also supported by the low negative values found for A in some cases (Figures 3 and 4).

Both A and B were very well correlated with the maximum rate of increase (MI). Since MI is a function of B (MI = B × C/4), it is logical that B and MI are correlated. The correlation between A and MI is likely to result indirectly from the very clear correlation between A and B. It is less likely that the correlation between A and MI resulted from larger ILAs and consequently higher growth rates, because both the correlations between ILA and A were not consistently found (Figures 3 and 4) and the correlations between A and C were weak.

*Relationship between C and MI.* Since MI is also a function of C (MI = B × C/4), it would be logical that C and MI are correlated. However, correlations between C and MI in the transplant and tuber production phase were usually not significant (although positive). Only those between B and MI were. Correlations between B and C tended to be negative. All this suggests that the relations between C and MI were mainly of a non-physiological nature.

*Relationships between M and C, A or B.* The positive correlation between M and C often found in the tuber production phase suggests that plants that were later in development achieved a higher leaf area increase. This is likely because (1) more advanced plants will show earlier tuber bulking, which restricts haulm growth (cf. Van Dam *et al.*, 1996), and (2) leaf senescence, that was already prominent at high temperature during tuber production (Tadesse *et al.*, 2001), will have been more severe in more advanced plants.

Especially at the higher temperature during tuber production, M and A were clearly negatively correlated, suggesting that plants with larger fitted minimum leaf areas were more advanced. Alternatively, because of the clear association between A and B, it might also suggest that plants with a clearer S-shaped curve were more advanced. The latter, however, seems less likely because the association between M and B within some treatments was positive but negative in others. Direct associations between A or B and C in the tuber production phase, however, were usually not significant, though negative.

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