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Shoot Growth Parameters of Potato Seedlings are Determined by Light and Temperature Conditions

Olivia C. Kacheyo[1,2] · **Hannah M. Schneider**[1] · **Michiel E. de Vries**[2] · **Paul C. Struik**[1]

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Abstract

Raising quality hybrid potato true seedlings — derived from hybrid true potato seeds (TPS) — for field transplanting is a critical aspect contributing to the success in cultivating field-transplanted hybrid potato seedling-based crops. Various critical seedling vigour attributes must be defined and enhanced to improve plant performance as well as plant response and adaptation to field conditions after field transplanting. Moreover, additional attributes besides leaf number and stem length should be defined to design selection criteria for "transplantability" of potato seedlings. To assess these attributes and their robustness under different environmental conditions, seedlings of hybrid genotypes were raised under contrasting daylengths, light intensities and temperature regimes, and combinations of these factors, and the effects of these factors (or factor combinations) on seedling vigour and its attributes were quantified. Increased light intensity and daylight integral (DLI) enhanced leaf parameters and increased biomass under long-day conditions, but seedlings did not show conclusive responses under short-day conditions in most attributes. Higher temperatures increased biomass and constant day and night temperatures enhanced compaction of shoot architecture as did higher light intensities — to a greater extent. These results highlight additional seedling vigour attributes including leaf area parameters and seedling biomass which should be utilised to determine "transplantability" of hybrid potato seedlings. Additionally, the results provide a starting point for further research on optimising hybrid seedling production for field transplanting.

Keywords Greenhouse nursery · Hybrid potato seedlings · Seedling vigour · Solanum tuberosum · True potato seeds

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✉ Olivia C. Kacheyo
olivia.kacheyo@wur.nl

[1] Centre for Crop Systems Analysis, Wageningen University and Research, Bornsesteeg 48, 6708 PE Wageningen, The Netherlands

[2] Solynta, Dreijenlaan 2, 6703 HA Wageningen, The Netherlands

Introduction

Seedling production has been defined as a critical aspect and prerequisite for success in the field-transplanting system for hybrid potato seedling crops (Almekinders et al. 2009; van Dijk et al. 2021; Kacheyo et al. 2023). The system involves developing high-quality, nursery-raised true seedlings from hybrid true potato seeds (TPS) for field transplanting to produce seed or ware tubers (van Dijk et al. 2021; Kacheyo et al. 2023). These true seedlings are derived from botanical true seeds in contrast to in vitro potato plantlets or seedlings generated from various other potato propagules (Almekinders 1995; Struik and Wiersema 1999; Kacheyo et al. 2021). The seedling nursery phase is a new aspect in potato cultivation due to the introduction of the novel cultivation systems for hybrid potato, thereby raising the urgency to investigate the optimal growing conditions and the contribution of various factors to seedling growth and development (Stockem et al. 2023).

Seedling vigour is a critical component of seedling quality and is defined as comprising of traits that define the potential for field establishment, growth and development under several field conditions (see Ros et al. 2003; Kacheyo et al. 2023). Although the end result of seedling vigour is defined as field performance, the whole early seedling stage under nursery conditions is the primary determinant. Vigour traits — hereafter described as vigour attributes — can be defined as observable characteristics on seedlings as a result of seedling growth and development under varying environmental conditions. These attributes include number of leaves, leaf area (cm^2) and other leaf area parameters; stem length (cm) and sturdiness of the stem — hereby defined by compactness of the shoot (mg shoot dry weight per cm of stem) — as well as root to shoot ratio and others. The attributes can be used to quantify seedling vigour in transplanted potato seedlings by (1) defining the growth potential of a seedling through attributes that are part of the production apparatus of the plant — such as leaf number, leaf area (cm^2) and leaf area parameters (cf. Tadesse et al. 2000) — as well as (2) defining the seedlings architecture — through attributes such as stem length, compactness of the shoot and root traits — which facilitate morphological adaptation to field conditions after transplanting (Teichmann and Muhr 2015). Faster leaf area development is one indicator of early vigour in seedlings since it results in greater light capture (cf. Tadesse et al. 2000), and greater leaf area and shoot dry weights increase resistance to field conditions (cf. Cantliffe 1993).

Various factors contribute to seedling vigour in seedlings during the nursery phase, and of these factors, the physical, climatic and management conditions of the nursery significantly affect seedling growth and performance. For hybrid potato true seedling production, the effects of these factors, more specifically climatic factors, on general seedling quality have not been substantially explored or quantified. This is a result of the contrasting propagule types between hybrid seedlings and seed tubers and the novelty in use of hybrid seedlings in potato cultivation (Kacheyo et al. 2021). In this study, we therefore (1) explored the effects of various climatic factors on selected seedling — specifically shoot — vigour attributes and (2) described additional selection criteria for transplantable hybrid potato seedlings. Defining the various influences of selected climatic factors on shoot growth and development in the nursery will contribute to the currently limited knowledge on hybrid true seedling production, assist in improving selection criteria for field transplanted potato seedlings as well as quantify seedling responses to various critical climatic factors that influence shoot vigour in hybrid potato seedlings. Moreover, while seedlings were not field transplanted in this study, vigorous seedlings are expected to contribute to reduced risks of transplanting shock and enhance field establishment since seedlings are preselected for favourable traits before field transplanting (van Dijk et al. 2021; Kacheyo et al. 2023). Additionally, preselection reduces the chance of selecting seedlings that are advanced to tuberisation relatively shortly after transplanting, as that would make the crop cycle too short for high yields (see Almekinders et al. 2009; van Dijk et al. 2022; Kacheyo et al. 2023).

Prominently, almost all currently available research on early growth response to various climatic factors in potato is based on tuber-grown plants or on in vitro plantlets (cf. Lommen 1999; Tadesse 2000; Tadesse et al. 2001; Struik 2007). Some work on true potato seedlings has been carried out by researchers of the International Potato Center and others with tetraploid TPS material (e.g., Malagamba 1985; Wiersema 1984; Gupta et al. 2004 etc.). However, application to diploid TPS based propagules will hereby be first explored. Generally, during early growth in potato, temperature is known to significantly influence leaf parameters such as appearance rates, number and size of leaves (Struik 2007). Additionally, both average day and night temperatures, their diel amplitude and longer-term changes significantly influence early growth in potato (Wolf et al. 1990; Struik 2007). High temperatures lead to prolonged sympodial growth and may lead to an increase in number of leaves, which are however generally smaller, resulting in a lower leaf to stem ratio (Struik and Ewing 1995). Under low temperatures, restricted shoot development is expected (Struik and Ewing 1995). Potato is considered a photoperiod-sensitive plant and similar differences in assimilate partitioning and other components of seedling quality — such as individual leaf growth, leaf area and specific leaf area (SLA) — are generally expected between long-day and short-day conditions. Additionally, Engels et al. (1994; 1995) reported influence of photoperiod on "true" potato seedling early growth and development with effects on shoot development and tuberisation.

The interaction of the factors temperature and photoperiod also influence shoot development by influencing the number of leaves, shoot life span and leaf parameters (Wolf et al. 1990; Struik and Ewing 1995). Low temperatures ($\leq$ 15 °C) and short-day conditions ($\leq$ 12 h) are known to contribute to restricted shoot development and early tuberisation — also in "true" seedlings — through advanced accumulation of dry matter into tubers (see Menzel 1985; Struik and Ewing 1995; Engels et al. 1995; van Dam et al. 1996). Light intensity and daylight integral (DLI) also influence early growth and development, and the effects of these have been quantified for most vegetable crops and in vitro potato plantlets (Plantenga 2019; Aguirre-Becerra et al. 2020; Hwang et al. 2020; Carotti et al. 2021), but not true potato seedlings. Low light intensities are associated with higher specific leaf area (SLA) and elongated stems to maximise light capture while higher light intensities lead to increased leaf thickness and often high compaction in the shoot (Carotti et al. 2021).

For transplanted hybrid potato, van Dijk et al. (2021) reported the use of 5-week-old transplantable seedlings of 7–12 cm in stem length with 5–8 fully developed leaves; this description is currently the only available criterion to determine "transplantability" of hybrid potato seedlings as no other additional seedling attributes have been reported yet. For in vitro potato plantlets, leaf area and other attributes such as leaf appearance rate and accumulated leaf dry matter have been used as indicators of vigour (Tadesse et al. 2000). Plantlet age has also been considered as a proxy for fitness to transplant for both true seedlings (van Dijk et al. 2022) and in vitro plantlets (Lommen 2023). While no differences were observed between younger and older seedlings in true seedlings (van Dijk et al. 2022), older in vitro plantlets — which were associated with a larger leaf area — performed better than younger plantlets under field conditions (Lommen 2023). It is therefore important to explore additional seedling vigour attributes that can contribute to enhance seedling vigour, describing selection criteria for transplantable hybrid seedlings and, overall, contribute to seedling quality at field transplanting.

For field-transplanted potato cultivation systems, it is critical to raise vigorous seedlings; as such, it is paramount to define the environmental conditions under which seedling quality is optimal. Boundary conditions for seedling growth and development must be defined such that seedlings can be produced under optimal ranges of these factors to ensure seedling vigour and quality. This paper, therefore, describes the independent contributions of photoperiod, temperature, light intensity and DLI as well as some of their interactions to seedling growth and development parameters and specifically to seedling vigour attributes. Additionally, the allocation and partitioning of seedling biomass as influenced by the aforementioned factors will be assessed. Through the use of climate rooms, climatic factors could be regulated to ascertain the effects of one or more factors independently and interactively.

Materials and Methods

In this research, four trials were conducted under climate room conditions to investigate effects of genotype, photoperiod, light — intensity and DLI —, temperature and selected interactions of these factors.

Location, Growing Conditions and Plant Materials

Trials were conducted in climate rooms at the Solynta facility in Ressen, Gelderland, The Netherlands, during different periods in 2021. Seeds of diploid hybrid potato genotypes were used in all trials. These diploid hybrid potato genotypes were sourced from the breeding programme of the Dutch breeding company Solynta in Wageningen, The Netherlands.

Seeds were sown and germinated in 104-plug sowing trays (13 × 8 rows; 54 × 31 × 40 cm; Obturo Plugs® (Van der Knaap, Kwintsheul, The Netherlands), with a single seed sown per plug, until fully emerged with both cotyledon leaves fully visible (approximately 9–12 days after sowing (DAS), BBCH stage 009; Kacheyo et al. 2021). During germination, the climate cells were set to a 16-h daylength, 18 °C constant temperature and 80% humidity. Light intensity was set to 300 μmol·m^{-2}·s^{-1}. After germination, seedling trays were checked, and un-germinated plugs were replaced with fully germinated seedling plugs to form completely filled 104-plug trays at the start of the treatments. At the start of the treatments, various climate conditions were set to treatment levels and were maintained throughout the course of the treatments in all trials (Table 1). Daylengths of 16 h, 60% humidity, light intensity of 300 μmol·m^{-2}·s^{-1} and 28/24 °C day/night temperature settings were standard settings for all trials unless otherwise specified. For both germination and growth, seedlings were daily watered with nutrient solution using the Peters Professional 16-11-32 water soluble fertiliser (ICL Fertilisers Europe, Amsterdam, The Netherlands). The germination and growing conditions for each trial were as defined in this section and Table 1.

Experimental designs, Treatments and Genotypes

In the trials, sampling plots were allocated to either 16 plants (*Trial 1* and *Trial 2*) or to 12 plants (*Trial 3* and *Trial 4*) — eight or six plants per row respectively — within the tray with gross plots of four rows of eight plants each (see Figure S1). Sampling plots were therefore bordered by single rows of eight plants on length sides and either no rows or a single plant per row on both width sides for *Trials 1* and *2* or *Trials 3* and *4*, respectively. The border rows within the trays were shared by the sampling plots (Figure S1). Treatment combinations within the climate rooms were replicated in blocks resulting into "pseudo replicates" and the number of pseudo replicates differed depending on the trial (Table 1). In the "Statistical Analysis" section, a description of how the pseudo replicates were dealt with is provided. Additionally, the trials were also replicated in time, such that each trial was conducted twice unless specified otherwise (Table 1). The repeats were conducted consecutively and when more than one climate room was used, allocation of treatments was randomised to ensure different climate rooms were used per treatment. Additionally, the setup of the climate room allowed treatments allocated within the climate rooms to be randomised over runs by placing trays at different positions.

Trial 1: Genotype (G)

A panel of 20 genotypes was first assessed for seedling vigour attributes under climate room conditions. A single trial run was conducted for this trial. The trial was laid out in a randomised complete block design (RCBD) with two pseudo-replicated blocks, with the genotype randomised within each block. The blocks were assigned to two distinct tables in the climate room. The genotype was assigned to whole seedling trays to avoid error when randomising within the sowing trays. A destructive harvest was conducted to quantify genotypic differences in biomass allocation, leaf area and other seedling vigour attributes within the genotypes in the trial, over time, and function as a basis for selection of genotypes to use in subsequent trials.

Table 1 Descriptions of treatment factors in *Trials 1* through *4*

Trial	Factors	No. true replicates	Photoperiod h	Temperature °C	Light intensity $\mu mol \cdot m^{-2} \cdot s^{-1}$	DLI $mol \cdot m^{-2} \cdot d^{-1}$	No. of pseudo replicates	No. of sampled plants per net plot	Genotype	Sowing date	Days at destructive harvest DAS
Trial 1	Genotype	1	16 h	28/24	300	17.28	2	16	Panel of 20	07/06/2021	24
Trial 2	Photoperiod, DLI, genotype	1	10 h	28/24	240, 400, 480	8.64, 14.40, 17.28	3	16	SR04, SR07	30/06/2021	22
		1	16 h	28/24	150, 250, 300	8.64, 14.40, 17.28	3		SR04, SR07		22
Trial 2a	Light intensity, genotype	1	10 h	28/24	240, 300, 400, 480	8.64, 10.80, 14.40, 17.28.	3	16	SR04, SR07	30/06/2021	22
Trial 2b	Light intensity, genotype	1	16 h	28/24	150, 250, 300, 400	8.64, 14.40, 17.28, 23.04.	3	16	SR04, SR07	30/06/2021	22
Trial 3	Temperature, genotype	2	16 h	24/24; 28/20; 28/24	300	17.28	4	12	SR01, SR04, SR05, SR07	20/09/2021 for 24/24 Rep 1; 11/10/2021 for 28/24 and 28/20 Rep 1; 08/11/2021 for 28/24 and 28/20 Rep 2; 15/11/2021 for 24/24 Rep 2	25
Trial 3a	Night temperature, genotype	2	16 h	28/20; 28/24	300	17.28	4	12	SR01, SR04, SR05, SR07		25
Trial 3b	Day temperature, genotype	2	16 h	24/24; 28/24	300	17.28	4	12	SR01, SR04, SR05, SR07		25
Trial 4	Temperature, light intensity, genotype	2	16 h	24/24; 28/20; 28/24	300	17.28	4	12	SR01, SR04, SR05, SR07		25

Trial 2: Photoperiod (P), Light Intensity (LI) and Day Light Integral (DLI)

To assess the effects of photoperiod, light-intensity and daylight integral on seedling growth and development, trials were conducted under climate room conditions. *Trial 2: Photoperiod and DLI* assessed the combined effects of photoperiod and DLI using two photoperiods — long day (LD) and short day (SD) —, three common DLI treatments (8.64, 14.40 and 17.28 mol·m^{-2}·d^{-1}) within the two photoperiods and two genotypes. The trial utilised data from *Trials 2a* and *2b* which specifically assessed the effects of increasing light intensity within contrasting photoperiods (LD and SD). *Trial 2a: Short day (SD)* was laid out in a split-split plot design with the photoperiod assigned to the main plot and light intensities assigned to the sub plots and the genotype randomised within the light intensities in the sub-sub plots. Three pseudo replicates were also applied in the trial and were nested within the light intensities. Two genotypes were then allocated to each of the pseudo replicates. Four light intensities were used in the trial: 240, 300, 400 and 480 µmol·m^{-2}·s^{-1} which translated to daylight integrals (DLIs) of 8.64, 10.80, 14.40 and 17.28 mol·m^{-2}·d^{-1} (Table 1). The light treatments were allocated to four sections of the same climate room as the structure of the rooms allowed for up to four possible light intensities within the same room without interference between the light treatments. *Trial 2b: Long day (LD)* was laid out in a split-split plot design, similar to *Trial 2a*, with similar allocation of photoperiod and light intensities and pseudo replicates. The daylength was set to 16 h and the four light intensities used were: 150, 250, 300 and 400 µmol·m^{-2}·s^{-1}, which translated to daylight integrals (DLIs) of 8.64, 14.40, 17.28 and 23.04 mol·m^{-2}·d^{-1}. The trials (*2a* and *2b*) were therefore run concurrently in two separate climate rooms and the common DLI(s) between the two photoperiods were included for purposes of *Trial 2*.

Trial 3: Temperature (T)

Trial 3 was conducted to assess the effects of three temperature regimes on seedling growth and development. The trial was laid out in a split-plot design with three temperature regimes (28/24, 28/20 and 24/24 °C day and night temperatures) assigned to the main plot — which was a single climate room for each temperature treatment — and four genotypes assigned to the sub plot (Table 1). The trial was also pseudo replicated, with the pseudo replicates nested and randomised within the temperature treatments. Four genotypes were then assigned to the sub plots level within the pseudo replicates. Additionally, the trial was replicated twice, in time. Based on the temperature treatments in the trial, additional analyses could be made based on similar day temperatures (28 °C) and similar night (24 °C) treatments in *Trial 3a* and *Trial 3b*, respectively. *Trial 3a* compared seedling parameters at constant day (28 °C) but varying night temperatures (24 and 20 °C) whereas *Trial 3b* compared seedling parameters at varying day (28 and 24 °C) and constant night temperatures (24 °C) (Table 1).

Trial 4: Temperature and Light Intensity (T × L)

Trial 4 ran concurrently with *Trial 3* but assessed the influence of temperature regimes and light intensity and their interactions on seedling growth and development. The trial was arranged in a split-split plot design with temperature treatments (28/24, 28/20 and 24/24 °C day and night temperatures) assigned to the main plots, three light intensities (150, 300, 450 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) assigned to the sub plots and the four genotypes assigned to the sub-sub plots. The temperature and genotype treatments as well as the light intensity 300 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ were similar to those of *Trial 3*, but individual trials were setup and conducted and independent datasets were generated from both trials. The treatment combinations were replicated in four pseudo replicates in each climate room, within which the sub plots were assigned, and the trial was replicated in time (Table 1).

Destructive Harvests and Measurements

Destructive harvests were conducted at the end of each trial where biomass was partitioned into leaves in all trials, stems and stolons in *Trials 1* and *2*, individual stems and individual stolons in *Trials 3* and *4*. This is because, in later trials, we recognised the need to quantify individual biomass of the stems and stolons due to the differences in the number of stolons between the genotypes. Total biomass, in all trials, excluded roots as the type of plug used did not allow for extraction of roots. Shoot biomass in *Trials 3 and 4* comprised of leaf and stem biomass. Fresh weights of all fractions of biomass were assessed on the sampling date and samples were oven dried at 70 °C for 48 h for dry weights. Additionally, the length of stems (cm) and leaf area (cm^2) were measured. For leaf area, the WinDIAS Leaf image analysis system (Delta-T Devices, Cambridge, UK) was used in *Trial 1* and *Trial 2*, and for *Trial 3* and *Trial 4*, the LI-3100C (LI-COR Biosciences, Lincoln, NB, USA) was used. The following formulas were used to calculate leaf mass per area (LMA), specific leaf area (SLA) and compactness of the shoot:

$$\text{LMA} = \frac{\text{Leaf dry weight (mg)}}{\text{Leaf area (cm}^2\text{)}}$$

$$\text{SLA} = \frac{\text{Leaf area }\left(\text{cm}^2\right)}{\text{Leaf dry weight (mg)}}$$

$$\text{Compactness} = \frac{\text{Total shoot biomass}\left(\frac{\text{mg}}{\text{plant}}\right)}{\text{Stem length}\left(\frac{\text{cm}}{\text{plant}}\right)}$$

Statistical Analysis

Data were analysed using R statistical software (R Core Team 2023). In *Trial 1*, one-way analysis of variance (ANOVA) was used to analyse differences among genotypes in the measured traits. In *Trial 2* the lm function in base R was used to conduct two-way and three-way analysis of variance (1) to assess the significance ($p < 0.05$) of main factors and interactions of light intensity and genotype within each daylength and (2) to assess the significance of main factors and interactions of photoperiod, DLI (common DLI in the two photoperiods) and genotype. In *Trial 2*, treatment combinations were only pseudo replicated, therefore, the pseudo replicates were nested in the light intensity factor for *Trial 2a* and *2b* as well as *Trial 2* when assessing the effects of the main factors and their interactions. For *Trial 3* and *Trial 4*, true replicates were conducted over time for each temperature treatment in addition to the four pseudo replicates applied within each temperature treatment. The nlme package (Pinheiro et al. 2022) was therefore used to employ a mixed model for these trials to capture the true replicate as a random factor and also cater for the heterogeneity of the true replicates, as trials were replicated in time. The significance of main factors and their interactions of temperature, light and genotype were therefore assessed in a mixed model with the pseudo replicate included as nested in the temperature factor in *Trial 3* (temperature and genotype) and as nested in the light treatment in *Trial 4* (temperature, light and genotype) to account for the differences in the pseudo replicates in the model. Comparisons of means of main factors and their interactions in all trials were done using Tukeys' test ($\alpha = 0.05$) in the emmeans package (Lenth 2023).

Results

Variation in Genotype Performance Under Climate Room Conditions

Genotypes significantly varied in allocation of dry biomass (g) to leaves, stems and stolons and in the total plant biomass as well as in leaf area (cm^2) and LMA (mg cm^{-2}) in *Trial 1* (Table S1). Based on their performance — mostly higher leaf area, lower LMA and intermediate stem lengths — the genotypes SR01, SR04 and SR07 were selected for use in subsequent trials, and an additional genotype SR05 was also added.

Influence of Increasing Light Intensity Under Short and Long Photoperiods

Variations in seedling performance, including partitioning of biomass and leaf area parameters were observed under different light intensities under long- and short-day conditions in *Trials 2a* and *2b* (Fig. 1; Table 2; Table S2; Table S3). Genotypes SR04 and SR07 differed significantly in the amount of dry biomass accumulated in stems and stolons as well as in total plant biomass (g) under short-day conditions. SR04 had significantly higher biomass in all plant parts than SR07. Leaf area (cm^2) was influenced by light intensity and the genotype under short-day conditions. SR04 had significantly more leaf area than SR07 and the increase in light intensity led to a significant increase in leaf area, with the light intensity (400 μmol·m^{-2}·s^{-1}) resulting in the largest leaf area, and no significant differences between leaf area at 300 and 480 μmol·m^{-2}·s^{-1}. SLA was significantly influenced by the interaction between light intensity and genotype. Additional light intensity led to lower SLA beyond 300 μmol·m^{-2}·s^{-1} (Fig. 1; Table 2; Table S3). Under long-day conditions, light intensity significantly influenced all measured parameters except for the number of leaves and the stem and stolon dry biomass (g). The highest light intensity, 400 μmol·m^{-2}·s^{-1}, resulted in significantly more total biomass with a larger proportion of biomass allocated to the leaves than the stems and stolons (Fig. 1; Table 2; Table S2). At 150 μmol·m^{-2}·s^{-1}, total and leaf biomass were significantly lower than at all other treatments. Leaf area parameters and stem length differed with change in light intensity. Leaf area (cm^2) and stem length (cm) were also influenced by the genotype. At 400 μmol·m^{-2}·s^{-1}, the amount of leaf area and SLA were reduced; however, this treatment had the highest LMA. Lower light intensities generally resulted in seedlings with more leaf area, but higher SLA. Stems were more elongated at lower light intensity (Table 2; Table S2). The genotypes also significantly differed in means of all measured parameters except for the amount of biomass partitioned to the stems and stolons as well as the SLA and LMA.

Effects of Day Light Integral Under Contrasting Photoperiods

The response of seedlings to changes in DLI under short- and long-day conditions was assessed in *Trial 2*. Seedlings responded differently to an increase in DLI between short- and long-day conditions in terms of leaf area, SLA, LMA and stem length. The leaf area was not different between DLIs under long-day conditions (Fig. 2). Seedlings under short day had significantly greater SLA than those under long day except at the lowest DLI (8.64 mol·m^{-2}·d^{-1}). The inverse of SLA, LMA, was influenced by the interaction between daylength and DLI, where seedlings grown at the moderate and highest DLI under long day gave the highest mean LMAs. Seedlings gave significantly more elongated stems at the lowest DLI under long day, similar to those at moderate DLI under short day (Table S4). More light gave significantly shorter stems under long day but there was no trend under short-day conditions. Increasing DLI therefore significantly decreased stem length and SLA but increased LMA under long day with no clear response in these parameters under short-day conditions (Table S4). Leaf area increased with increasing DLI under short day, but no changes were observed under long-day conditions (Table S4; Fig. 2).

Partitioning of biomass to leaves as well as the total seedling biomass was influenced by the main factors photoperiod and DLI, and additionally, genotype for leaf dry biomass. Seedlings had significantly greater leaf and total dry biomass under long day than under short day. Increases in DLI resulted in significantly greater leaf biomass but no differences were observed between the intermediate and higher DLI. The greatest total dry weight was attained under the intermediate DLI followed by

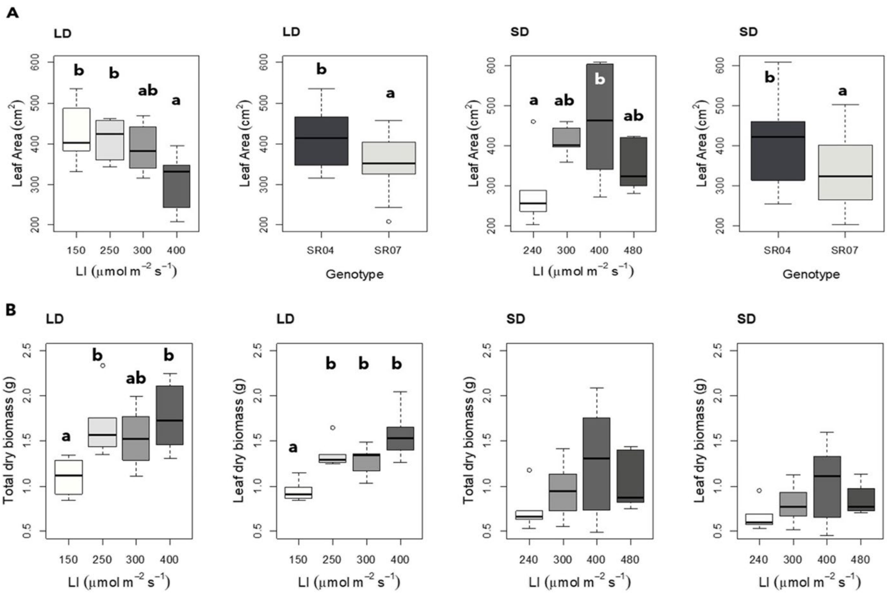

Fig. 1 Influence of light intensity (LI) and genotype under contrasting photoperiods (LD, long day; SD, short day) on measured parameters in *Trials 2a* and *2b*. Trends in leaf area (**A**) and biomass allocation (**B**) under contrasting photoperiods and increasing light intensities within the respective photoperiods are shown. Letters indicate significant differences between mean values of measured parameters ($\alpha < 0.05$). Whiskers indicate the range of the data

that of seedlings grown at the highest DLI with the lowest dry weight at the lowest DLI (Fig. 2). SR04 had significantly more leaf biomass than SR07 but no differences were observed for the other organs or the overall total biomass. The number of leaves on the other hand was significantly influenced by genotype, with more leaves in SR07 than in the other genotypes (Table 2; Table S4).

Temperature and Light Effects on Seedling Parameters

The main effects of temperature and genotype as well as the effects of temperature, light intensity and genotype and their interactions were assessed on seedling parameters of four different genotypes in *Trial 3* and *Trial 4*, respectively.

Temperature Effects on Seedling Biomass Allocation

At constant light intensity (300 $\mu mol \cdot m^{-2} \cdot s^{-1}$), the overall comparison of the three temperature regimes and genotypes as well as their interactions was conducted. Significant main effects of temperature regimes and genotype were observed on seedling biomass allocation in *Trial 3* (Table 3). In general, the 28/24 °C regime resulted in significantly the greatest dry weight across the three temperature regimes

Table 2 Significances ($p < 0.05$) of treatment factors in *Trials 2*, *2a* and *2b*. The factors are abbreviated LI for light intensity, Gen for genotype, P for photoperiod and DLI for daylight integral. P. rep represents the pseudo replicate factor. p values in bold indicate significance of the main factor or interactions of main factors for the measured parameters

Trial	Factors	Leaf number	Leaf area	SLA[1]	LMA[2]	Leaf	Stem and stolon	Total	Stem length
		No. per plant	cm^2	cm^2 mg^{-1}	mg cm^{-2}	g dry biomass	g dry biomass	g dry biomass	cm
Trial 2a	LI	0.116	**0.023**	**0.039**	0.075	0.143	0.192	0.131	0.591
Short day	Gen	0.831	**0.041**	0.292	0.473	**0.054**	**0.020**	**0.032**	**0.003**
	LI × P. rep	0.171	0.718	0.809	0.903	0.830	0.667	0.802	0.820
	LI × Gen	0.509	0.590	**0.039**	0.962	0.574	0.266	0.443	0.889
Trial 2b	LI	0.437	**0.013**	**< 0.001**	**< 0.001**	**< 0.001**	0.558	**0.017**	**< 0.001**
Long day	Gen	**0.018**	**0.017**	0.648	0.610	**0.033**	0.130	**0.028**	**< 0.001**
	LI × P. rep	0.320	0.384	0.417	**0.037**	0.772	0.863	0.899	0.327
	LI × Gen	0.654	0.214	0.472	0.103	0.951	0.690	0.824	0.882
Trial 2	P	0.392	0.093	**< 0.001**	**< 0.001**	**< 0.001**	0.236	**0.002**	0.204
	DLI	0.561	**0.040**	**0.011**	**0.004**	**< 0.001**	0.117	**0.004**	**0.042**
	Gen	**0.004**	**0.015**	0.880	0.509	**0.016**	0.060	0.157	**< 0.001**
	DLI × P. rep	0.647	0.348	0.771	0.539	0.703	0.828	0.888	0.576
	P × DLI	0.193	**0.029**	**0.004**	**0.003**	0.648	0.990	0.883	**0.026**
	P × Gen	0.676	0.496	0.685	0.934	0.588	0.978	0.730	0.749
	DLI × Gen	0.109	0.299	0.201	0.067	0.760	0.750	0.770	0.970
	P × DLI × Gen	0.925	0.826	0.926	0.738	0.873	0.417	0.677	0.948

[1] *SLA specific leaf area* (cm^2 mg^{-1})

[2] *LMA leaf mass per area* (mg cm^{-2})

(Table S5). The amount of dry biomass partitioned to the leaf, shoot and total biomass was, however, similar for 24/24 °C and 28/20 °C, which are the regimes with similar average temperature (24 °C). Between the three temperature regimes, SLA and LMA were similar, but the leaf area responded to changes in temperature regimes. The leaf area was the highest at 28/24 °C and lowest at 28/20 °C (Table S5). Stems, across the genotypes, were significantly longer in treatments with higher day temperatures (6.30 and 6.71 cm on average for 28/24 and 28/20 °C, respectively) and shorter and more compact at the lowest day temperature (3.69 cm at 24/24 °C) (Table S5). The biomass partitioned to the stolons was significantly greater under constant temperature than at the combination of high day and low night temperatures (28/20 °C). To further dissect these effects, diurnal temperature changes were assessed for their impact on seedling growth parameters between the temperature regimes.

Effects of Day Temperature Changes Under Similar Night Temperature

At the same night temperature (24 °C), day temperatures (28 and 24 °C) differed significantly in biomass partitioning in seedlings as well as in stem length and compactness. Seedlings had significantly more biomass at higher day temperature (28 °C) than at lower day temperature (24 °C) (Table 3; Table S6). Stem dry weight, however, was significantly influenced by the interaction between temperature and genotype, where in general, under lower day temperatures, less

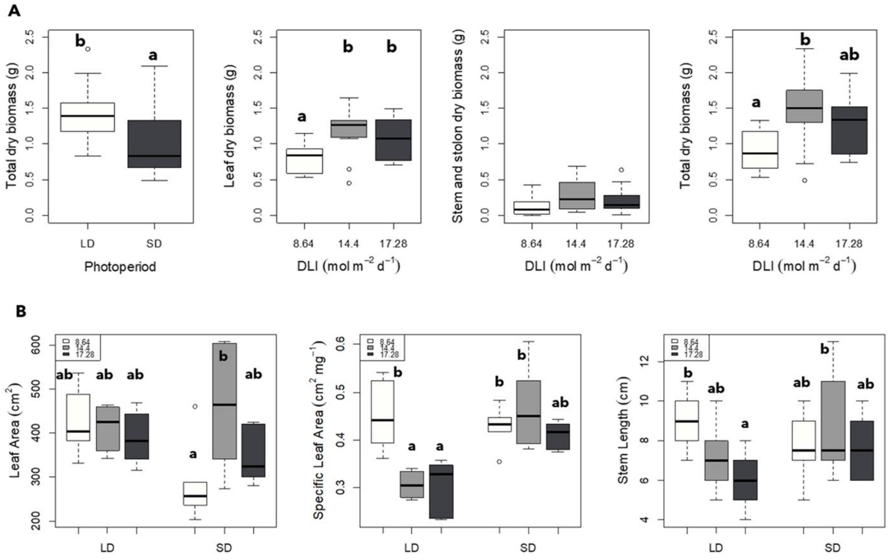

Fig. 2 Influence of photoperiod (LD, long day; SD, short day), light integral (DLI), genotype and their interactions on measured parameters in *Trial 2*. The responses of seedlings to photoperiod and DLI in terms of biomass partitioning (**A**), as well as leaf area, SLA and stem length (**B**) are indicated. The legend in **B** indicates the three DLI levels in the trial. Letters indicate significant differences between mean values of measured parameters ($\alpha < 0.05$). Whiskers indicate the range of the data

biomass was partitioned to stems in SR07, SR01 and SR05 as opposed to the same genotype at higher day temperature. Additionally, for the genotype with the most biomass partitioned to the stem (SR04), significantly larger amounts were partitioned at higher day temperature than at lower day temperature (Table S6). Interestingly, temperature did not significantly influence the leaf area and its parameters but significantly influenced stem length and compactness of the shoot. Seedlings had significantly shorter stems at low day temperature (24 °C) but allocated significantly more biomass per cm of stem at such temperature (Fig. 3B; Table S6). The inverse was true under higher day temperature, where significantly less biomass was allocated per unit stem length when the seedlings had significantly longer stems in comparison to those of the lower day temperature.

Effects of Night Temperature Changes Under Similar Day Temperature

Night temperature significantly influenced biomass allocation to seedling organs as well as leaf area parameters and stem length. Lower night temperature gave significantly less biomass than higher night temperature. Genotypes grown at a higher night temperature significantly outperformed the lower night temperature in leaf area and SLA. The inverse was true for LMA, where lower night temperatures led to higher LMA (Table 3; Table S6).

Stem length (cm), on the other hand, was significantly influenced by the interaction between temperature and genotype. For SR01, the stem length was significantly lower under low night temperature than under higher night temperature. For SR07 and SR05, the length of the stems did not differ significantly between night temperatures, and SR04 and SR05 were more compact than SR01 and SR07 (Fig. 3B, Table S6).

Temperature Effects Under Increasing Light Intensities

The combined effects of temperature and light on seedling growth parameters were assessed in *Trial 4*. Seedling attributes and biomass allocation to seedling organs were influenced by one or more of the two-way interactions of temperature (T), light (L) and genotype (G) (Fig. 4; Table 3). In the case of leaf area and LMA, a significant influence of the main factors (T, L and G) was observed in the trial (Table 3; Table S8).

T Leaf area was significantly higher in the high-day and high-night temperature treatment (28/24 °C; Fig. 4). The lowest leaf area was obtained under the treatment with the lowest night temperature (28/20 °C) and did not differ from that of seedlings under constant day and night temperature (24/24 °C). LMA, on the other hand, was significantly lower at higher night temperatures than at the lower night temperature (Table S8).

L Intermediate light intensity gave significantly more leaf area than the lowest and highest light intensity which were at par. For LMA, an increase in light intensity gave a significant linear increase in the leaf mass per unit leaf area of the seedlings (Table S8).

Table 3 Significances ($p < 0.05$) of treatment factors in *Trials 3*, *3a* and *3b* and *Trial 4*. The factors are abbreviated as LI for light intensity, Gen for genotype, P for photoperiod and DLI for daylight integral. P. rep represents the pseudo replicate factor. *p* values in bold indicate significance of the main factor or interactions of main factors for the measured parameters

Trial	Factors	Leaf	Stem	Shoot	Stolon	Total plant	Leaf number	LA[1]	SLA[2]	LMA[3]	Stem length	Compactness
		g dry biomass	g dry biomass	g dry biomass	g dry biomass	g dry biomass	no. per plant	cm^2	$cm^2\ mg^{-1}$	$mg\ cm^{-2}$	cm	$mg\ cm^{-1}$
Trial 3	T	**0.009**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**0.006**	0.101	0.075	**< 0.001**	**< 0.001**
	Gen	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	0.217	0.092	**< 0.001**	**< 0.001**
	T × P. rep	0.651	0.421	0.476	0.230	0.510	**0.022**	0.071	0.252	0.336	0.877	0.321
	T × Gen	0.949	0.246	0.958	0.879	0.948	**0.034**	0.987	0.956	0.531	**0.002**	0.195
Trial 3a	T	**0.002**	**0.013**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**0.002**	**0.003**	0.478	0.855
	Gen	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**0.004**	**0.010**	**< 0.001**	**< 0.001**
	T × P. rep	0.196	0.091	0.065	0.297	0.079	0.419	0.268	0.508	0.551	0.698	0.250
	T × Gen	0.624	0.334	0.857	0.480	0.830	0.089	0.719	0.527	0.322	**0.039**	0.106
Trial 3b	T	**0.012**	**< 0.001**	**< 0.001**	0.655	**< 0.001**	**< 0.001**	0.339	0.952	0.367	**< 0.001**	**< 0.001**
	Gen	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**0.003**	**< 0.001**	0.740	0.714	**< 0.001**	**< 0.001**
	T × P. rep	0.441	0.356	0.475	0.163	0.454	0.383	0.014	0.079	0.277	0.186	0.671
	T × Gen	0.809	**0.017**	0.731	0.829	0.724	**0.041**	0.875	0.946	0.465	0.158	0.113
Trial 4	T	0.468	**< 0.001**	**0.002**	**< 0.001**	**0.006**	**< 0.001**	**< 0.001**	**0.014**	**< 0.001**	**< 0.001**	**< 0.001**
	LI	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**
	Gen	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**0.004**	**0.003**	**< 0.001**	**< 0.001**
	LI × P. rep	0.096	0.550	0.094	0.892	0.109	0.153	0.402	0.906	0.662	0.366	**0.003**
	Gen ×T	0.112	**< 0.001**	**0.002**	0.056	**0.001**	**0.001**	0.226	0.214	0.172	**< 0.001**	0.057
	T × LI	0.160	**0.035**	0.074	**< 0.001**	0.066	0.662	0.088	**0.044**	0.055	0.068	**0.012**
	Gen × LI	**< 0.001**	**0.003**	**< 0.001**	**< 0.001**	**< 0.001**	**0.038**	0.135	0.050	0.516	**0.002**	**< 0.001**
	T × LI × Gen	0.587	0.240	0.488	0.334	0.486	0.172	0.959	0.978	0.626	0.287	0.370

[1] *LA: Leaf area* (cm^2)

[2] *SLA: Specific leaf area* ($cm^2\ mg^{-1}$)

[3] *LMA: Leaf mass per area* ($mg\ cm^{-2}$)

G The four genotypes responded differently in leaf area and LMA. SR01 and SR05 significantly gave the lowest leaf area of all genotypes. SR07 had a larger leaf area than both SR01 and SR05, which was, however, significantly lower than that of SR04. SR04 and SR07 gave significantly lower LMA but did not differ from SR05. SR01 gave the highest LMA but was similar to SR05. SLA was the highest under constant day and night temperatures at the highest light intensity but was similar to the SLA of seedlings of the other temperature treatments under the same light intensity (Table S8).

G × T The genotype and temperature interaction influenced the number of leaves, stem length and the amount of biomass partitioned to the shoot and total dry biomass significantly. For both shoot and total dry biomass, the greatest biomass was attained in SR04 at the highest average temperature 28/24 °C (Table 3; Table S7). Temperature regimes did not influence biomass in SR01 and SR04 as both genotypes maintained the lowest biomass across all temperature and genotype treatment combinations. The highest leaf numbers were attained at 28/24 °C in SR07 and the lowest were attained in SR05 at 28/20 °C. Interestingly, these low leaf numbers did not differ from those observed in all genotypes and 24 °C average temperature treatment combinations (24/24 and 28/20 °C) but SR07 at 28/20 °C. While leaf numbers increased with increasing day temperatures (28 °C) (Table S8), SR05 still produced low leaf numbers at 28/24 °C. Stem length, on the other hand, was highest in SR04 at high-day temperature treatments (28/20 and 28/24 °C) and was lowest in SR01

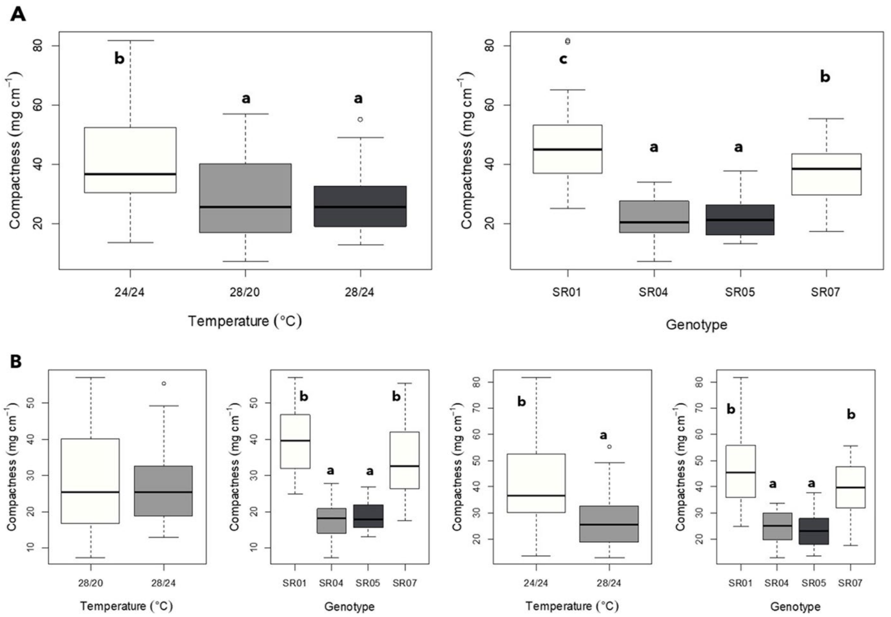

Fig. 3 Effects of temperature (°C) and genotype on seedling compactness in *Trials 3*, *3a* and *3b*. **A** Compactness as influenced by three temperature regimes in *Trial 3* and **B** as influenced by similar day but contrasting night (*Trial 3a*) as well as contrasting day and similar night temperature (*Trial 3b*) conditions. Letters indicate significant mean differences between factors in measured parameters ($\alpha = 0.05$). Whiskers indicate the range of the data

at all temperature treatments as well as SR05 and SR07 at constant day and night temperature (24 °C; Table S8). Generally, stem lengths increased with increase in day temperatures (24 to 28 °C; Table S8) and were often lowest at constant day and night temperature (24 °C) across the genotypes.

G* × *L The interaction between light and genotype also influenced both the shoot and total biomass in the treatment combinations. With increasing light intensity, shoot and total biomass increased across all genotypes with variations in additional biomass with additional light. The lowest light intensity (150 µmol·m^{-2}·s^{-1}) produced seedlings with, generally, the lowest shoot and total biomass within each genotype but across all genotypes significantly greater biomass was attained in SR04 and the lowest in SR05. The largest biomass was attained at the highest light intensity (450 µmol·m^{-2}·s^{-1}) in SR04 (Table S7). Interestingly, between the moderate and highest light intensities, dry matter significantly increased with increasing light intensity in all genotypes but SR01. Seedlings were most compact in SR01 and SR07 at the highest light intensity across all genotype and light intensity combinations and interestingly within all genotypes, but SR04, for each additional unit light intensity a significant — almost linear — increase in compaction was observed.

T* × *L Combined effects of the temperature and light treatments influenced biomass allocation into stems and stolons as well as SLA and compactness of the shoots. Stem dry biomass was significantly highest at 300 µmol·m^{-2}·s^{-1} under the 28/24 °C regime across all light intensity and temperature regime combinations (Table S7).

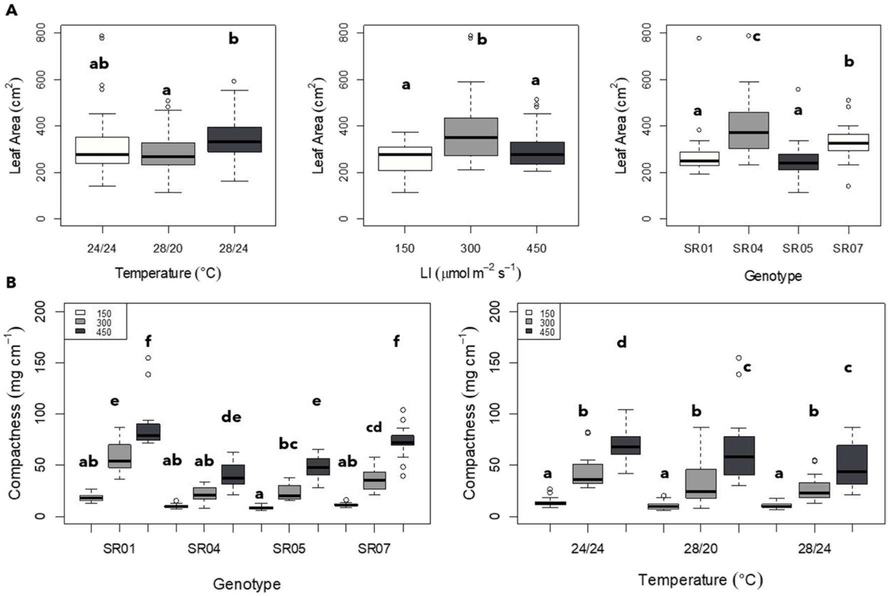

Fig. 4 Effects of temperature (°C), Light intensity (LI: µmol·m^{-2}·s^{-1}), Genotype and their interactions on seedling parameters in *Trial 4*. **A** Leaf area as influenced by temperature, light intensit, and genotype. (**B**) Compactness of seedling shoots as a function of genotype and light intensity as well as temperature and light. Letters indicate significant mean differences between factors and their interactions in measured parameters ($\alpha = 0.05$). Whiskers indicate the range of the data

Under constant day and night temperatures (24 °C), stem biomass was significantly lowest at the lowest light intensity (150 μmol·m^{-2}·s^{-1}) and did not respond to increase in light intensity from 150 to 450 μmol·m^{-2}·s^{-1}. Additionally, across all temperature regimes, increasing light intensity from the moderate to the highest intensity did not lead to a significant response in stem biomass. SLA also responded to the temperature and light intensity combinations by generally decreasing with increasing light intensity (Table S8; Table 3). Increase in light intensities led to significantly lower SLA across all temperature treatments and SLA was the highest and did not significantly differ in seedlings at 150 μmol·m^{-2}·s^{-1} across all temperature regimes. Compactness was also influenced by the temperature and light interaction where the most compact seedlings were attained at the highest light intensity and constant day and night temperature regime (24/24 °C). Additional light intensity led to a significant and similar mean increase in compaction in seedlings in the temperature treatments with high-day temperature. The increase in light intensity leads to significantly more biomass allocated per unit stem length. Additionally, at the moderate (300 μmol·m^{-2}·s^{-1}) and lowest (150 μmol·m^{-2}·s^{-1}) light intensities, seedlings did not differ significantly in compactness across the temperature treatments indicated by similar means at the two respective light intensities (Table S8).

Discussion

The objective of these trials was to quantify the effects of temperature and light conditions and their interactions on attributes that contribute to seedling vigour of different potato hybrids. Additionally, the contribution of the aforementioned factors to enhancement of shoot growth and development was assessed and additional selection criteria for transplantable seedlings have been defined. Selected attributes such as leaf parameters, stem vigour as well as allocation of seedling biomass into seedling organs and their general responses to changes in photoperiod, light intensity, temperature and combinations of these factors will hereby be first reported. These assessments will contribute to defining a protocol for production of hybrid true potato seedlings fit for various purposes.

Daylength Influences Seedling Response to Increasing Light Intensity and Day Light Integral

Increasing light intensity under short day did not result in any differences in seedling leaf, stem and stolon or total dry biomass (Table S3). Additionally, increasing light intensity under short or long day did not influence the number of leaves per plant (*Trial 2*, *2a* and *2b*; Table 2, Table S2; Table S3) but increased the leaf area under short day. Leaf area under short day, however, was not different between light intensities greater than 300 μmol·m^{-2}·s^{-1} (Fig. 1; Table 2) and low light conditions resulted in both a small leaf area and low SLA (*Trial 2a*; Figure S2; Table S3). Short days are known to decrease leaf growth rate in potato in the long term (Lorenzen and Ewing 1990) and often lead to slow seedling development and a shift of biomass from above ground organs to tubers (Engels et al. 1994), findings which were confirmed in *Trial 2a*. Under long day, seedlings responded to increasing light intensity by increasing dry biomass up to 300 µmol·m^{-2}·s^{-1}, after which an increase in light intensity no longer increased the amount of biomass invested to the leaves (Fig. 1; Table S2). Under low light intensity, seedlings were more stretched and less compact (Figure S3) which is in line with the findings in lettuce (Sago 2016) where increasing light intensity generally increased the amount of biomass, and no clear differences were found between higher light intensities. Malagamba (1985) also reports that in potato seedlings, low light intensities — caused by excessive shading — resulted in weaker seedlings while increased shading resulted in elongated stems, but with higher seedling emergence. Increasing light intensity led to shorter stems in seedlings under long day with no clear distinction between the intermediate light intensities 250 and 300 µmol·m^{-2}·s^{-1} (Table S2). Leaf area decreased with increasing light intensity but was also steady at intermediate light intensities. Under long day, SLA was, as expected, highest under low light intensities (cf. Poorter et al. 2019; Liu et al. 2016), which is, however, not desirable since a higher SLA is associated with a lower leaf thickness. Under long day, biomass and leaf area, therefore, did not scale linearly with increasing light intensities and the effect of increasing light was increasingly lower (Fig. 1). A clear contrast between seedling response to increasing light intensity between the contrasting daylengths indicates the photoperiod sensitivity in hybrid seedlings even in early growth phases.

Higher Temperatures Positively Influence Most Seedling Parameters

Under different temperature regimes and the same light intensity (*Trial 3*), a higher average temperature (26 °C; 28/24 °C) led to more leaves than lower average temperatures and constant temperature resulted in the least number of leaves. Higher day temperatures also resulted always in a greater average number of leaves and leaf area also responded positively to higher night temperatures (24 °C) as opposed to low-night temperatures (20 °C) (Table S6, Table S8). A build-up in leaf area with higher day and night temperatures relates to the high growth rates under higher average temperatures and is in line with the findings of Tadesse et al. (2000) where leaf area increased more at higher than at lower temperatures during transplant production through in vitro potato plantlets. Additionally, shoot biomass is known to respond more to higher, than to lower night temperatures (Malagamba 1985), and for seedlings, the most shoot biomass is observed to be partitioned to the leaves (Table S5, Table S6). The dry matter partitioned to the shoot was greatest in the seedlings grown under high day and night temperature (28/24 °C) compared to the rest of the temperature treatments. Interestingly, increasing both day and night temperatures (24 °C to 28 °C and 20 to 24 °C, respectively) significantly increased biomass. This could be attributed to the higher average temperature in the high-day and high-night temperature treatments as opposed to the slightly lower temperature in the other temperature treatments (cf. Malagamba 1985; Struik 2007). Therefore, a slightly higher increase in average temperatures will lead to significant increase in leaf and total biomass.

Under high temperatures, SLA is expected to be higher than under cool temperatures in tuber-grown plants (Struik 2007); however, it was observed that between the three temperature regimes, no differences in SLA were observed at 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$. SLA and LMA were only influenced by increasing night temperatures at constant day temperature (Table S6). Seedlings indeed had the highest SLA at the higher average temperature. A high amplitude (8 °C) resulted in a lower SLA or higher LMA on average, in line with the finding reported by Struik (2007) (Table S6). At constant day and night temperature (24 °C) seedlings also had reduced stem lengths, but more dry matter per unit stem length, indicating more compacted seedlings (Table S6, Table S8). Considering the similar trends between LMA and compaction with increasing light intensity (see Table S8; Fig. 4), seedling compactness occurred similarly in both leaves and stems. Therefore, with increasing compactness — due to increasing light intensity — leaves and stems equally downsize as expressed through an increase in LMA and reduced stem lengths, respectively (Table S8). In potato, effects of temperature on shoot development show that most expected temperature effects are on leaves with clear influences on leaf appearance rate, number of leaves as well as leaf size and expansion (Struik and Ewing 1995; Struik 2007) leading to a higher overall leaf and total biomass. The difference, however, between the highest and lowest leaf dry weight was small: 1.54 g for 26 °C and mean 1.38 g for both treatments with an average 24-h temperature of 24 °C.

Combined Effects of Climatic Factors Contribute Relatively Less to Seedling Attributes than Combined Effects of Genotype and Climatic Factors

Considering the interactions between various factors during nursery seedling production, the combined effects of photoperiod and DLI as well as temperature and light intensity were also assessed. Increase in DLI led to increase in leaf area only under short-day conditions (Table S4; Fig. 2) and to increase leaf area, a higher light intensity and DLI is therefore more suitable under short than under long day. The highest mean SLAs were generally found under short day indicating that seedlings had much thinner leaves and maintained similar biomass with increasing DLI (Table S4). An increase in LMA was observed only under long day (Table S4), in agreement with Poorter et al. (2019) where increasing DLI led to a significant decline in SLA. Since no response was observed under short day, it can be concluded that for leaf parameters, long days favour leaf-area production and responses in leaf parameters to increasing light intensity and DLI, whereas under short day, additional light does not result in changes in SLA or LMA (Table S4). Both the photoperiod and increase in DLI singularly influenced the partitioning of biomass to both the leaves and total plant but interestingly, seedlings under short-day conditions gave significantly lower biomass than those under longer days, which is expected since short-day conditions promote decreased leaf growth rate, slower and reduced shoot growth as well as a lower increase in weight (Lorenzen and Ewing 1990; Ewing and Struik 1992; Kooman 1995). Under longer days, therefore, a higher seedling biomass is expected. Generally, long-day conditions gave significantly more favourable seedlings for transplanting and clear responses to light intensity and DLI increases in measured parameters as opposed to short-day conditions (Table S2, Table S3, Table S4; Figure S2).

In *Trial 4*, higher average temperatures also resulted in more leaves in seedlings (Table S6; Table S8) but did not translate to more leaf biomass (Table S7). Generally, the number of leaves may increase with increasing temperature and higher air temperatures can be effectively used in increasing the number of leaves (Struik 2007). Seedlings under 300 µmol·m^{-2}·s^{-1} had more leaves and leaf area than those grown at 150 or 450 µmol·m^{-2}·s^{-1} (Table S8). Additionally, seedlings showed an increase in biomass with increasing light intensity, regardless of the temperature regime indicating no combined effect of temperature and light intensity on total biomass accumulation (Table 3; cf. Malagamba 1985).

Genotypic differences also influenced the response of seedlings to climatic conditions and the genotype contributed almost always significantly to variation in seedling vigour attributes. Large variations between genotypes were observed in the partitioning of biomass to various seedling organs as well as in leaf area parameters — except for SLA — and number of leaves (Table S1). Biomass partitioning into various organs in seedlings grown at different temperatures and varying light intensities (*Trial 4*, Table S7) showed that the seedlings grown under lowest light intensities resulted in the lowest shoot biomass in all genotypes and dry matter in the leaf and shoot increased with increasing light intensity in all genotypes and the increase was not linear with additional light. This shows definitively that above 300 µmol·m^{-2}·s^{-1}, the increase in light intensity does not result in significantly more assimilate production for higher biomass in the seedlings of hybrid genotypes. Therefore, higher light intensities up to 300 µmol·m^{-2}·s^{-1} as opposed to higher average temperatures are more favourable for higher biomass accumulation in both leaves and total seedling biomass in hybrid genotypes.

Under increasing light intensity and contrasting temperature regimes, the genotype also contributed to variations in seedling attributes through interactions with light (G × L) and temperature (G × T) (Table S8). The variation in the mean number of leaves within and between genotype and light as well as genotype and temperature regime combinations, although significant, is however minimal as the mean number of leaves ranged from 6 to 7. These results are in line with the results reported by Struik (2007) and Almekinders and Struik (1994), although using different starting material.

Temperature and light intensity contributed significantly to only seedling SLA and compactness in the shoot (Table S8). SLA declined with increasing light intensity within the various temperature regimes with lower light resulting in increased SLA with, generally, very minor differences within the temperature treatments. Carotti et al. (2021) reported that an increase in light reduced the influence of air temperature on SLA which explains the small differences in mean SLA at each respective light intensity between temperature regimes (Table S8). LMA on the other hand, increased significantly with each additional increase in light intensity but was not different between temperature regimes with higher night temperatures (24 °C) (Table S8). Low night temperatures led to higher LMA as was also reported by Poorter et al. (2009). SLA and LMA, therefore, responded more to light-intensity treatments than to temperature treatments overall. Within the different temperature regimes, increasing light intensity also resulted in more compact seedlings with the highest compaction under constant day and night temperature (24/24 °C) and the highest light intensity (Fig. 4), which is in line with the results of Sago (2016) in lettuce. Stem vigour was also additionally associated with the combined effects of genotype and either light or temperature (Table S8) indicating that responses to both light and temperature may enhance stem vigour in some genotypes. Although genotypes contributed significantly to the variations in seedling vigour attributes within seedlings, clear responses to climatic factors were also observed independent of the genotype and in addition to the combined effects of the genotype and other factors. Under nursery conditions, therefore, where climatic conditions are more variable, combined effects of genotype and climatic factors should be expected and seedling production should, to an extent, take into consideration the genotype and its performance in relation to climatic factors.

Field Conditions and Seedling Phenotypes

Reports on successful establishment of field transplanted hybrid potato seedlings defined seedlings to have been raised for 5 weeks, with a stem length of 7 to 12 cm and 5 to 8 fully developed leaves (van Dijk et al. 2021; 2022), which were, on average, similar ranges to those attained in these trials. Ideally, based on this assessment and these criteria, most of the seedling types developed and reported in this study would be suitable for transplanting. However, further assessment of the seedling traits indicates that seedling performance would be varied under diverse field conditions, due to other attributes associated with the seedling types such as stem vigour, leaf area parameters as well as seedling biomass. For hybrid potato, a desirable seedling for transplanting would be that with a large leaf area coupled with short and thick stems and an appropriate root to shoot ratio (see Kozai et al. 1995). These criteria eliminate seedlings generated — in this study — under short-day conditions which were characteristically stretched and less compact (Figure S2) as well as seedlings developed under low light intensities (150 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) in the long-day treatment. From 300 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ under long-day conditions, seedlings were therefore mostly sturdy with higher leaf area and additional light led to significant compaction and shorter stems, affirming the potential of these seedlings for field transplanting. Low SLA could be attributed to higher resistance to field conditions (Masson et al. 1991), and higher light intensities in this study were mostly associated with low SLA further affirming the suitability for transplanting of seedlings raised under higher light intensities. Furthermore, consideration should be placed on stem length since seedlings require enough stem length to circumvent the risk of being damaged during mechanical transplanting exercises. Long and elongated (stretched) seedlings (SD: at all light intensities and LD: < 300 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) possess a high risk of damage during mechanical transplanting (van Dijk et al. 2022) and breakage in the event of strong winds. With increasing light intensity under long-day conditions, stem lengths reduced with increasing sturdiness (from 300 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ and higher); however, a threshold should be met to also avoid very short seedlings (> 450 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) that tend to be buried during mechanical transplanting.

Another critical factor to consider is the possibility of premature tuberisation and subsequently, poor canopy development especially when temperatures are lower and/or photoperiods are shorter. While stolon availability was not extensively reported in this study, the presence of stolons during transplanting increases the risk of mechanical damage and increasing susceptibility of seedlings to soil-borne pests and diseases by creating entry points (see Kerbiriou et al. 2013; van Dijk et al. 2022). Lorenzen and Ewing (1990) reported that under short day, only when a larger tuber-sink is developed, leaf growth starts to be affected; as such, possibilities for transplanting when stolon development has commenced — but is not yet advanced — are available until before tuber formation, after all, stolon development is also observed to be genotype dependent in hybrids (Tables 2, 3). When transplanted in contrasting conditions, seedling performance is also expected to be varied even for seedlings here deemed transplantable. A transplant shock period is expected and the recovery of seedlings in these diverse conditions mostly depends on environmental conditions (see Engels et al. 1995; van Dijk et al. 2022; Kacheyo et al. 2023).

Short-Day Conditions

Under short day (10 h) conditions, additional factors such as temperature and possibly nutrients, should be used to control the quality of seedlings in the nursery and ensure sturdy seedlings with enough leaf area at the moment of transplanting. When transplanting under short-day conditions, seedling development is likely to be temperature dependent (Engels et al. 1995). Higher temperatures will accelerate growth and development of both above and below ground organs and lower temperatures tend to shift biomass production to below ground organs.

Long-Day Conditions

Long day (16 h) conditions favour seedlings response to climate factors and subsequently seedling vigour. Transplanting under long-day conditions is also temperature dependent and high temperatures mostly favour faster recovery from transplant shock as opposed to low temperatures. Low temperatures which are likely to occur in temperate regions in spring are unfavourable for seedling growth as they lead to seedlings progressing towards tuberisation and extended growing periods (Engels et al. 1994).

Further Studies and Implications for Hybrid Seedling Production for Field Transplanting

Considering seedling production for hybrid potato is a rather novel but critical aspect of the field transplanting system, knowledge of boundary conditions for seedling production is required for prospective users of the system (Kacheyo et al. 2023). The variation of conditions in the prospective nurseries where seedling production of hybrid potato seedlings for field transplanting will be produced, e.g. in East-Africa

(Den Braber et al. 2023), Sub-Saharan Africa (Gildemacher and ter Steeg 2023; ter Steeg and Gildemacher 2023) and The Netherlands (de Vries et al. 2023); the treatments under which these trials were conducted did not capture all possible nursery conditions. Further studies should therefore focus on tailoring seedling production to various regions to develop protocols adapted to the specific conditions under which seedling production is conducted. Success in seedling raising — for both commercial and subsistence production (see Kacheyo et al. 2023; Den Braber et al. 2023) — will require knowledge of the boundary conditions for hybrid seedling production. Therefore, these results should be considered a start at understanding the influence of climatic conditions on seedling growth and development. Root development, in these studies was not quantified, but was observed to be influenced by light intensity increase (Figure S3); therefore, further studies should quantify root to shoot ratios in seedlings and their contribution to seedling field establishment. In this study, under short-day conditions, seedlings were elongated and less compact as such characterised not fit for transplanting (Figure S3). This could be attributed to the artificial conditions under climate room settings, as such, similar studies under greenhouse conditions in normal short-day regions should be undertaken to set boundary conditions for seedling production in these regions. Other non-climatic factors such as nutrients and watering regimes and quantities, sowing substrates, their composition, and volume among other factors, should be explored to quantify their contribution to seedling vigour attributes since these factors can also be utilised to manipulate seedling growth (cf. Tuku 1994; Malagamba 1983). Therefore, a balance between climatic and crop management decisions should be defined to optimise seedling production in the nursery. This will further contribute to the current knowledge on factors contributing to seedling quality in hybrid potato seedling production systems.

Conclusion

Individual and combined effects of climatic factors influence seedling vigour attributes and ultimately seedling quality in hybrid potato seedlings. Variations in number of leaves were significant but minimal, and number of leaves could be, to some extent, manipulated with increased average temperatures and/or day temperatures. For leaf area parameters, a clear influence of daylength on leaf development — leaf thickness, allocation of biomass per unit leaf area (LMA) — was observed. Long-day conditions favoured positive responses in leaf area parameters. Higher day and/or average temperatures positively influenced seedling biomass as well as most leaf parameters. Seedling SLA and LMA responded more to light intensity treatments than to temperature treatments with increases in light intensity leading to a decrease and increase in SLA and LMA, respectively. Stem vigour was more influenced by daylength — with more compaction under long day than short day — increasing light intensity and, to some extent, temperature, specifically constant day and night temperatures. While increasing average temperatures and diurnal temperature changes influenced stem length, the response was mostly genotype dependent. The goal for hybrid seedling production should be to attain seedlings with large leaf area, short and sturdy stems — through higher average temperatures and light intensities — to increase the success of establishment under field conditions. As these studies were conducted under selected climate conditions and limited interactions of growth conditions, results should be used as a stepping stone in studies on optimisation of nursery hybrid seedling production. Additionally, further research is required to assess more levels and/or ranges of the factors influencing seedling vigour and seedling quality since these conditions differ depending on location.

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Author Contribution OCK designed the experiments and analysed the data. The manuscript was drafted by OCK and was revised based on the input of all authors. All authors approved the final version.

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Data Availability Yes

Code Availability Not applicable.

Declarations

Ethics Approval Not applicable.

Consent to Participate Not applicable.

Consent for Publication Yes

Competing Interests OCK and MEdV work at a hybrid potato breeding company; PCS is Editor-in-Chief of Potato Research.

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