Speed breeding in growth chambers and glasshouses for crop breeding and model plant research

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Abstract

'Speed breeding' (SB) shortens the breeding cycle and accelerates crop research through rapid generation advancement. SB can be carried out in numerous ways, one of which involves extending the duration of plants' daily exposure to light combined with early seed harvest to cycle quickly from seed-to-seed, thereby reducing the generation times for some long-day or day-neutral crops. In this Protocol, we present glasshouse and growth chamber-based SB approaches with supporting data from experimentation with several crops. We describe the conditions which promote the rapid growth of bread wheat, durum wheat, barley, oat, various Brassica species, chickpea, pea, grasspea, quinoa and Brachypodium distachyon. Points of flexibility within the protocols are highlighted, including how plant density can be increased to efficiently scale-up plant numbers for single seed descent. Additionally, instructions are provided on how to perform SB on a small-scale in a benchtop growth cabinet enabling optimization of parameters at a low cost.

KEYWORDS Speed breeding, Rapid generation advancement, Photoperiod, Glasshouse, Greenhouse, Growth chamber, Growth cabinet, Wheat, Barly, Pea, *Brachypodium*, Quinoa, Oat, Brassica, Single seed descent.

EDITORIAL SUMMARY This protocol describes the procedures for implementing speed breeding approaches using growth cabinets and LED-supplemented glasshouses. The approaches can be used to accelerate crop research and is compatible with a wide variety of crops.

TWEET A new protocol describing speed breeding approaches to accelerate crop breeding and plant research using growth cabinets and LED-supplemented glasshouses.

COVER TEASER Speed breeding for accelerated crop research

Please indicate up to four primary research articles where the protocol has been used and/or developed.

1. Watson, A. and Ghosh, S. et al. Speed breeding is a powerful tool to accelerate crop research and breeding. Nature Plants, 1 (2018).

2. Pretorius, Z.A., et al. An accelerated method for evaluating adult-plant resistance to leaf and stripe rust in spring wheat. Acta Phytopathologica et Entomologica Hungarica 35, 359-364 (2000).

3. Hickey, L.T., et al. Grain dormancy in fixed lines of white-grained wheat (*Triticum aestivum* L.) grown under controlled environmental conditions. Euphytica 168, 303-310 (2009).

4. O'Connor, D., et al. Development and application of speed breeding technologies in a commercial peanut breeding program. Peanut Science 40, 107-114 (2013).

Introduction

To improve the productivity and stability of crops there is pressure to fast-track research and increase the rate of variety development. The generation time of most plant species represents a bottleneck in applied research programs and breeding, creating the need for technologies that accelerate plant development and generation turnover. Recently we reported an approach for 'speed breeding' (SB), which involves extending the photoperiod using supplementary lighting and temperature control, enabling rapid generation advancement in glasshouses with sodium vapour lamps (SVL) or growth chambers fitted with a mixture of metal halide and light-emitting diode (LED) lighting[1]. By adopting a 22-hour photoperiod and controlled temperature regime, generation times were significantly reduced for spring bread wheat (*Triticum aestivum*), durum wheat (*T. durum*), barley (*Hordeum vulgare*), chickpea (*Cicer arietinum*), pea (*Pisum sativum*), canola (*Brassica napus*), the model grass, *Brachypodium distachyon* and the model legume, *Medicago truncatula*, in comparison to the field or a glasshouse with no supplementary light. Under the rapid growth conditions, plant development was normal, plants could be easily crossed (wheat and barley), and seed germination rates were high. We also demonstrated that SB can be used to accelerate gene transformation pipelines and adult plant phenotyping could be performed under SB conditions for traits such as flowering time, plant height, and disease resistance in wheat, leaf sheath glaucousness in barley, and pod shattering in canola[1].

The use of extended photoperiod to hasten plant growth is not novel. Sysoeva et al. (2010)[2] provides an extensive review of the literature surrounding this subject, published within the last 90 years, which outlines successful attempts using spring wheat, barley, pea, chickpea, radish (*Raphanus sativus*), alfalfa (*Medicago sativa*), canola, flax (*Linum usitatissimum*), arabidopsis (*Arabidopsis thaliana*), apple (*Malus domestica*) and rose (*Rosa x hybrida*), among others. More recent examples of photoperiod manipulation to hasten flowering time of crop species include lentil (*Lens culinaris*)[3,4], pea (*P. sativum*), chickpea (*C. arietinum*), faba bean (*Vicia faba*), lupin (*Lupinus angustifolius*)[5] and clover (*Trifolium subterraneum*)[6].

Here, we provide a standardised SB procedure for use in a glasshouse, or a growth chamber with additional data-supported modifications. We provide details for scaling-up plant numbers in the glasshouse, suitable for single seed descent (SSD) to generate large populations. Since plant species, indeed even cultivars within a species, are highly diverse in their response to photoperiod, a universal procedure for all plant species and traits is not possible. We therefore provide instructions for building a low-cost benchtop SB cabinet with controlled lighting and humidity monitoring, suitable for small-scale research projects and trialling SB parameters. Notwithstanding, we have

observed that the procedures are flexible and can be tailored to fit a wide range of breeding or research objectives and crop species. By sharing these procedures, we aim to provide a pathway for accelerating crop research and breeding challenges.

Overview of the procedure

In this protocol, we describe how to implement SB in existing growth chambers (see *Box 1*), and in temperature-controlled glasshouses using supplementary LED lighting, which provides significant cost savings over traditional SVLs (see Equipment setup, *LED-Supplemented glasshouse setup*). The procedures have been tested in the UK and Australia, with lights from the same company, but with slightly different models. We also outline compatible soil mixes for various crops when growing them under these lighting regimes (see Reagent Setup, *Soil*), along with advice for early harvest to reduce generation time further (see Procedure, *step 3: Harvesting the seed*). We provide supporting data to demonstrate the suitability of these setups (see Anticipated results) to significantly decrease the number of days to flowering and overall generation advancement for spring wheat, barley, canola, chickpea, pea, *B. distachyon*, *M. truncatula,* oat (*Avena strigosa*), grasspea (*Lathyrus sativus*) and quinoa (*Chenopodium quinoa*). We also include the design, step-by-step construction procedure, and operation of a small growth cabinet (see Equipment and Equipment Setup, *Benchtop Growth Cabinet*), which allows control over the light quality, intensity and photoperiod to help optimize the SB recipe for different crops and cultivars before implementing a large-scale glasshouse experiment.

Crop breeding programs commonly use SSD for several generations, on large numbers of segregating plants, to generate homozygous lines with fixed traits[7]. A glasshouse is often preferred for SSD because plant populations can be grown year-round. This process involves both a large investment in time as well as space within the glasshouse. Following the crossing of two homozygous lines, six generations of self-pollination are required to produce progeny that are 98.4% homozygous, which, at a rate of two generations per year, would take three years to complete. While only one or two seeds are needed from each plant to begin the next generation, plant researchers and breeders seek to maximise the number of plants within a restricted space. Plant density can be scaled-up under SB to enable concurrent rapid cycling of large plant populations, which is ideal for SSD programs. To demonstrate this, we evaluated spring wheat and barley sown at different plant densities in a glasshouse fitted with LED supplementary lighting (see Box 1). By comparing the physiological, morphological and yield parameters, we illustrate the normal development of these plants and highlight how this SB approach can save time and resources for SSD programs (see Anticipated Results, *Speed breeding in single seed descent (SSD) programs*).

Development of the approach

The SB concept was inspired by the efforts of NASA to grow crops in space, using an enclosed chamber and extended photoperiod[8]. In recognising the opportunity to more rapidly produce adult wheat and barley plants and allow faster selection and population development, SB became the norm in cereal research activities at the University of Queensland (UQ), Australia, thanks to Dr Ian Delacy and Dr Mark Dieters. The original approach was first described and implemented for wheat[9] and peanut (*Arachis hypogaea*)[10]. Variations of this approach have been demonstrated to be an efficient system for rapid screening of wheat germplasm for adult plant resistance to various diseases[11-14] and also for pyramiding multiple disease resistance in barley[15]. The appraoch has also been adapted for high-density plant production systems for SSD programs. The current SB approach described in this Protocol was developed from the initial implementation described for wheat to include a two-hour dark period that improved plant health[1]. This change was made following experiments in a controlled environment chamber at the John Innes Centre (JIC), UK, and was demonstrated to be suitable for accelerating research activities involving adult plant phenotyping, genetic structuring, and molecular studies like gene transformation in wheat and barley. It was further demonstrated to be suitable for rapid generation advancement for durum wheat (*T. durum*), pea, the model grass, *B. distachyon* and the model legume, *M. truncatula*, and could be scaled up in the SB glasshouse system at UQ, to be made suitable for rapid generation advancement of wheat, barley, canola and chickpea.

Comparison with other approaches

Perhaps the most well-known strategy to increase generation turnover is 'shuttle breeding', introduced by Dr Norman Borlaug in the 1950s at the international Centre for Maize and Wheat Improvement (CIMMYT), which enabled growing two generations per year by sowing wheat populations at field locations differing in altitude, latitude, and climate in Mexico[16]. There is also a long history of extensive efforts to accelerate plant growth of many species by manipulating photoperiod under artificial conditions, as briefly outlined above.

Supplementary lighting is not the only basis for rapid generation advance in plants. A common approach involves exerting physiological stress to trigger flowering and earlier setting of seed. This may involve restricting plant growth area (by growing plants at high densities), nutrient and water access[17] and/or intense light. Such a method is well-established and documented for rice[18] and has also been demonstrated for pea (Supplementary Figure 1) and canola[19]. Embryo rescue is another common feature in many rapid cycling methods where immature seed is harvested and induced to

175 germinate on culture media, with or without the addition of plant growth regulators (PGR), to
176 negate the waiting time for seed to mature. Bermejo et al. (2016)[20] used PGR in embryo culture
177 media to promote germination of immature lentil seed to achieve 4 generations annually. Mobini et
178 al. (2015)[21] sprayed lentil and faba bean plants with PGR to promote early flowering and applied
179 embryo rescue with PGR-enriched agar media to achieve up to 8 and 6.8 generations per year,
180 respectively. Castello et al. (2016)[22] reported 3-4 generations per year in subterranean clover
181 (*Trifolium subterraneum*), also with PGR in the culture medium. Application of PGR is not required
182 for SB, which may be desirable considering the additional time and effort required for handling
183 these and working out the logistics of their application at specific times. In addition, if a species-
184 specific protocol is not available, extensive testing would be needed to optimise such applications.
185 There are also examples of embryo rescue without PGR to shorten generation time. Zheng et al.
186 (2013)[23] and Yao et al. (2017)[24] reported up to 8 generations per year for wheat and Zheng et al.
187 (2013) [23] reported up to 9 generations per year for barley. Both Ochatt et al. (2002)[25] and Mobini and
188 Warkentin (2016)[5] reported up to 6.9 and 5.3 generations of pea per year respectively, and Roumet
189 and Morin (1997)[26] reported 5 cycles per year in soybean (*Glycine max* L.), all with embryo rescue
190 without PGRs. Other methods to reduce generation time have involved combining embryo rescue
191 with other techniques. In addition to hastening flowering through stress, Liu et al. (2016)[27] used
192 embryo rescue to achieve shorter generation times in oat (*Avena sativa*) and triticale (*Triticosecale*)
193 and Ribalta et al. (2017)[28] in pea. Yao et al. (2016)[19] reported 7 generations per year in canola when
194 combining stress and embryo rescue. Ribalta et al. (2014)[29] used the PGR Flurprimidol to reduce
195 plant growth and induce early maturation in pea, followed by embryo rescue to achieve over 5
196 generations per year. Without embryo rescue, SB conditions are capable of producing 6 generations
197 per year for spring wheat, barley, chickpea and pea, and 4 generations per year for canola[1]. Testing
198 is needed for any plant species prior to implementation, but this approach is promising for other
199 cereal, pulse and legume crops. Seed of wheat and barley produced under SB conditions can be
200 harvested prematurely at two weeks post-anthesis, followed by a short period of drying and chilling
201 to achieve high and uniform germination rates and healthy plants[1]. Approaches involving embryo
202 rescue are important and useful for breeding and research programs if the required infrastructure is
203 available[30], particularly for species that are recalcitrant to other parameters used to accelerate
204 generation advancement such as temperature or photoperiod manipulation[31-33]. In comparison, the
205 SB approach outlined here are less labour intensive, especially with large populations, and
206 laboratory facilities are not required, making the procedures more accessible.
207

Plant growth can also be promoted by increasing the CO_2 concentration. For example, for C_3 plants like rice and wheat, photosynthetic efficiency increases with increasing CO_2 levels, leading to an increase in biomass and early flowering. In fact, there are documented methods for rapid generation advance in rice that combine restricted root growth and canopy thinning with high CO_2 concentration, followed by early harvest and embryo rescue to cut down generation times of many rice varieties[34].

Doubled haploid (DH) technology, where haploid (*n*) embryos are rescued and undergo chromosome doubling (2*n*), is extensively and routinely used in the breeding of several crop species, thus reducing the number of generations required to achieve homozygous lines from six or more to just two generations[35]. Despite this, DH technology has some disadvantages: it can be expensive, requires specialist skills, restricts recombination to a single round of meiosis, and has a variable success rate that may be genotype-dependant[36]. The approach can also be labour intensive for large populations, especially those requiring removal of the embryos from the seed coat. Notably, there is the potential for SB to further accelerate the production of DH lines by speeding up the crossing, plant regeneration and seed multiplication steps.

We have presented a design for building a low-cost benchtop growth cabinet to trial SB. Compared to other published approaches for self-made growth chambers[37,38], our design makes use of a more widely available control system using a Raspberry Pi and compatible sensors, with codes for the user interface (UI) freely available from GitHub (https://github.com/PhenoTIPI/SpeedSeed3/wiki). The cabinet was trialled for the 22-hour SB lighting, temperature and photoperiod regime (22 °C/17 °C (22 hours/2 hours)), and successfully reproduced the accelerated development of one rapid-cycling variety of each of wheat and pea (Supplementary Tables 1, 2). The component costs for constructing such a cabinet are provided in Supplementary Table 3.

Limitations of the approach

Different plant species can have markedly different responses when exposed to extended photoperiods. For long-day (LD) plants, time to flowering is often accelerated under extended photoperiods since the critical day length is generally exceeded. This is also the case with day-neutral plants, where flowering will occur regardless of the photoperiod. In contrast, short-day (SD) plants require the photoperiod to be below the critical daylength to flower[39], which could be at odds with SB conditions. However, there are exceptions and some species show a facultative response where, although flowering is promoted by a particular photoperiod, flowering will still occur in the

opposite photoperiod. Furthermore, the time difference between being a SD or LD plant can be a matter of minutes[40]. These factors highlight both a limitation of SB and a point of flexibility. In cases where the photoperiod response is unknown or complex in nature, experimentation of light and temperature parameters is required to optimise a SB strategy, for example, by using the benchtop growth cabinet. For instance, applying extended light prior to and following a shortened photoperiod to induce flowering, could hasten initial vegetative growth and accelerate maturity, respectively, thus producing an overall shorter generation time. Such an approach has been successfully applied to amaranth (*Amaranthus* spp. L), a SD species, where a 16-hour LD photoperiod was used to initiate strong vegetative growth after which plants were transferred to an 8-hour SD photoperiod to induce flowering[41]. The overall effect was a shorter lifecycle and ability to produce eight generations per year rather than two in the field. The need for vernalisation, such as in winter wheat, creates a situation similar to above. Young plants require chilling for a number of weeks to trigger the transition to flowering. Once the vernalisation requirement is met in winter wheat, exposing the plants to extended photoperiod is likely to accelerate growth[42,43]. Overall, the 'SB recipe' is more straight forward and easier to implement for LD and day neutral species which do not require vernalisation. Experimentation and optimisation of parameters are highly recommended for each species.

The SB procedures presented here take place in an enclosed, artificial environment, which differs significantly from the field where eventual crop production may occur. While this is acceptable for many activities, such as crossing, SSD and screening for some simple traits[1], other activities, such as selection for adaptation in the target environment must still occur in the field. Nevertheless, programs alternating between SB and the field save time overall. The ability to shorten generation time further through early harvest of immature seed can interfere with the phenotyping of some seed traits. For this reason, in spring wheat breeding programs where dormant and non-dormant genotypes need differentiating, phenotyping grain dormancy under SB conditions is limited to only four generations per year[9].

The initial investment to build a glasshouse or purchase a growth chamber with appropriate supplementary lighting and temperature control capabilities is substantial if these facilities are not already available. However, depending on the budget of the research or breeding program, the benefits may outweigh the costs. For instance, an economic analysis performed by Collard et al. (2017)[44] compared the rapid generation advance (i.e., no phenotypic selection at each generation) with the pedigree-based breeding method (i.e., with phenotypic selection at each generation) for

rice and determined that rapid generation (achieved through restricted soil access and canopy thinning) was more cost-effective and advantages would be realized after one year even if new facilities were constructed. Nevertheless, most breeding programs have pre-existing glasshouse facilities that can be converted for SB applications, but careful selection of energy efficient lighting and temperature control systems are needed to minimise operating costs. Research activities often do not require the high plant numbers needed in breeding, so growth chambers are common. The cost of these start at tens of thousands of dollars, making them inaccessible for many projects and a barrier for implementing SB. In addition, the energy to provide extended supplementary lighting is significant. A cost-benefit analysis should be carried out to determine feasibility although there are areas where cost-savings can be made. Supplemental LED lighting provides more efficient power usage and reduced heat than other lighting types, such as SVLs. An estimate of the maintenance and energy costs associated with LED lighting is provided in the supplementary material of Watson and Ghosh et al. (2018)[1]. Investing in solar panels is another strategy to offset the increased energy costs, depending on availability and location.

The investment in SB needs to be weighed in terms of the potential benefits to variety development and research output. As with most technologies, determining the optimal way to integrate SB in a crop improvement program needs careful consideration and may require significant re-design or restructure to the overall program. Prior to implementing such changes, computer simulations are a good way to evaluate the different breeding programs incorporating SB.

Experimental Design

To set-up an effective SB system, certain factors require careful consideration. These include:

Lighting requirements: Many lighting sources are appropriate for SB, including SVLs and LEDs[1]. Even incandescent lighting has been shown to accelerate flowering in clover[6]. However, selection should be based on the space available, plant species and energy resources. For example, LED lighting may be preferred due to its energy efficiency although simple incandescent lighting may be suitable within a smaller area, with sufficient cooling to counteract the higher heat output. Plant species may also differ in their response to the different spectra of wavelengths emitted by different lighting sources so this should be carefully considered. The lighting setup for glasshouses and growth chambers detailed in this protocol can act as a starting point but is by no means the final conditions that may be optimum for another situation. The procedures outlined here have been successful for the species trialled but a modified approach may be more suitable for another crop. We recommend

mining existing literature and studies on suitable light spectra (particularly with regard to blue to red ratios, red to far-red ratios, and the proportional level of UV light that may be introduced into the system) for the crop and trait of interest.

Initial light calibrations: Requirements in terms of light quality and intensity for a particular species, cultivar of that species, and desired phenotype, should be determined prior to application on a large scale or use within an experiment. Several 'dummy' or 'test' growth cycles are recommended to initially assess the rate of growth and quality of the plants so that alterations can be made to enable optimal outcomes (see **Box 1**). For this purpose, we recommend starting with the benchtop growth cabinet option – the costs of which are low enough to build several and trial, in parallel, different light-combinations, photoperiods and temperatures to determine the optimal conditions to implement on a larger scale, such as a glasshouse, for your crop and trait.

Germplasm: As detailed above, not all plant species (or indeed cultivars within a species) are amenable to extended photoperiod. Care should therefore be exercised in selection of the germplasm to be grown under SB and appropriate modifications implemented to ensure optimal conditions for each species.

End-use requirements: The intended end-use of the resultant plants can affect all aspects of the initial set-up of the SB approach, such as glasshouse space and sowing density. For example, within an SSD program large numbers of plants are grown within a defined space, so an appropriate sowing density needs to be determined. Conversely, a small number of plants needed for a research experiment under variable lighting parameters is more appropriate for a small growth chamber experiment with flexible settings.

Control conditions: Before beginning a SB experiment, it is important to have replicates of your germplasm growing under the conditions you would normally use in your breeding program or institute. This will allow you to directly compare plant growth parameters (including generation time), operational costs (e.g. electricity) and plant quality. For popular varieties grown for many generations in the field or glasshouses, the control data may be readily available.

Materials

Reagents

- **Soil**
  - UQ Compost Mix, designed by Mr K. Hayes, Central Glasshouse Services, University of Queensland, Australia (composition outlined in Supplementary Table 4)
  - JIC Cereal Compost Mix (prepared by Horticulture Services at the John Innes Centre, composition outlined in Supplementary Table 5)
  - JIC Peat and Sand Mix (prepared by Horticulture Services at the John Innes Centre, composition outlined in Supplementary Table 6)
- **Nutrient**
  - Vitafeed Balanced 1-1-1, Vitax (http://www.vitaxgrower.co.uk/product/vitafeeds/)
  - Calcium nitrate (Sigma, cat. no. C1396)
  - Gibberellic acid (GA_3)(Sigma, cat. no. G7645)
- **Seeds**

  If the reader wishes to replicate any of our experiments with the same germplasm, information on where the relevant seed can be obtained is listed in Supplementary Table 7.

Equipment

Benchtop growth cabinet

CRITICAL This section provides an overview of the equipment required for constructing a small benchtop cabinet for SB, which may be used for small-scale pilot trials before investing in a larger system, such as a glasshouse. The cabinet has a footprint of 0.225 m^2 and comfortably accomodates eight 1 L square pots. To construct your low cost growth cabinet the components listed below are required.

- **Hardware**
  - 12 V, 50 A DC power supply 600 W (Amazon, cat. no. B072M7P7QJ)
  - 12 V to 5 V, 3 A DC/DC converter module (Amazon, cat. no. B00G890MIC)
  - USB extension cable – 30 cm (Amazon, cat. no. B002M8RVKA)
  - Ethernet extension cable – 30 cm (Amazon, cat. no. B077V421QH)
  - Arduino UNO (Amazon, cat. no. B00CGU1VOG)
  - Raspberry Pi 3 model B (CPC, cat. no. 2525225)
  - Raspberry Pi display 7 inch touchscreen (CPC, cat. no. 2473872)
  - Arduino base shield v2 – SeeedStudio (CPC, cat. no. SC13822)
- **Cabinet structure**

- Aluminium composite panel, 757 X 307 X 3 mm, quantity = 6 (Cut Plastics, cat. no. CP027-03)
- Aluminium composite panel, 757 X 357 X 3 mm (Cut Plastics, cat. no. CP027-03)
- Aluminium composite panel, 757 X 107 X 3 mm (Cut Plastics, cat. no. CP027-03)
- Aluminium composite panel, 757 X 757 X 3 mm (Cut Plastics, cat. no. CP027-03)
- PVC foam board, 757 X 157 X 3 mm, quantity = 2 (Cut Plastics, cat. no. CP015-03)
- PVC foam board, 757 X 141 X 3 mm (Cut Plastics, cat. no. CP015-03)
- PVC foam board, 757 X 307 X 3 mm, quantity = 2 (Cut Plastics, cat. no. CP015-03)
- Perspex clear acrylic sheet, 757 X 307 X 3 mm (Cut Plastics, cat. no. CP001-03)
- OpenBeam, 1000 mm, quantity = 4 (Technobots Online, cat. no. 4451-900)
- OpenBeam, 750 mm, quantity = 13 (Technobots Online, cat. no. 4451-750)
- OpenBeam, 300 mm, quantity = 10 (Technobots Online, cat. no. 4451-300)
- Corner bracket – MakerBeam, quantity = 4 (Technobots Online, cat. no. 4446-013)
- L-joining plate – OpenBeam, quantity = 36 (Technobots Online, cat. no. 4450-003)
- T-joining plate – OpenBeam, quantity = 2 (Technobots Online, cat. no. 4450-004)
  - **Lighting system**
- Full spectrum grow light LED bulb, quantity = 16 (Amazon, cat. no. 071J3BC1W)
- E27 lamp holder, quantity = 16 (Sinolec Components, cat. no. E27-SD04-2)
- Solid state relay – grove SeedStudio (Mouser, cat. no. 713-103020004)
  - **Temperature and humidity control system**
- 12 V, 10 A thermoelectric cooler, quantity = 3 (Amazon, cat. no. B01M2ZBBVM)
- Temperature and humidity sensor pro–grove SeeedStudio (CPC, cat. no. MK00343)
- Relay – grove SeedStudio, quantity = 4 (CPC, cat. no. MK00330)
- 12 V cooling fan, 50 mm (Amazon, cat. no. B00HPKC5MO)

Software

- Arduino IDE (v1.8.5, https://www.arduino.cc/en/Main/Software)

LED-supplemented glasshouse setup

CRITICAL: This section provides an overiew of the equipment required for setting up SB in a glasshouse using LED lamps for supplementary lighting. Its efficacy is demonstrated for a range of crop species, along with some examples of how single-seed descent for wheat and barley can be carried out.

- **Glasshouse**: A well-located glasshouse with the required space and sufficient ambient lighting. We recommend fitting a temperature control system and programmable lights.

Controllable blinds are also optional if blocking out high irradiance on very sunny days is required.

- **LED lamps**: While any kind of lighting system can be used to supplement the ambient lighting in the glasshouse, we recommend LED lamps above all because of the significant savings these provide in terms of maintenance and energy consumption. The glasshouse-based SB experiments detailed in our previous paper[1] were based on SVLs, but we have obtained similar results with LED-lighting at both UQ and JIC. The LED supplemental lighting within glasshouses at JIC (UK) and UQ (Australia), were supplied by the same company, Heliospectra (Göteborg, Sweden). Details of both setups are provided, along with the results of experiments carried out at both locations.The lighting system configuration, make and model of the lights for both locations are provided in Equipment setup.
- **SSD trays**: For demonstration, at UQ, three seedling tray types with increasing sowing densities were used. The dimensions and volumes are given in Supplementary Table 8. The soil media composition is given in Supplementary Table 4.

CRITICAL: Energy tariffs can vary according to the time of day, depending on peak energy usage patterns in the location. Substantial savings can be achieved by programming the dark period to coincide with the energy tariff imposed during peak electricity consumption.

Additional equipment needed

- **PAR meter**: The PAR is measured in either PPFD or Lux. Any off-the-shelf PAR meter can be used, as long as it provides PPFD levels and relative wavelength composition. We used the MK350S Spectrometer from UPRtek and the Spectrum Genius Essence Lighting Passport light sensor from AsenseTek Inc. (Taiwan) at JIC and UQ, respectively.
- **Energy meter**: This allows measuring the energy consumption for lighting and temperature maintenance thereby providing insight into SB operational costs. Any off-the-shelf energy meter can be used for this purpose. To obtain energy consumption data for both the lights employed and the Controlled Environment Rooms (CERs) at JIC, we utilised a clamp-on Current Transformer meter with the capacity to store and download data. The instrument provided half hourly readings and as such was highly accurate in determining energy costs

Reagent Setup

Soil. Soil mixtures which have previously been shown to work for certain crops in SB conditions are provided in Table 1. Please refer to this table to pick the most appropriate mix for your crop and prepare the mix using the necessary components in the required proportions. Details of the soil

mixture composition, along with information on proportions and suppliers, can be found in Supplementary Tables 4, 5 and 6. Some components, for example, the wetting agent, may need to be adjusted depending on the local watering regimes and practices.

CRITICAL: The JIC Cereal Mix and Peat and Sand Mix composts must be prepared fresh in order to eliminate the potential for inconsistent fertiliser spread through the soil and a build up of salts occurring in the stored compost, as the slow realise fertiliser starts to break down and leaches to the bottom.

Nutrient feed. Depending on the size of the pots and the type of soil, the plants may need a nutrient feed. If the pots are small (~100 ml), a single or fortnightly application of a liquid nutrient feed should be considered to prevent the plant leaves from turning yellow prematurely with concomitant reduced vigour and seed set. In the JIC glasshouses and growth chambers, we have successfully used Vitafeed Balanced 1-1-1 from Vitax (http://www.vitaxgrower.co.uk/product/vitafeeds/) for wheat growing in high density trays.

CRITICAL: Due to the rapid growth of plants under SB, fertiliser application and swift amelioration of nutrient deficiencies are of utmost importance. Appropriate slow-release fertiliser within the soil media is recommended for growth to maturity, and maintenance of soil pH is important to avoid restriction of nutrient absorption; e.g. a pH that is too acidic can inhibit calcium uptake. Foliar fertiliser applications may be required for rapid access of nutrients to the leaves although some level of calcium deficiency is common. See Supplementary Figure 2 for common symptoms of calcium deficiency. In our experience, for wheat, barley and *Brachypodium*, symptoms are more common at early growth stages during the period of prolific vegetative growth and are relieved at later growth stages. See ?TROUBLESHOOTING for specific suggestions on calcium applications.

Equipment setup

Benchtop growth cabinet

- **Hardware:** Connect the display to the Raspberry Pi using the provided cables as instructed by the manufacturer. The Arduino connects to the Raspberry Pi via USB ports. Sensors and relay modules are connected using the Grove system (SeedStudio).
- **Cabinet structure:** Assemble the beam profile using the joining plates. Slide the panels, boards and sheets before fully assembling each side.
- **Lighting system:** The photoperiod with the full-spectrum LED light bulbs is controlled by a solid-state relay connected to the Arduino microcontroller. Sixteen 57 mm diameter holes need to be drilled in one of the 757 x 307 x 3 mm aluminium composite panels, to fit the E27 lamp holders. The lamp holders are then inserted and wired in parallel.

- **Temperature and humidity system:** Pre-assembled thermoelectric cooling modules are used to simplify the construction of the benchtop growth cabinet. These are composed of fans, aluminium heat sinks, and Peltier elements. The cooling modules are controlled by relays connected to the Arduino. Airflow is used to control the humidity, *i.e.* the humidity sensor will trigger the 12 V fan to circulate air from outside the cabinet in order to reduce the humidity inside.
- **Software installation and setup:** The speed breeding cabinet is controlled by three main subsystems: The arduino micro controller that monitors and controls the environment according to a desired optimal; a python daemon that stores the current conditions and reads the expected conditions from a MongoDB database and; a graphical interface written in ReactJS that allows the users to set up the expected conditions in a 24-hour range. The circuit diagram for making the connections are provided in Supplementary Figure 3 and a photograph of the assembled cabinet is provided in Supplementary Figure 4. The cabinet has an available area of 0.225 m^2 . For the lamps we have used, the spectrum is provided in Supplementary Figure 5, with the light levels in PPFD (Photosynthetic Photon Flux Density) being on an average about 120 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at 16 cm above the base where the pots are kept, and about 320 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and 220 $\mu mol \cdot m^{-2} \cdot s^{-1}$ from a 10 cm and 20 cm distance respectively from the top of the cabinet where the lights are situated. The energy consumption of the mini cabinet is 6.24 kWh per day. A step-by-step guide for constructing the cabinet and installing the software is available at https://github.com/PhenoTIPI/SpeedSeed3/wiki, along with troubleshooting tips.
  Caution: The construction of the cabinet requires the use of sharp cutting and drilling tools that may cause physical injury if handled improperly. Many steps involve electrical components, which can cause fire if operated without being earthed. Ensure all necessary safety steps are followed and use personal protective equipment when constructing the cabinet.

LED-supplemented glasshouse

Table 2 provides the lighting arrangement in two glasshouse configurations. Both setups have been demonstrated to successfully support SB for the species listed.

A summary of the crops for which we have successfully demonstrated a shortening of generation time using SB, including information on which specific SB setups were used, and where the reader can find more information on the key growth stages and other growth parameters of the crop grown under those conditions is provided in Table 3.

CRITICAL: Weather and ambient light varies by location and season, especially at higher latitudes. Thus, for the glasshouse setups listed here, the light spectrum is determined not just by the presence of the LED lights but also by the ambient light. To ensure reproducibility, consider setting up your experiment in a way that mitigates these environmental variables. For example, use programmable lights that allow intensity modification based on sensor feedback, or controllable blinds to regulate photoperiod. Provision of a short dark-period is recommended for optimum plant health. We highly recommend setting up a temperature monitoring and control system.

Procedure

Preparing seed for sowing.

1. To increase germination efficiency some seeds may need a pre-treatment either by cold stratification (prolonged imbibition in the cold) or scarification (physical or chemical weakening of the seed coat). In case pre-treatment is required, follow Option A, if pre-treatment is not required, follow Option B.

Option A: Germination with pre-treatment to break seed dormancy. [TIMING 5-7 days]

CRITICAL The requirements for germination pre-treatments are specific for each species, and accessions of that species, and should be determined on an individual basis.

i. Imbibe dormant seed on moistened filter paper in a Petri dish for 24 hours and then chill at 4 °C for approximately three days (longer times may be required depending on the level of dormancy) in the dark. In a large-scale scenario, directly sow seeds into high density trays and place the tray in a cold-room.

ii. Leave the seeds at room temperature (~20-25°C) for one to three days to germinate in the dark prior to transferring to soil. In the large-scale scenario, trays can now be moved to the growing environment in the glasshouse. (see ?TROUBLESHOOTING for tips on handling seed germination issues)

iii. Grow the plants under the desired speed breeding conditions (see **Box 1**).

Option B: Germination without pre-treatment to break seed dormancy. [TIMING 3-5 days]

i. If pre-treatment is not required, germinate the seed in a Petri dish on moistened filter paper in the dark before transferring to soil. In a large-scale scenario, seed may be sown directly into soil in the glasshouse/growth chamber. Note that for some crop species like pea or grasspea, you need to scarify seeds by chipping off a tiny bit of the seed coat with a scalpel to facilitate better imbibition. Take care not to chip on or around the hilum of the seed, to avoid damaging the embryo.

CRITICAL STEP: If seeds germinate in a Petri dish and become too well established (i.e. develop green leaves) before transplanting to soil, the shift to SB conditions, especially the presence of intense light, can shock the plants, resulting in a strong hypersensitive response and possibly death. Take care to prick them out early, or if they are already established, transfer them to soil and place a mesh over the plants to reduce light intensity while they adapt to the new environmental conditions.

ii. Grow the plants under the desired speed breeding conditions (see **Box 1**).

Monitoring key growth stages, growth parameters, and phenotyping: [TIMING: depending on crop, cultivar/genotype, and SB setup used. Refer to Table 3 for guidance timelines in associated supplementary data]

2. To enable comparison to normal development, monitor the key growth stages of the plants. For many crops, defined growth stages have been published; for example, cereal crops[45], canola[46], quinoa[47] and legumes[48]. Take note of the heading times and earliest time point to harvest viable seeds. We also advise monitoring the height and general physiology of the plants. Plants growing at such a rapid pace may start to exhibit micronutrient deficiencies. The manifestation of some of these deficiencies can interfere with plant phenotyping, and reduce seed set. Some of these issues (particularly for wheat and barley) are highlighted in ?TROUBLESHOOTING .

~~**CRITICAL STEP**~~*:* Experiments performed using a LED-supplemented glasshouse setup at the JIC, UK, involved a SB glasshouse compartment (i.e. 22 h day length; as detailed in **Table 2**)), and a twin compartment with a 16 h day length to measure the effect and value of increased day length. Growth parameters and harvest times are provided for both lighting regimes where available.

CRITICAL STEP*:* For wheat and barley, we have previously demonstrated how SB conditions do not interfere with the phenotyping of a number of key traits[1], and how variations of the SB approach can be used to rapidly screen wheat and barley for resistance to a number of major diseases or disorders (Table 4).

Harvesting the seed: [TIMING: depending on crop, cultivar/genotype, and SB setup used. Refer to Table 3 for guidance timelines in associated supplementary data]

3. Shortened generation times can also be achieved in some species by harvesting premature seed. In order to do this, one should first wait until the seeds have set in the plant (indicated by filled seed in spikes for wheat, or filled pods for legumes). After this has occurred, either increase the temperature or withhold water from the plant to hasten seed ripening and drying. After a week of this stress application, harvest the seeds.

   ~~**CRITICAL STEP:**~~ For experiments performed using the LED-supplemented glasshouse setup (at the JIC, UK), early harvest times are provided for both lighting regimes where available. If not indicated, the harvest time outlined is for harvest at physiological maturity.

   CRITICAL STEP: Freshly harvested seed may display dormancy. See ?TROUBLESHOOTING for more details on how to overcome this issue.

Monitoring the energy use: [TIMING: Once at the end of every cycle]

~~4.~~ At the end of one cycle, review the energy costs for your SB system. This is particularly useful to evaluate the generation time vs cost trade-off where multiple conditions have been tested concurrently (e.g. different day lengths). For the LED-Supplemented glasshouse setup in JIC, there were two rooms set up concurrently with 16-hour and 22-hour photoperiods. An example of the energy calculations for running each of these setups per month is given in Supplementary Table 9, along with a comparison of how much it would cost to run a similar setup with Sodium Vapour Lamps.

Timing

Step 1 Option A, Germination with pre-treatment. **5-7 days**.

Step 1 Option B, Germination without pre-treatment. **3-5 days**.

Step 2, Monitoring key growth stages, growth parameters, and phenotyping. **(depending on crop, cultivar/genotype, and SB setup used. Refer to Table 3 for guidance timelines in associated supplementary data).**

Step 3, Harvesting the seed. **(depending on crop, cultivar/genotype, and SB setup used. Refer to Table 3 for guidance timelines in associated supplementary data).**

Step 4, Monitoring the energy use. **Once at the end of every cycle**.

Troubleshooting

Troubleshooting guidance can be found in **Table 5**.

Anticipated Results

As demonstrated in our previous study, under SB conditions with a 22-hour photoperiod, it should be possible to produce up to 6 generations per year in spring wheat and barley and up to 4 and 4.5 generations per year in canola and chickpea, respectively[1]. However, it is important to remember that results are highly dependent on the crop species and can vary greatly between cultivars. The light quality, duration of the photoperiod and temperature regime also impact the extent to which the generation time is reduced. It should also be noted that ambient sunlight strength and duration will vary with location and season, thus resulting in differences in rate of development. These factors, in addition to basic growing conditions, such as soil type, can be manipulated to obtain the optimal parameters for the crop of interest. The various procedures outlined above are designed to facilitate this process.

Speed breeding using the bench-top cabinet

The self-made, bench-top speed breeding cabinet will facilitate identification of conditions that enable rapid-cycling of wheat and pea, and by extension, the other crops listed (Supplementary Figure 4). We demonstrated the efficacy of this cabinet design by growing rapid-cycling varieties of pea (*P. sativum* cv. JI 2822) and wheat (*T. aestivum* cv. USU Apogee) and showing the shortened time from seed to seed, without compromising the viability of early harvested seed (Supplementary Tables 1, 2). This is comparable with data from our previous study[1] where we evaluated the same pea variety (JI 2822) under SB conditions using a commercial CER.

Speed breeding using LED-supplemented glasshouses

The time taken for reproductive development to occur for a range of crop species under the LED-fitted, SB glasshouse (JIC, UK) is provided in Table 6. Two extended photoperiods are represented to give an approximate expectation of the rapid development of these species under SB, and to give the reader an idea of what a 6-hour difference in photoperiod can produce in a range of crops and cultivars. The much slower rate of development under control or regular glasshouse conditions without supplemental lighting was reported for some of these species in our previous study[1] . Plants grown under SB can be expected to look healthy (Figure 1) with minor reductions in seed set (refer to Table 3 in order to view the related data for the crop of interest) and spike size (Supplementary Figure 6) or pod size (Supplementary Figure 7 and Supplementary Figure 8). In some crop species, the SB conditions can produce a slight reduction in height and/or internode length. In our experience, while working on *M. truncatula* and *P. sativum*, we found the plants grown under SB produced leaves with much smaller surface areas. Occasionally, micronutrient deficiencies manifest

themselves because of the rapid growth and change in soil pH – some of these issues (particularly for wheat and barley) are highlighted in the ?TROUBLESHOOTING section. Despite efforts to optimise soil composition, there may be a cultivar that responds very poorly to the long-photoperiod and high irradiance.

We have previously demonstrated that wheat, barley and canola plants grown under SB are suitable for crossing and phenotyping a range of adult plant traits[1]. That said, complex phenotypes such as yield and abiotic stress resilience (heat or drought stress) are best evaluated in the field, particularly for breeding objectives. We have also demonstrated how SB can be combined with transformation of barley to speed up the process of obtaining transformed seeds[1].

Speed breeding in single seed descent (SSD) programs

In breeding programs, SSD is often an important step in cultivar development that requires high-density plantings. The SB approach provided for glasshouses are ideal for SSD programs, particularly cereal crops. Increasing sowing density under SB can enable rapid cycling of many lines with healthy plants and viable seed. Figure 2 shows an example of the plant condition, spike lengths and seed sizes that could be expected at various sowing densities in SB. Under the UQ-GH-LED approach, at a density of 1000 plants/m^2, up to 6 generations of wheat and barley can be expected per year (Supplementary Tables 10, 11, 12, and 13). At higher densities, plant height and seed numbers can be reduced due to the greater competition and low soil volume. Despite this, even at the highest sowing density shown here, all plants produced a spike with at least enough seed to perform SSD, and in most cases many more. Large differences in the speed of development can be achieved by extending the photoperiod from 16 to 22 hours. Under the JIC-GH-LED approach, spring and durum wheat were over ten days faster in development with an additional 6 hours of photoperiod. Table 7 provides the approximate development times for several cereal crops at a range of sowing densities, appropriate for intensive SSD. The SSD SB approach was performed under two extended photoperiod and temperature regimes at either JIC, UK, or UQ, Australia. These results demonstrate that plants can be grown at high densities under SB conditions to produce plants suitable for effective and resource-efficient generation turnover in SSD programs.

Figure 1. Accelerated plant growth and development under speed breeding compared to standard long-day conditions. Plants on the left are grown under speed breeding (22-hour photoperiod conditions) and plants on the right are grown under standard long-day (16-hour photoperiod) conditions in LED-supplemented glasshouses at John Innes Centre, UK. **a,** Winter growth-habit wheat (*T. aestivum* cv. Crusoe) at 112 days after sowing (DAS), including 12 days of growth under 16-hour photoperiod conditions followed by 56 days of vernalisation at 6 °C with 8 hour photoperiod; **b,** Spring wheat (*T. aestivum* cv. Cadenza) at 57 DAS; **c,** Spring barley (*H. vulgare* cv. Manchuria) at 35 DAS; (scalebar is 20 cm for a, b, c) **d,** Grasspea (*L. sativus* cv. Mahateora) at 35 DAS (red arrows indicate position of flowers); **e,** *B. distachyon* (accession Bd21) at 34 DAS; **f,** Pea (*P. sativum* accession JI 2822) at 34 DAS; (scalebar is 20 cm for d, e, f) **g,** Quinoa (*C. quinoa* accession QQ74) at 58 DAS; **h,** *Brassica oleracea* (line DH1012) at 108 DAS**; i,** *Brassica napus* (line RV31) at 87 DAS **; j,** *Brassica rapa* (line R-0-18 87) at 87 DAS**; k,** Diploid Oat (*A. strigosa* accession S75) at 52 DAS (scalebar is 60 cm for g, h, i, j). All plants were sown in October or November 2017, except for the quinoa, which was sown in February 2018.

Figure 2 | Single seed descent sowing densities of spring wheat (bread and durum) and barley. All plants were grown under LED-Supplemented Glasshouse setup at JIC, UK or UQ, Australia. Durum wheat (*T. durum* cv. Kronos) grown under the LED-Supplemented Glasshouse setup, JIC, UK, in 96-cell trays: **a**, Forty-three days after sowing under 16-hour photoperiod; **b**, Forty-three days after sowing under 22-hour photoperiod; **c**, Seventy-nine days under 16-hour photoperiod; **d**, Seventy-nine days under 22-hour photoperiod. Scale bar is 20 cm. Spring wheat (*T. aestivum* cv. Suntop) grown under LED-Supplemented Glasshouse setup, UQ, Australia, at 37 days after sowing: **e**, plants in a 30-cell tray; **f**, plants in a 64-cell tray; **g**, plants in a 100-cell. Barley (*H. vulgare* cv. Commander) grown under LED-Supplemented glasshouse setup, UQ, Australia, at 34 days after sowing: **h**, plants in a 30-cell tray; **i**, plants in a 64-cell tray; **j**, plants in a 100-cell. Scale bar is 20 cm. Mature spikes of spring wheat (*T. aestivum* cv. Suntop) grown under LED-Supplemented glasshouse setup, UQ, Australia: **k**, plants in a 30-cell tray; **l**, plants in a 64-cell tray; **m**, plants in a 100-cell. Mature spikes of barley (*H. vulgare* cv. Commander) grown under LED-Supplemented glasshouse setup, UQ, Australia: **n**, plants in a 30-cell tray; **o**, plants in a 64-cell tray; **p**, plants in a 100-cell. Scalebar is 3 cm. Mature seeds of spring wheat (*T. aestivum* cv. Suntop) grown under LED-Supplemented glasshouse setup, UQ, Australia: **q**, plants in a 30-cell tray; **r**, plants in a 64-cell tray; **s**, plants in a 100-cell. Mature seeds of barley (*H. vulgare* cv.

711 Commander) grown under LED-Supplemented glasshouse setup, UQ, Australia: **t**, plants in a 30-cell
712 tray; **u**, plants in a 64-cell tray; **v**, plants in a 100-cell. Scalebar is 1 cm.
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Competing Interests – All the authors declare that they have no competing financial interests.

Data and code availability statement - The authors confirm that all relevant data are included in the paper and/or its supplementary information files as summary statistics. Any request for raw data collected by researchers should be made to the corresponding authors. All relevant code required for running the small customised speed breeding growth cabinet are provided in the public GitHub link: https://github.com/PhenoTIPI/SpeedSeed3/wiki.

Table 1 | List of soil mixes that have been demonstrated to be compatible for speed breeding using our approach. For details on the soil media composition, see Supplementary Tables 4, 5, 6.

Species	Compatible soil mixes
Bread wheat (*T. aestivum*)	JIC Cereal Compost Mix, UQ Compost Mix
Durum wheat (*T. durum*)	JIC Cereal Compost Mix, UQ Compost Mix
Barley (*H. vulgare*)	JIC Cereal Compost Mix, UQ Compost Mix
Pea (*P. sativum*)	JIC Cereal Compost Mix
Chickpea (*C. arietinum*)	UQ Compost Mix
Brassica rapa	JIC Cereal Compost Mix
Brassica oleracea	JIC Cereal Compost Mix
Canola (*Brassica napus*)	JIC Cereal Compost Mix, UQ Compost Mix
Quinoa (*C. quinoa*)	JIC Peat and Sand Mix
Oat (*A. strigosa*)	JIC Cereal Compost Mix

Grasspea (*L. sativus*)	JIC Cereal Compost Mix
Brachypodium distachyon	JIC Cereal Compost Mix, 50% JIC Cereal Compost Mix + 50% JIC Peat and Sand Mix
Medicago	JIC Cereal Compost Mix

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883 **Table 2 | LED-Supplemented Glasshouse setups for speed breeding at JIC and UQ**

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		John Innes Centre (JIC), United Kingdom	**University of Queensland (UQ), Australia**
LED lamp make and model		LX602C LED Grow Lights from Heliospectra (Göteborg, Sweden). More information can be found at: https://www.heliospectra.com/led-grow-lights/lx60/	E602G LED Grow Lights from Heliospectra (Göteborg, Sweden). More information can be found at: https://www.heliospectra.com/led-grow-lights/e60/
Glasshouse area		66.4 m^2	30 m^2
No. of fitted lights and arrangement	*No. of lights in the given area*	25 Heliospectra LX602C lights	8 Heliospectra E602G lights
	Distance between lights and bench	244 cm	155 cm
	Distance between lights and plant canopy/ sensor	144 cm (LICOR sensor, kept approximately at plant canopy height)	95 cm from approximately the spike-height of a tall, adult wheat plant.
	Approximate distance of canopy from bench surface	100 cm	60 cm
	Schematic	Supplementary Figure 9	Supplementary Figure 10

Light level monitoring and programmability	These fixtures can be programmed to emit custom spectra and light intensities.	These fixtures are not programmable and have a fixed spectrum and intensity.

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Lighting regime and PPFD levels	Two similar compartments within the same glasshouse were set up with two different photoperiod regimes: i) 22 hours of light, followed by 2 hours of darkness ii) 16 hours of light, followed by 8 hours of darkness The PPFD values and spectrum at various distances from the lights are provided in Supplementary Table 14 and Supplementary Figure 11.	Photoperiod of 22 hours, followed by 2 hours of darkness. The PPFD values and spectrum at various distances from the lights are provided in Supplementary Table 15 and Supplementary Figure 12.
Temperature Regime	20 °C as the maximum temperature to be operative during the photoperiod (16 or 22 hours depending on the photoperiod regime, *see above*). 15 °C as the minimum temperature to be operative during the dark period (8 or 2 hours depending on photoperiod regime, *see above*).	22 °C as the maximum temperature to be operative for 12 hours during the photoperiod. 17 °C as the minimum temperature to be operative during the dark period (2 hours).

Heating/Cooling system	*Heating*: gas-fired central heating *Cooling*: Cooling fans that go off when the temperature goes above a set-point. *Temperature monitoring and control*: Glasshouse temperature monitoring is carried out through TomTech (TomTech UK Ltd) which is a glasshouse specific business management system.	*Heating and cooling*: a 240 kW chilled water system that uses insulated aspirated temperature controller sensors with air handling units to each room with heaters and chilled water valves. *Temperature monitoring and control*: Glasshouse temperature automatically controlled using a business management system running on an Innotech system using Magellan Builder (Brisbane, Australia). The temperatures are controlled to ± 1 °C.

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890 **Table 3 | A list of speed breeding approaches that have been demonstrated for different**

891 **species along with pointers for locating the associated data.**

Species	**Demonstrated SB conditions and associated data**		
	This protocol	**Watson and Ghosh et al., 2018**	**Other**
Spring wheat *T. aestivum*	JIC-GH-LED[1] (Supplementary Tables 16 - 24) UQ-GH-LED[2] (Supplementary Tables 10 and 11)	UQ-GH-SVL[3] (Supplementary Tables 11, 15, 21, 28, 30, 31) CER-JIC[4] (Supplementary Tables 2, 5-8, 19, 27, 34-36)	
Winter wheat *T. aestivum*	JIC-GH-LED (Supplementary Tables 25 - 27)		

Durum wheat *T. durum*	JIC-GH-LED (Supplementary Tables 20 - 24)		Alahmad et al., 2018
Spring barley *H. vulgare*	JIC-GH-LED (Supplementary Tables 28 - 30) UQ-GH-LED (Supplementary Tables 12 and 13)	UQ-GH-SVL (Supplementary Tables 12, 16, 20, 22, 29, 30, 32) CER-JIC (Supplementary Tables 3, 6, 37, 38)	
Canola *Brassica napus*	JIC-GH-LED (Supplementary Tables 31 - 35)	UQ-GH-SVL (Supplementary Tables 13, 17, 23, 25, 30, 39)	
Brassica rapa	JIC-GH-LED (Supplementary Tables 31 - 35)		
Brassica oleracea	JIC-GH-LED (Supplementary Tables 31 - 35)		
Pea *P. sativum*	JIC-GH-LED (Supplementary Tables 36 and 37)	CER-JIC (Supplementary Table 10)	
Grasspea *L. sativus*	JIC-GH-LED (Supplementary Tables 38 - 40)		
Medicago		CER-JIC (Supplementary Table 9)	
Brachypodium distachyon	JIC-GH-LED (Supplementary Tables 41, 42)	CER-JIC (Supplementary Table 4)	
Quinoa *C. quinoa*	JIC-GH-LED		

	(Supplementary Tables 43 - 45)		
Oat *A. strigosa*	JIC-GH-LED (Supplementary Tables 46 - 48)		
Chickpea *C. arietinum*		UQ-GH-SVL (Supplementary Tables 14, 18, 24, 26, 30)	
Peanut *A. hypogaea*			O'Connor et al., 2013[10]
Amaranth *Amaranthus* spp.			Stetter et al., 2016[41]

[1] **JIC-GH-LED**: LED-Supplemented Glasshouse setup, JIC, UK (described in this Protocol, see Equipment Setup "LED-supplemented glasshouse setup").

[2] **UQ-GH-LED**: LED-Supplemented Glasshouse setup, UQ, Australia (described in this Protocol, see Equipment Setup "LED-supplemented glasshouse setup").

[3] **UQ-GH-SVL**: SVL-Supplemented Glasshouse setup, UQ, Australia (described in Box 1 as Speed Breeding II[1]).

[4] **CER-JIC**: Controlled Environment Room, JIC, UK (described in Box 1 as Speed Breeding I[1]).

Table 4 | Protocol modifications for phenotyping diseases and disorders under speed breeding conditions.

Disease / disorder	Species	Reference
Stripe rust (*Puccinia striiformis* f. sp. *tritici*)	Spring wheat (*T. aestivum*)	Pretorius *et al.* (2000). *Acta Phytopathologica et Entomologica Hungarica, 35*(1-4), 359-364[49] Hickey *et al.* (2012). *Plant Breeding, 131*(1), 54-61[14]
Leaf rust (*Puccinia recondita* f. sp. *tritici*, "brown rust") (*Puccinia triticina*, "black rust")	Spring wheat (*T. aestivum*)	Pretorius *et al.* (2000). *Acta Phytopathologica et Entomologica Hungarica, 35*(1-4), 359-364[49] Riaz *et al.* (2016). *Plant Methods, 12*, 17[14]

Yellow spot / Tan spot (*Pyrenophora tritici-repentis*)	Spring wheat (*T. aestivum*)	Dinglasan *et al.* (2016). *Euphytica, 209*(3), 693-707[12]
Leaf rust (*Puccinia hordei*) Net form net blotch (*Pyrenophora teres* f. sp. *teres*) Spot form net blotch (*Puccinia teres* f. sp. *maculate*) Spot blotch (*Cochliobolus sativus*)	Barley (*H. vulgare*)	Hickey *et al.* (2017). *Euphytica, 213*(3), 64[15]
Stem rust (*Puccinia graminis* f. sp. *tritici*)	Spring wheat (*T. aestivum*)	Riaz and Hickey (2017). *Wheat Rust Diseases: Methods and Protocols* (Vol. 1659, pp. 183-196)[50]
Crown rot (*Fusarium pseudograminearum*)	Durum wheat (*T. durum*)	Alahmad *et al.* (2018). *Plant Methods, 14*(1), 36[11]
Pre-harvest sprouting	Spring wheat (*T. aestivum*)	Hickey *et al.* (2009). *Euphytica 168,* 303-310[9]
Pod shattering	Canola (*B. napus*)	Watson and Ghosh et al. (2018). *Nature Plants, 4*(1), 23-29[1]

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905 **Table 5 | Troubleshooting Table**

Step	Problem	Possible Reason	Solution
Step 2	Plants exhibit tip-burn necrosis. The leaves curl inward or outward, and may have small, circular depressions or "bubbles"	Calcium deficiency – common in accelerated growth.	Apply a liquid fertiliser containing calcium as a foliar spray early in growth to control any developing deficiency. This may be a 1% (wt/vol) calcium nitrate

	(Supplementary Figure 2).		solution applied 2-3 times per week or as part of another broad-spectrum fertiliser. Acidic soil can interfere with calcium uptake – adding dolomite to the soil can reduce acidity if the base soil mix tends to a lower pH.
Step 2	Initially curling and death of young leaf-tips and down the leaf blade. Young leaves may also not emerge properly and form loops or twists. Later, spike top can wither, turn white and fail to produce grain. Spikes may also become twisted into curls (Supplementary Figure 13).	Copper deficiency – common in accelerated growth.	Apply a liquid fertiliser containing copper as a foliar spray early in growth to control any developing deficiency. Alkaline or waterlogged soil can affect copper uptake – do not over-water or add excessive dolomite when ameliorating calcium deficiency as described above.
Step 2	Young leaves appear striped with interveinal yellowing (Supplementary Figure 14).	Iron deficiency.	Apply a liquid fertiliser containing iron as a foliar spray early in growth to control any developing deficiency.
	Plants are weak and spindly or suffering chlorosis.	These are possible symptoms of a range of nutrient deficiencies.	Apply a liquid fertiliser with a broad range of nutrients to the soil and as a foliar spray.

Step 1, Step 3	Seeds do not germinate.	Seed harvested too early and are not viable. Seeds are dormant.	Harvest seed slightly later. Store the seeds for a few additional days or weeks before trying again. Alternatively, cold stratify the seed at 4-5 °C for several days and/or treat with a low concentration (~0.5 ppm) of gibberellic acid (GA3) by dipping the seeds into the solution or spraying.
Step 2	Plants did not cycle much faster than in the glasshouse with no supplemental lights and/or in field conditions, even though they are LD or day neutral plants.	The optimum conditions for rapid generation advancement have not been reached for the crop. The particular genotype may be recalcitrant to SB.	Make adjustments for temperature, light intensity, light quality and/or day length. Try other genotypes to explore if it is a genotype- or species-specific issue.
Step 2	LD or day neutral plants do not flower.	Vernalisation needed.	Depending on the species, vernalise the plants for up to 8 weeks at 4 to 10 °C.

Table 6 | Mean days to anthesis[1] under speed breeding using LED-supplemented glasshouses at JIC, UK. All plants had a temperature cycle regime of 22 hours at 22 °C and 2 hours at 17 °C to coincide with the light and dark period, respectively.

Species	Associated data	Photoperiod	Mean days to flowering[1]
Spring wheat *T. aestivum*	Supplementary Tables 10, 11, 16 - 24	22 h	49.6 ± 5.0
		16 h	62.5 ± 4.3
Winter wheat *T. aestivum*	Supplementary Tables 25 - 27	22 h	105.4 ± 1.7
		16 h	115.4 ± 1.9
Durum wheat *T. durum*	Supplementary Tables 20 - 24	22 h	46 ± 1.9[2]
		16 h	53.7 ± 1.0[2]
Spring barley *H. vulgare*	Supplementary Tables 12, 13, 28 - 30	22 h	38.4 ± 13.9
		16 h	46.6 ± 12.1
Canola *Brassica napus*	Supplementary Tables 31 - 35	22 h	34.5 ± 0.7[3]
		16 h	45.0 ± 0.0
Brassica rapa	Supplementary Tables 31 - 35	22 h	36.5 ± 2.5[3]
		16 h	41.0 ± 3.7
Brassica oleracea	Supplementary Tables 31 - 35	22 h	49.2 ± 1.8[3]
		16 h	61.2 ± 2.3
Pea *P. sativum*	Supplementary Tables 36, 37	22 h	32.2 ± 5.3[4]
		16 h	42.9 ± 5.3
Grasspea *L. sativus*	Supplementary Tables 38 - 40	22 h	31[3] ±
		16 h	ND
Brachypodium distachyon	Supplementary Tables 41, 42	22 h	31.5 ± 5.2
		16 h	44.0 ± 5.2
Quinoa *C. quinoa*	Supplementary Tables 43 - 45	22 h	54.6[5] ± 0.6
		16 h	61.1 ± 4.6
Oat *A. sativa*	Supplementary Tables 46 - 48	22 h	52 ± 0.0
		16 h	66 ± 0.0

911 [1]Days to flowering/anthesis (GS65) from sowing[45].

912 [2]Days to 50% ear emergence from sowing (GS55).

913 [3]Days to first flower opening from sowing.

914 [4]Days to the first flower bud from sowing.

915 [5]Days to anthesis (growth stage 6 according to BBCH scale[47]).

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Table 7 | Mean days to reproductive stages[3-5] of single seed descent (SSD) sowing densities under speed breeding using the JIC-GH-LED[1] or UQ-GH-LED[2] approach. JIC-GH-LED approach used a temperature cycle regime of 22 h at 22 °C and 2 h at 17 °C to coincide with light and dark times, respectively. The UQ-GH-LED approach used a temperature cycle regime of 12 h at 22 °C and 12 h at 17 °C.

Species	Approach	Sowing density	Photoperiod	Mean days to reproductive stage
Spring wheat *T. aestivum*	**JIC-GH-LED[1]**	96-cell (560 plants/m^2)	22 h	45.0 ± 0.0[3]
		96-cell (560 plants/m^2)	16 h	58.0 ± 0.0[3]
	UQ-GH-LED[2]	30-cell (300 plants/m^2)	22 h	31.3 ± 0.7[4]
		64-cell (640 plants/m^2)	22 h	30.0 ± 0.0[4]
		100-cell (1000 plants/m^2)	22 h	31.0 ± 0.0[4]
Tetraploid wheat *T. durum*	**JIC-GH-LED**	96-cell (560 plants/m^2)	22 h	42.0 ± 0.0[3]
		96-cell (560 plants/m^2)	16 h	50.0 ± 0.0[3]
Spring barley *H. vulgare*	**UQ-GH-LED**	30-cell (300 plants/m^2)	22 h	27.3 ± 1.2[5]

		64-cell (640 plants/m^2)	22 h	24.7 ± 0.3[5]
		100-cell (1000 plants/m^2)	22 h	24.0 ± 0.6[5]

[1] **JIC-GH-LED**: LED-Supplemented Glasshouse setup, JIC, UK (described in this paper, Equipment Setup Section c).

[2] **UQ-GH-LED**: LED-Supplemented Glasshouse setup, UQ, Australia (described in this paper, Equipment Setup Section c).

[3] Days to 50% ear emergence from sowing (GS55)[45].

[4] Days to mid-anthesis (GS65) from sowing.

[5] Days to awn-peep (GS49) from sowing.

BOX 1 Speed breeding setup

This box provides information to set up SB in an existing plant growth chamber or controlled environment room (CER). This section outlines the core "recipe" for programing an existing growth room to set up SB conditions.

- ***Lights***: We have shown in our previous studies[1], that any light that produces a spectrum which reasonably covers the photosynthetically active radiation (PAR) region (400-700 nm), with particular focus on the blue, red and far-red ranges, is suitable to use for SB. The referenced study has several examples of these spectra, and similar examples of possible SB spectra are provided here. An appropriate spectral range can be achieved through LEDs, or a combination of LEDs and other lighting sources (e.g. halogen lamps), or in the case of a glasshouse, by simply supplementing the ambient lighting with LEDs or SVLs. We highly recommend that measurements of the light spectrum are taken prior to commencement of the SB experiment. In addition to controlling the light quality, we recommend a photosynthetic photon flux density (PPFD) of approximately 450-500 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at plant canopy height. Slightly lower or higher PPFD levels are also suitable. Crops species vary in their response to high irradiance. However, the suggested level of 450-500 $\mu mol \cdot m^{-2} \cdot s^{-1}$ has been demonstrated to be effective for a range of crop species[1].
- ***Photoperiod***: We recommend a photoperiod of 22 hours with 2 hours of darkness in a 24-hour diurnal cycle. Continuous light is another option, but our experience has shown that the dark period slightly improves plant health. Gradually increasing light intensity to mimic dawn and dusk states should be done, if possible, but is not vital. In our previous paper, we have also provided an example where an 18-hour photoperiod was sufficient to achieve faster generation times for wheat, barley, oat and triticale[1].
- ***Temperature***: The optimal temperature regime (maximum and minimum temperatures) should be applied for each crop. A higher temperature should be maintained during the photoperiod, while a fall in temperature during the dark period can aid in stress recovery. At UQ, a 12 hour 22 °C / 17 °C temperature cycling regime with the 2 hours of darkness occurring within the 12 hours of 17 °C has proven successful (Speed breeding II)[1]. In contrast, a temperature cycling regime of 22 °C / 17 °C for 22 hours light and 2 hours dark, respectively, is used at JIC (Speed breeding I)[1]. In both scenarios, the generation times of all crops were successfully accelerated and comparable. In the controlled environment chamber in which this was demonstrated, the temperature was ramped up and down similarly to the lights, but this was subsequently found to not be of particular importance.

- ***Humidity***: Most controlled environment chambers have limited control over humidity but a reasonable range of 60-70% is ideal. For crops that are more adapted to drier conditions, a lower humidity level may be advisable.

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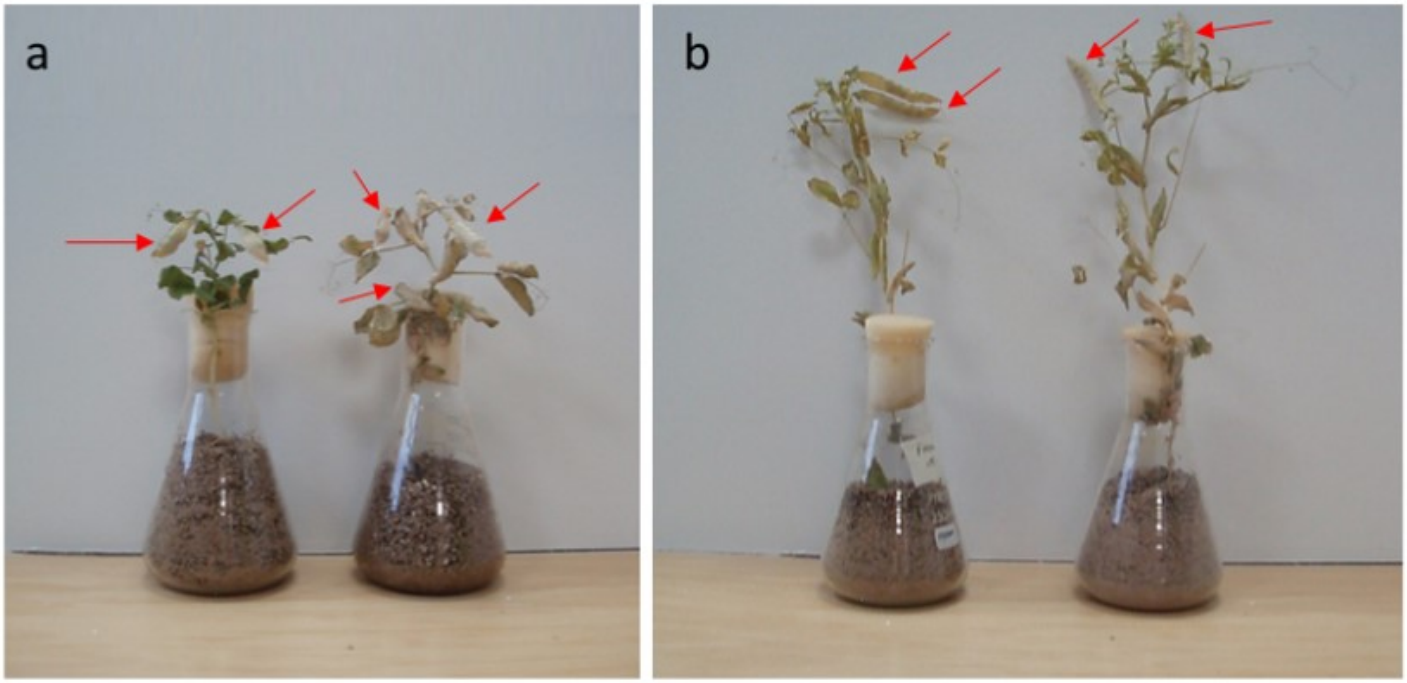


Supplementary Figure 1

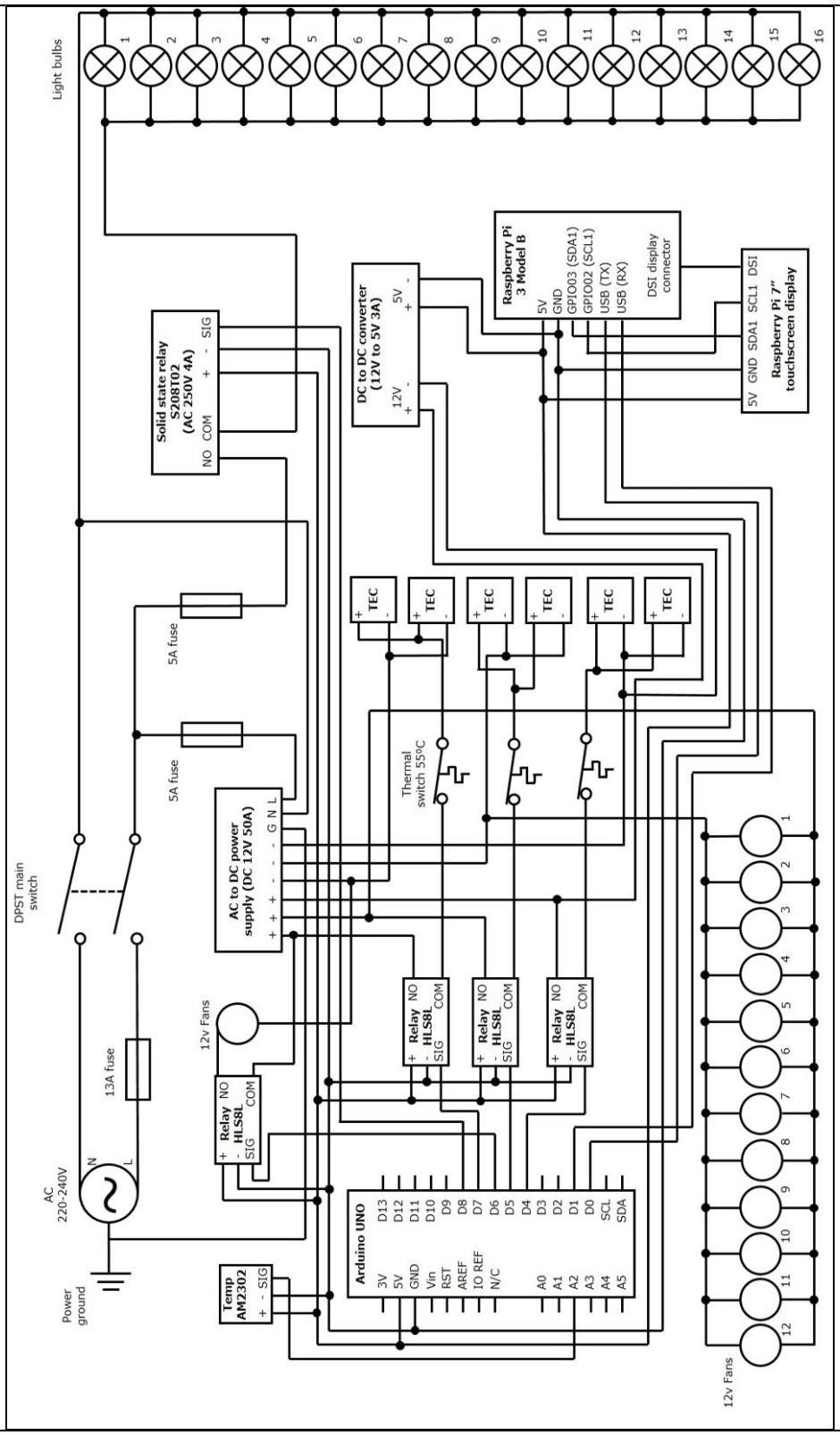
Mature eight-week-old pea plants grown in limited media and nutrition ("flask method") in order to achieve rapid generation advancement

Pisum sativum (a) accession JI 2822 and (b) cv. Frisson. Dry seeds were sterilised in 10% sodium hypochlorite, rinsed in sterile water, chipped and left to germinate in the dark for 3 days on sterile, wet filter paper. Germinating seeds were transferred to flasks containing 250 mL fine perlite and silver sand (mixed 50:50) and FP nutrient media which had been sterilised (composition described in Supplementary Table 49). Flasks were placed in the dark for a further 5 days. The seedlings were inoculated with *Rhizobium*, and the elongated shoot passed through the neck of the flask and held in place with a bung. The base of the flask was covered with a black plastic bag. Plants were grown in a Controlled Environment Room at constant 22 °C with a 16-hour photoperiod. After 3 weeks, flasks were watered with 50 mL FP media once a week. After 8 weeks post germination, plants had mature dry seed ready to harvest as shown (indicated by red arrows). JI 2822 plants grown in the glasshouse under lights required 12 weeks post sowing before mature dry seed were ready for harvest.

Supplementary Figure 2

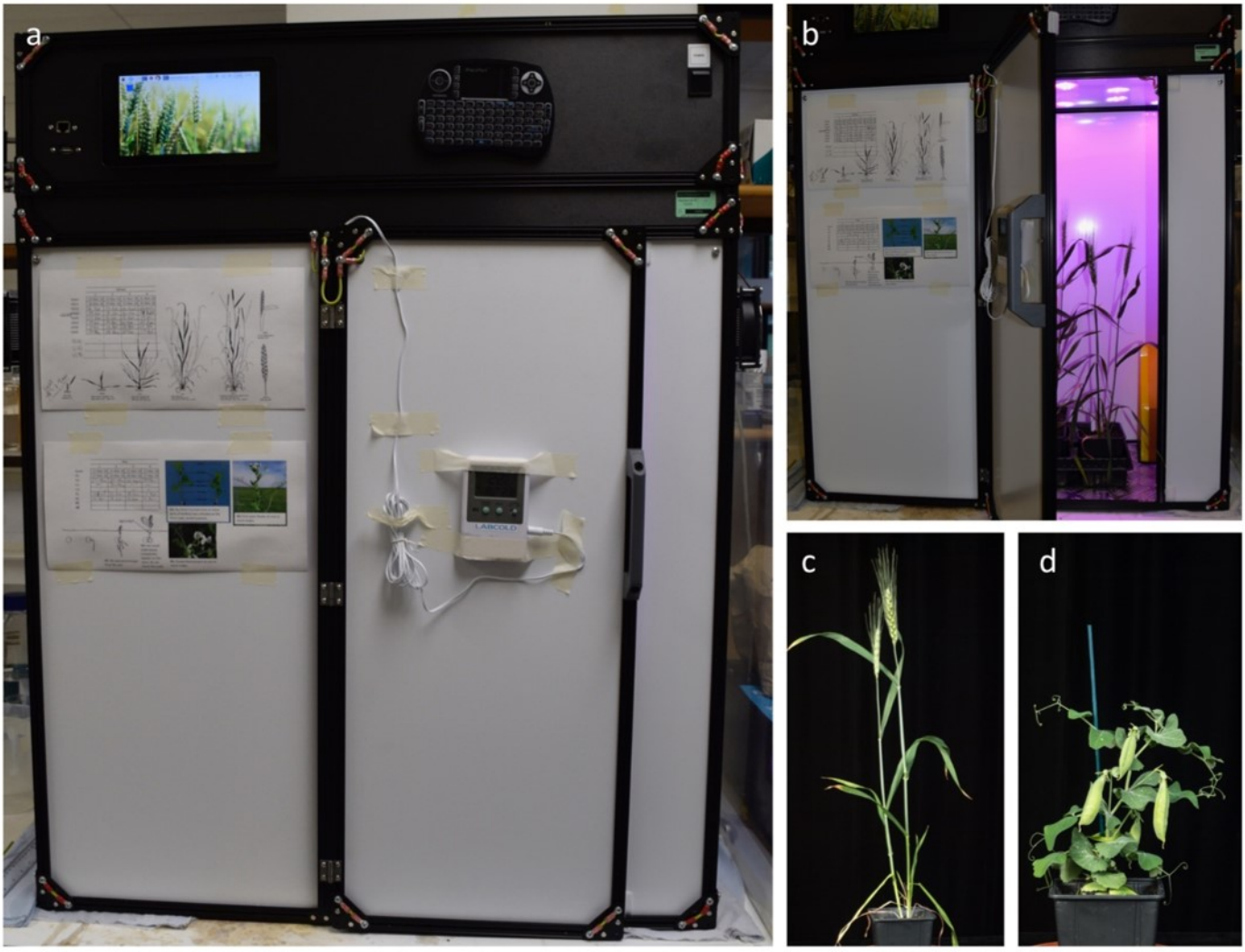
Symptoms of calcium deficiency in wheat grown under speed breeding conditions

Right: Small, circular depressions on the leaf blade; Left: Tip leaf necrosis.



Supplementary Figure 3

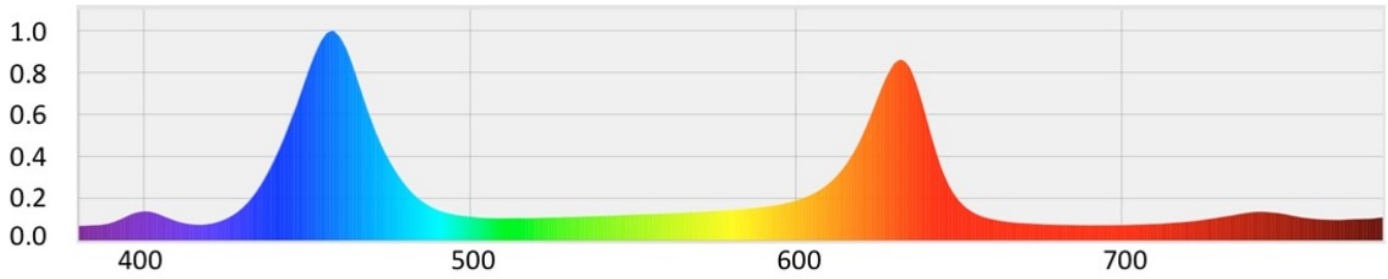
Circuit diagram of the monitoring and control system of the benchtop growth cabinet



Supplementary Figure 4

Benchtop Cabinet for conducting speed breeding

(a) Front view of the cabinet. **(b)** Front view of the cabinet with the door open to show the lighting and wheat plants (*Triticum aestivum* cv. Apogee) growing inside. **(c)** Apogee wheat plant grown in the cabinet, photographed at 55 DAS (Days after sowing). **(d)** Pea (*Pisum sativum*) variety JI 2822 grown in the cabinet, photographed at 50 DAS.



Supplementary Figure 5

Light spectrum measurements in in the benchtop growth cabinet 20cm below one of the LED bulbs

The x-axis represents the wavelength of light in nanometres, and y-axis is the normalised spectral power distribution. (Power distribution is measured in $mW \cdot m^{-2}$, and all values on y-axis are divided by the maximum value in the distribution in order to obtain normalised values). Graph was produced from measurements made by the MK350S LED meter from UPRtek, using the uSpectrum software produced by the same manufacturer.

Supplementary Figure 6

Barley spikes from plants grown under Heliospectra LED lights

Barley cv. Golden Promise from 22-hour light regime (left) and 16-hour light regime (right). Scale bar is 5 cm.

Supplementary Figure 7

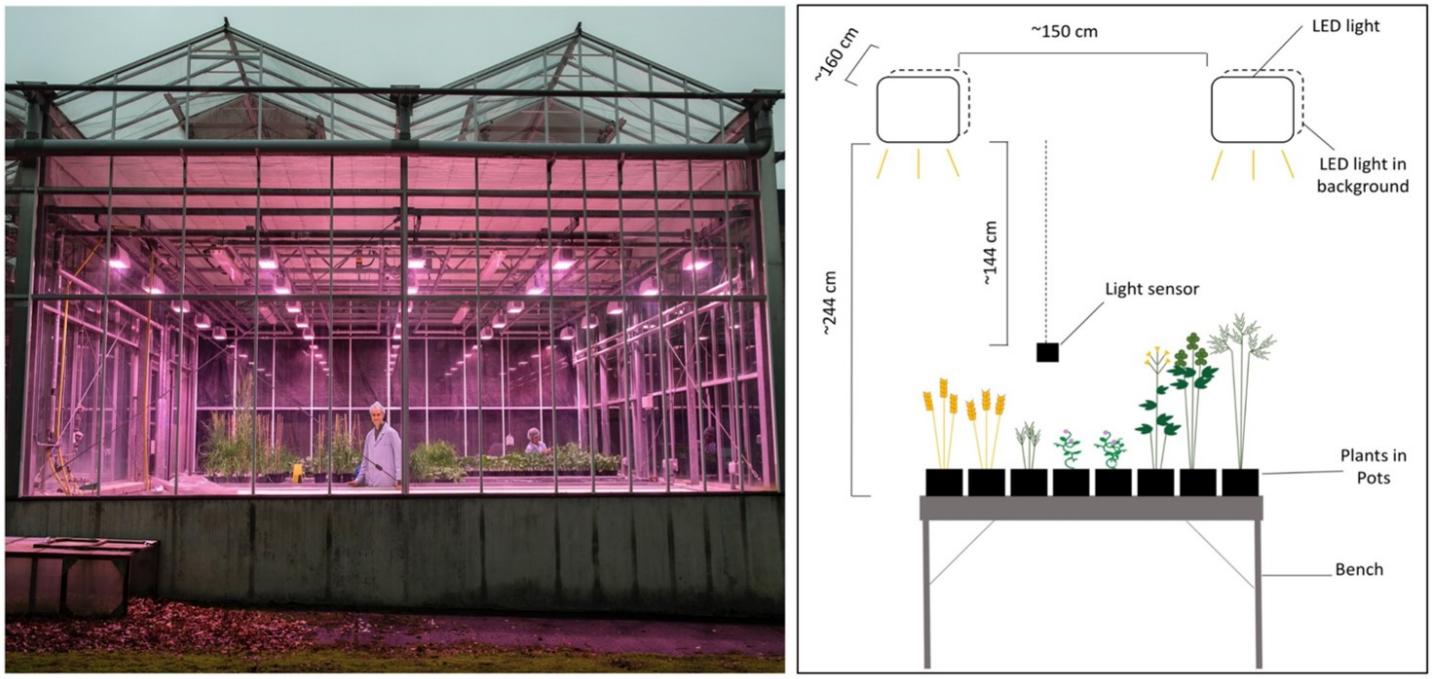
Pods from *Brassica rapa* R-0-13 grown in LED-supplemented glasshouses at the John Innes Centre, UK

Plants grown under (a) a 22-hour photoperiod or (b) a 16-hour photoperiod.

Supplementary Figure 8

Pods harvested from *Brassica napus* RV31 grown in LED-supplemented glasshouses at the John Innes Centre, UK

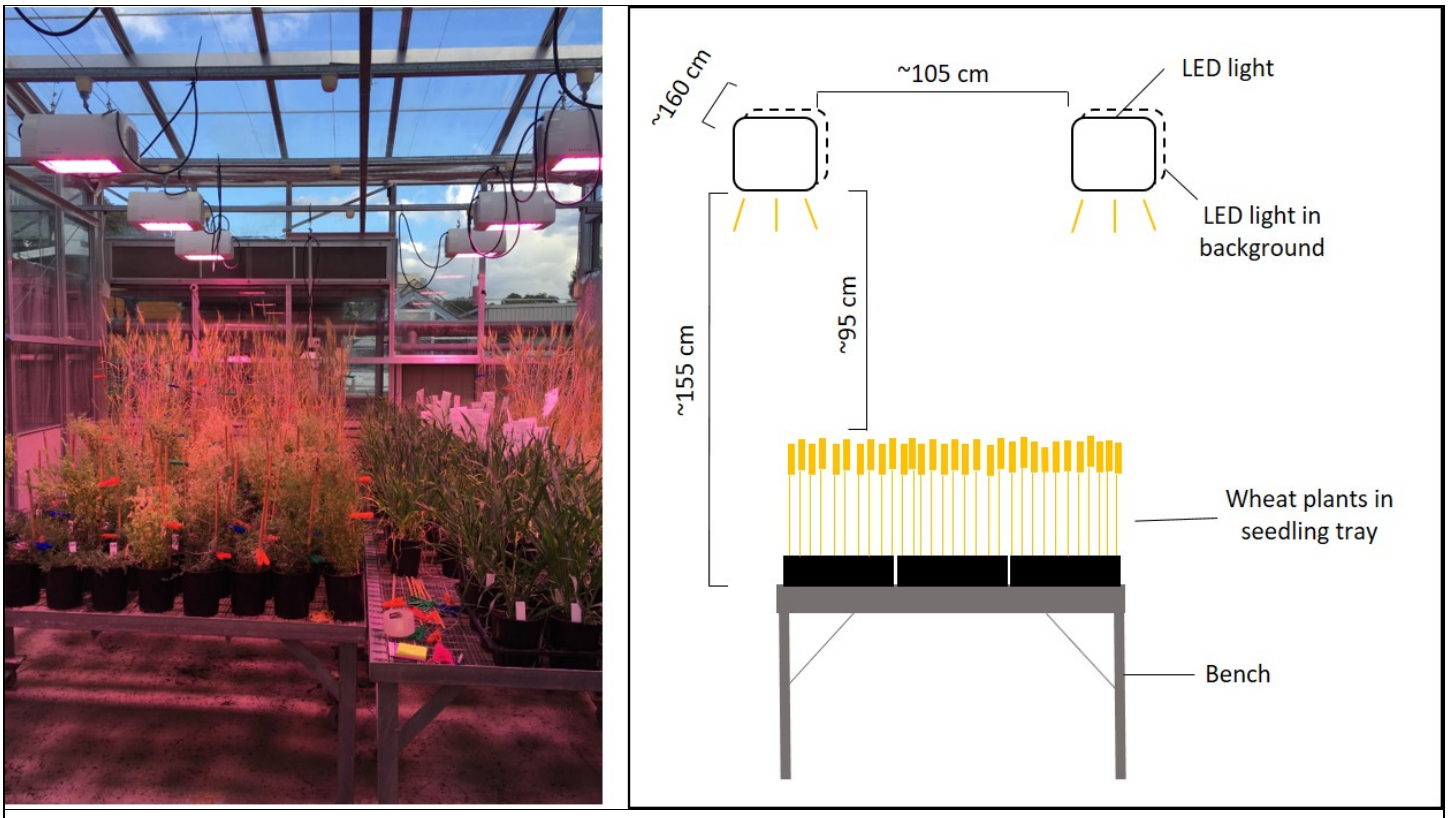
Plants grown under (a) a 22-hour photoperiod or (b) a 16-hour photoperiod.



Supplementary Figure 9

Layout of the glasshouse at John Innes Centre, UK, used for speed breeding

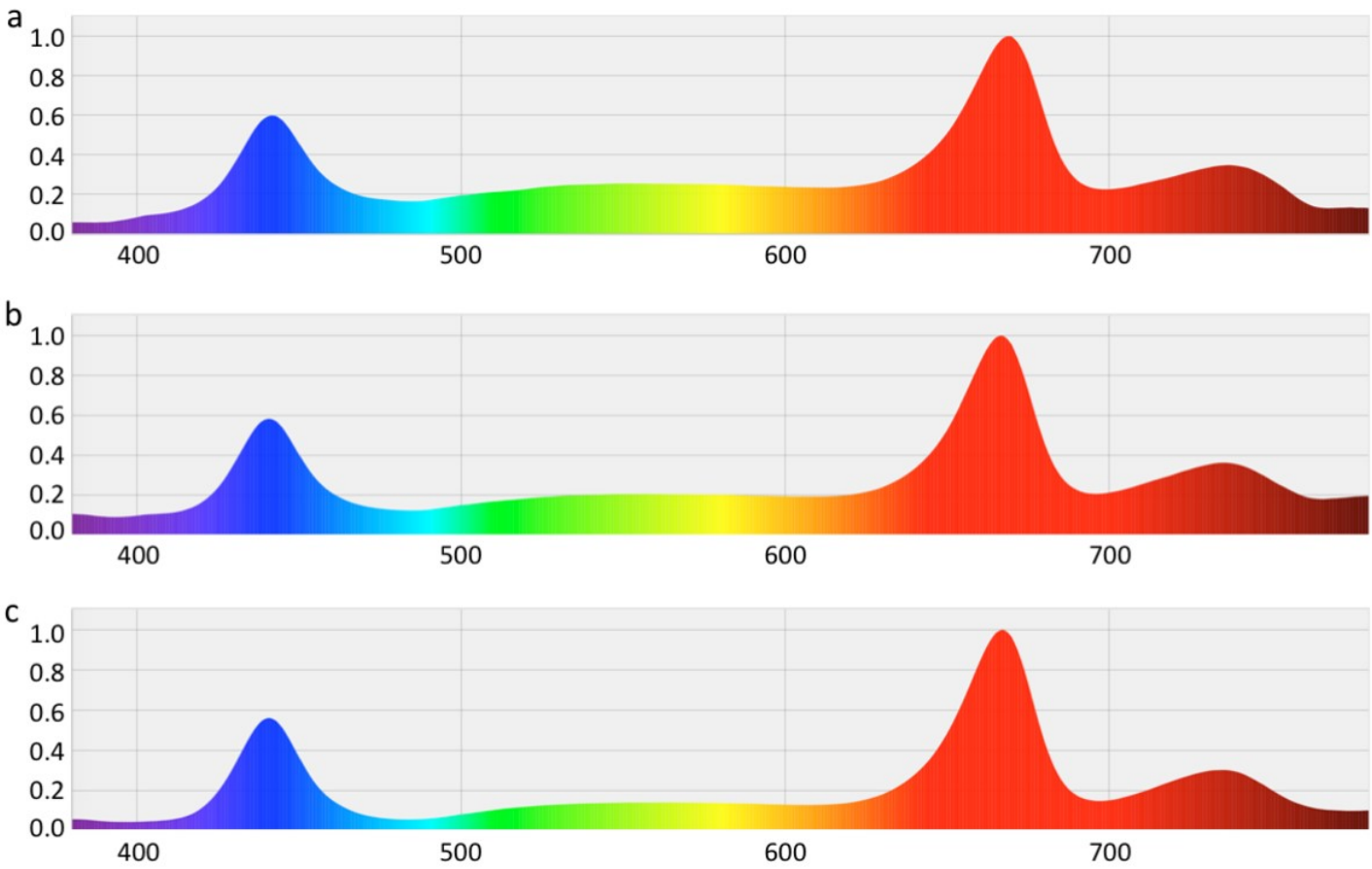
(Left) Photograph with Heliospectra LX60C2 LED supplementary lighting; (Right) Schematic of light positioning within the glasshouse relative to the bench, plants and other light fixtures



Supplementary Figure 10

Layout of the glasshouse at The University of Queensland, Australia, used for speed breeding

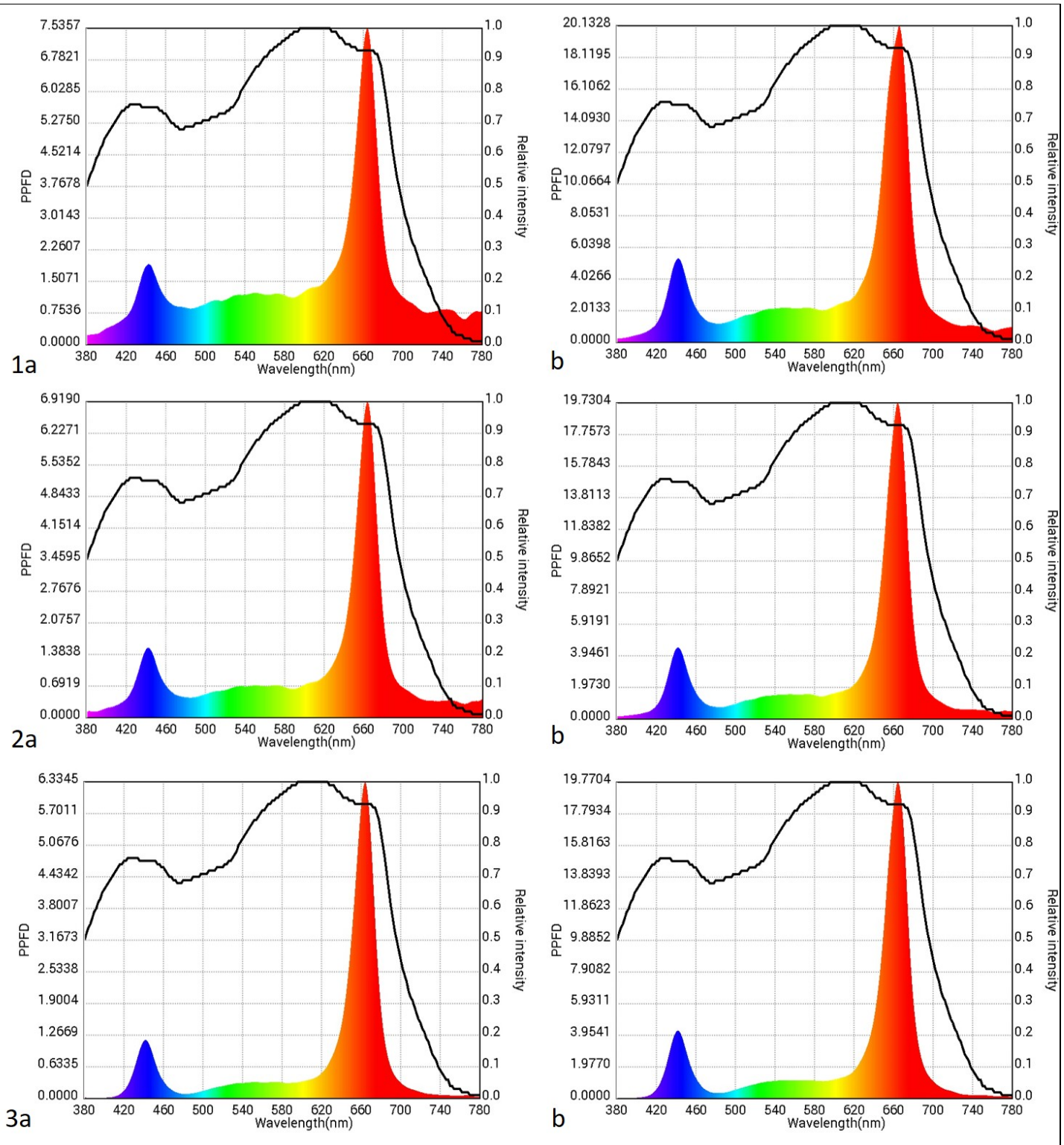
(Left) Photograph with Heliospectra E602G LED supplementary lighting; (Right) Schematic of light positioning within the glasshouse relative to the bench, plants and other light fixtures.



Supplementary Figure 11

Light spectrum measurements in JIC Glasshouses under a Heliospectra LX602C LED fixture

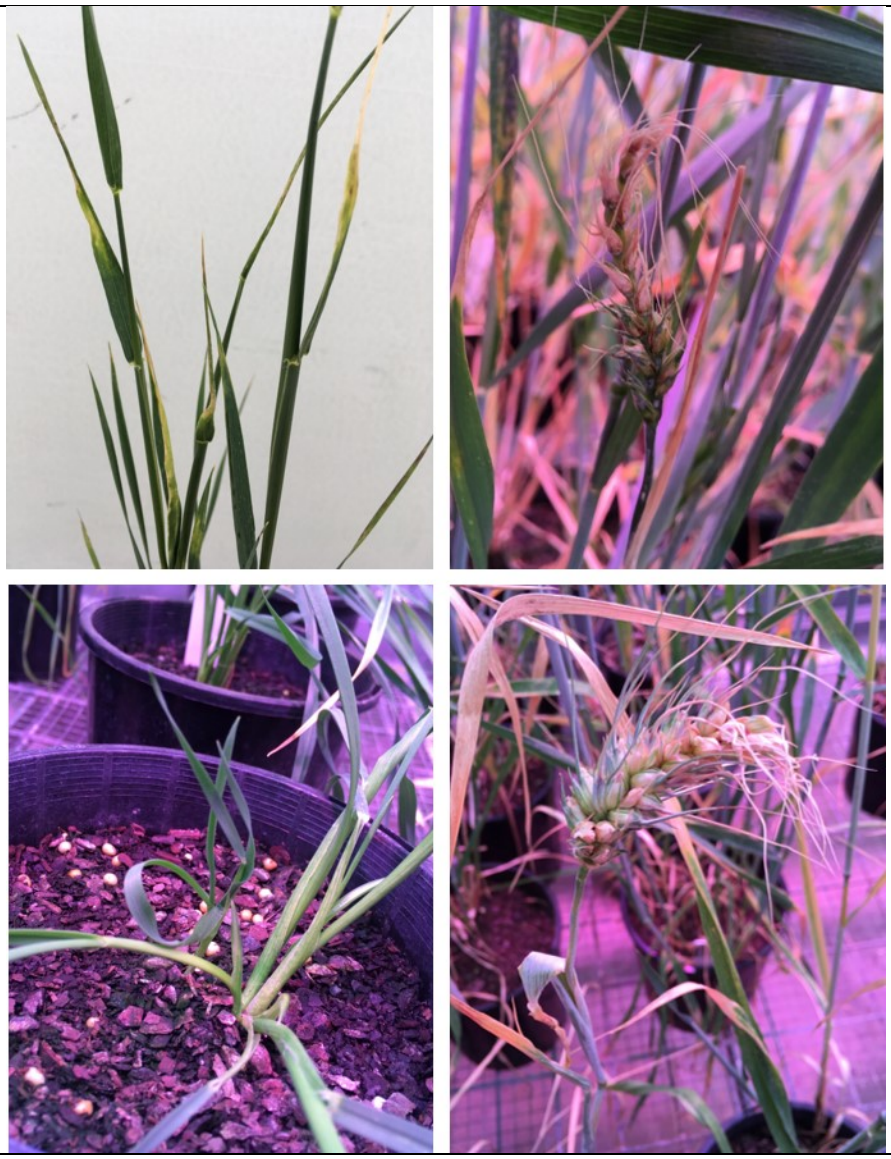
(a) Spectrum measurement in the glasshouse at bench level (244 cm from light fixture) on a clear, sunny day at 12 noon **(b)** Spectrum measurement in the glasshouse at bench level (244 cm from light fixture) on a cloudy day at 12 noon. **(c)** Spectrum measurement in the glasshouse at bench level (244 cm from light fixture) at night. The x-axis of all three graphs represents the wavelength of light in nanometres, and y-axis is the normalised spectral power distribution (Power distribution is measured in $mW \cdot m^{-2}$, and all values on y-axis are divided by the maximum value in the distribution in order to obtain normalised values). All graphs were produced from measurements made by the MK350S LED meter from UPRtek, using the uSpectrum software produced by the same manufacturer.



Supplementary Figure 12

Light spectrum measurements in the UQ glasshouse under a Heliospectra E602G LED fixture

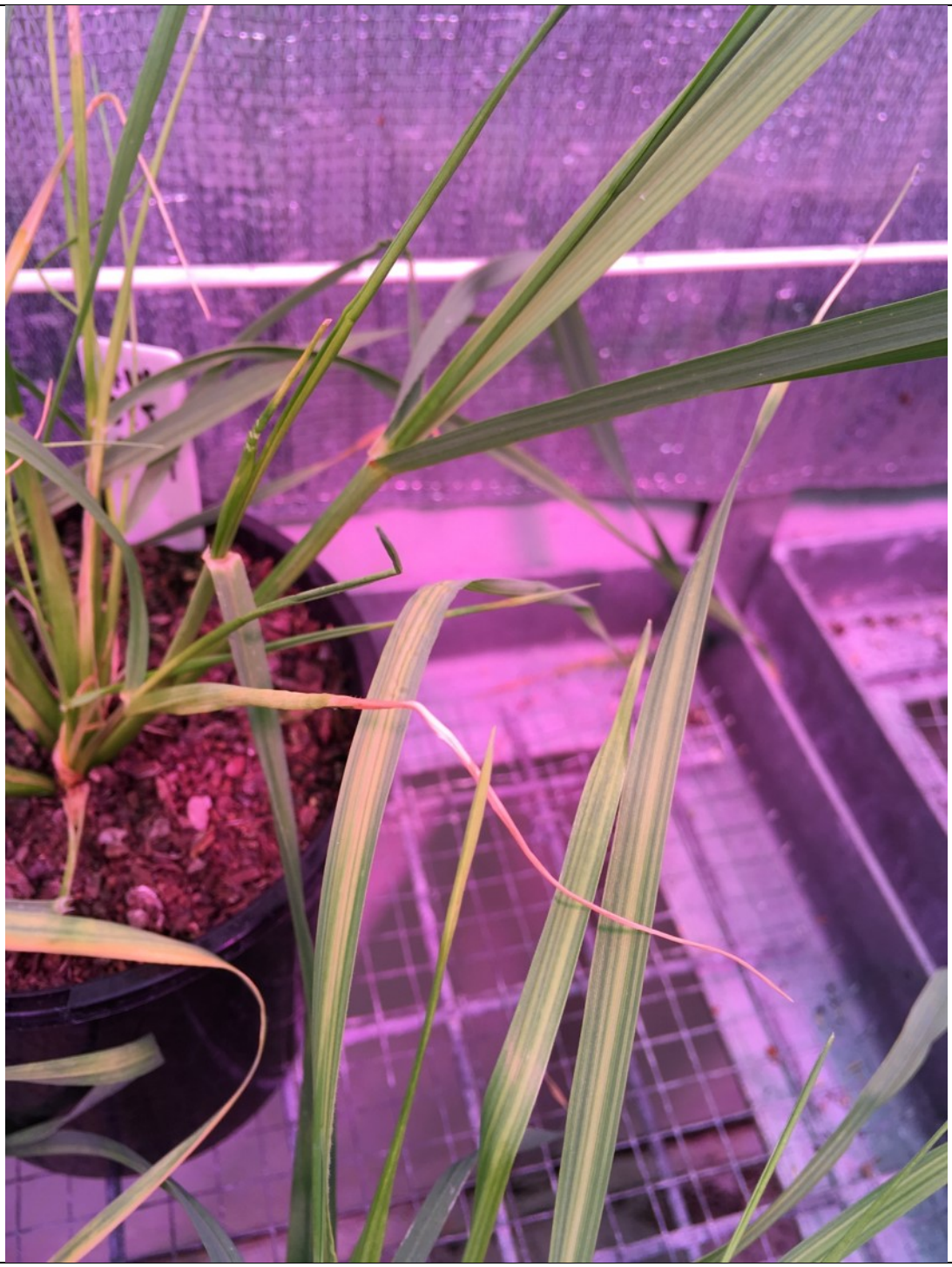
Weighted McCree action spectrum and photosynthetic photon flux density (PPFD; $\mu mol \cdot m^{-2} \cdot s^{-1}$) from under a Heliospectra E602G light using the Spectrum Genius Essence Lighting Passport light sensor and associated Spectrum Genius Agricultural Lighting app (AsenseTek Inc., Taiwan). (1) Centre measurement at 12 noon on a clear, sunny day, (2) Centre measurement at 12 noon on an overcast day and, (3) Centre measurement at night; a, bench level (155 cm from light) and b, approximate wheat spike height (95 cm from light). Figures were exported from the software.



Supplementary Figure 13

Symptoms of copper deficiency in wheat grown under speed breeding conditions

Left (top): Curling and death of young leaf tips and down the leaf blade; Left (bottom): Young leaves becoming stuck as they emerge and forming loops or curling; Right (top and bottom): Spikes wither and turn white at the tips. No seed is produced in these areas and spikes may be twisted.



Supplementary Figure 14

Symptoms of iron deficiency in wheat grown under speed breeding conditions

Young leaves appear striped with yellowing of the interveinal spaces.

Supplementary Tables

Supplementary Table 1 | **Growth rate of spring wheat (cv. Apogee) in a benchtop speed breeding cabinet.** Days to key growth stages and measurement of key growth parameters of wheat (*T. aestivum* cv. USU-Apogee) grown in a small benchtop cabinet set up for speed breeding (22-hour photoperiod with 22 °C during the photoperiod and 17 °C during the 2-hour dark period). Seeds were germinated for 4 days and sown on 22 March 2018 in 600 mL of JIC Cereal Compost Mix. Values indicated are mean ± standard deviation based on four replicates.

[1]GS11 – Emergence of first leaf (DAS)[2]	4.3 ± 0.5
GS13 – Emergence of third leaf (DAS)	13.0 ± 1.2
GS39 – Flag Leaf Emergence (DAS)	28.0 ± 0.8
GS45 – Mid-boot (DAS)	31.3 ± 1.7
GS55 – 50% ear emergence (DAS)	36.3 ± 1.3
GS59 – full ear emergence (DAS)	38.3 ± 1.3
GS65 – mid-anthesis (DAS)	40.5 ± 1.3
GS77 – Grain milk (DAS)	51.0 ± 0.0
GS85 – Grain Dough (seed harvested)[3] (DAS)	63.0 ± 0.0
No. of tillers	2.0 ± 0.0
100 seed weight (g)	2.1 ± 0.3
Germination percentage of 30 harvested seeds (%)	90.8 ± 8.8

[1] Growth stages (GS) measured for the first tiller according to the Zadoks scale (Zadoks et al., 1994).

[2] DAS, days after sowing. Seeds were stratified at 4 °C in the dark for two days and germinated at room temperature on Petri dishes for two days before being sown.

[3] Plants were subjected to seven days of water stress before seeds were harvested. Seeds were not at physiological maturity (GS90) when harvested.

Supplementary Table 2 | **Growth rate of pea (accession JI 2822) in a benchtop speed breeding cabinet.** Days to key growth stages and measurement of key growth parameters of pea (*Pisum sativum* accession JI 2822) grown in a small benchtop cabinet set up for speed breeding (22-hour photoperiod with 22 °C during the photoperiod and 17 °C during the 2-hour dark period). Seeds were scarified and sown on 22 March 2018 in pots containing 600 mL of JIC Cereal Compost Mix. Values indicated are mean ± standard deviation based on four replicates.

Epicotyl emergence from soil (DAS)[1]	6.0 ± 0.8
Appearance of scale leaves (DAS)	10.0 ± 1.4
Flower bud appearance at one or more nodes (DAS)	24.8 ± 1.3
First open flower at one or more nodes (DAS)	30.5 ± 1.3
Node number at first flower	7.3 ± 0.5
No. of side shoots	0.0 ± 0.0
Node no. at maturity	12.0 ± 0.0
Harvest DAS[2]	62.0 ± 0.0
No. of pods	3.8 ± 0.5
No. of seeds	9.8 ± 1.7
Germination percentage of harvested seed[3] (%)	97.2 ± 5.6

[1]DAS, days after sowing, with Day 1 being the day the seeds were sown.

[2]Plants were subjected to seven days of water stress (no watering) before pods were harvested. Pods were not harvested at physiological maturity, but slightly earlier.

[3]All seeds harvested were subjected to germination tests as each plant produced <30 seeds.

Supplementary Table 3 | **Components and costs of the speed breeding benchtop growth chamber.**

Qt.	Catalogue No.	Description	Unit Cost (£)	Total Cost[1] (£)	Supplier
1	B072M7P7QJ	Power Supply Unit 600 W (12 v, 50 A Constant Voltage)	28.99	28.99	Amazon
1	B00G890MIC	Power Supply 12 V to 5 V 3 A DC/DC Buck Converter Module	6.49	6.49	Amazon
1	B002M8RVKA	USB Extension Cable (30 cm)	4.69	4.69	Amazon
1	B077V421QH	Ethernet Extension Cable (30 cm)	5.99	5.99	Amazon
1	B00CGU1VOG	Arduino UNO	6.95	6.95	Amazon
3	B01M2ZBBVM	Thermoelectric Cooler (120 W power) 12 v @10A	23.99	71.97	Amazon
16	B071J3BC1W	LED Full Spectrum Grow Light	6.95	111.20	Amazon
16	E27-SD04-2	E27 Lamp Holder	0.93	14.88	Sinolec Components Ltd
1	2525225	Raspberry Pi 3 Model B	28.49	28.49	CPC-Farnell
1	2473872	Raspberry Pi Display 7" Touchscreen	51.19	51.19	CPC-Farnell
1	MK00343	Grove Temperature & Humidity Sensor Pro	11.99	11.99	CPC-Farnell
1	SC13822	Arduino Base Shield v2	8.99	8.99	CPC-Farnell
4	MK00330	Grove Relay	3.01	12.04	CPC-Farnell
1	713-103020004	Grove Solid State Relay	18.38	18.38	Mouser
6	CP027-03	White Aluminium Composite panel (757 x 307 x 3 mm)	8.59	51.54	Cut Plastics Ltd
1	CP027-03	White Aluminium Composite panel (757 x 357 x 3 mm)	9.99	9.99	Cut Plastics Ltd
1	CP027-03	White Aluminium Composite panel (757 x 107 x 3 mm)	3.00	3.00	Cut Plastics Ltd
1	CP027-03	White Aluminium Composite panel (757 x 757 x 3 mm)	21.19	21.19	Cut Plastics Ltd
2	CP015-03	Black PVC Foam Board (757 x 157 x 3 mm)	1.95	3.90	Cut Plastics Ltd

1	CP015-03	Black PVC Foam Board (757 x 141 x 3 mm) to be further cut	1.75	1.75	Cut Plastics Ltd
2	CP015-03	Black PVC Foam Board (757 x 307 x 3 mm)	3.82	7.64	Cut Plastics Ltd
1	CP001-03	Clear Perspex Acrylic Sheet (757 x 307 x 3 mm)	3.91	3.91	Cut Plastics Ltd
4	4451-900	OpenBeam – 1000 mm Long Black Anodised Beam	8.27	33.08	Technobotsonline Group Unit
13	4451-750	OpenBeam – 750 mm Long Black Anodised Beam	6.59	85.67	Technobotsonline Group Unit
10	4451-300	OpenBeam – 300 mm Long Black Anodised Beam	3.01	30.1	Technobotsonline Group Unit
4	4446-013	MakerBeam – 90 Degree Corner Bracket	0.58	2.32	Technobotsonline Group Unit
36	4450-003	OpenBeam – 'L' Joining Plate	1.87	67.32	Technobotsonline Group Unit
2	4450-004	OpenBeam – 'T' Joining Plate	1.87	3.74	Technobotsonline Group Unit

[1]Grand total cost £707.39.

Supplementary Table 4 | **UQ Compost Mix composition.** Compost mix components and fertilisers designed by Mr K. Hayes, Central Glasshouse Services, University of Queensland, Australia. The pH is balanced with either FeSO (when pH is high) or Dolomite (when pH is low).

Component Measure
Composted pine bark (0-5 mm) 70% (Fernland Agencies, Queensland, Australia)
Coco peat 30% (Fernland Agencies, Queensland, Australia)
Fertilizer
Yates Flowtrace® (Yates, Padstow, NSW, Australia) 1 kg m^{-3}
Iron sulphate heptahydrate 1 kg m^{-3} (Amgrow Specialty, New South Wales, Australia)
Superphosphate 0.4 kg m^{-3} (Swancorp, Queensland, Australia)
Copper sulphate 0.03 kg m^{-3} (Searles, Queensland, Australia)
Gypsum 1 kg m^{-3} (Qld Organics, Queensland, Australia)

Supplementary Table 5 | **JIC Cereal Compost Mix composition.** Compost supplied by Petersfield Growing (Leicester, UK).

Component	**Measure**
Medium Grade Peat (Brinkman (Horticultural Service) UK Ltd)	40%
Sterilised Soil (horticultural grade) (Petersfield Growing Mediums – Leicester, UK)	40%
Horticultural Grit (grade 3 -7mm washed grit – Composts Direct)	20%
Fertilizer	
PG Mix™ 14-16-18 + Trace Elements (TE) Base Fertiliser	1.3 kg/m^3
Osmocote® Exact Mini 16-8-11+ 2MgO + TE 0.02% Boron	1.0 kg/m^3
H2Gro® (Wetting Agent) from ICL Specialty Fertilizers (Ipswich, UK)	
Maglime (dolomitic limestone) (Berrycroft Horticultural Sundries)	3.0 kg/m^3
Insecticide	
Exemptor® from ICL Specialty Fertilizers (Ipswich, UK)	300 g/m^3

Supplementary Table 6 | JIC Peat and Sand Mix composition. Compost supplied by Petersfield Growing (Leicester, UK).

Component	**Measure**
Fine peat (Bulrush 0 -12 mm fine peat) (Brinkman (Horticultural Service) UK Ltd)	85%
Grit (grade 3 -7mm washed grit – Composts Direct)	15%
Fertilizer	
PG Mix™ 14-16-18 + Trace Elements (TE) Base Fertiliser	1.0 kg/m^3
Osmocote® Exact Mini 16-8-11 + 2MgO + TE 0.02% Boron	2.7 kg/m^3
H2Gro® **(**Wetting Agent) from ICL Specialty Fertilizers (Ipswich, UK)	
Maglime (dolomitic limestone) (Berrycroft Horticultural Sundries)	4.0 kg/m^3

Supplementary Table 7 | Sources and contact information for germplasm used in speed breeding experiments in this paper.

Crop and Cultivar	Germplasm collection/ References/ Contact information
Spring bread wheat (*Triticum aestivum*)	
-cv. Paragon	https://www.seedstor.ac.uk/ (entry number WBCDB0040)
-cv. Cadenza	https://www.seedstor.ac.uk/ (entry number W9368)
-cv. Fielder	https://www.seedstor.ac.uk/ (entry number W8354)
-cv. Suntop	commercial variety, Australian Grain Technologies
-cv. Apogee	https://www.seedstor.ac.uk/ (entry number W10285)
-cv. BR18	Embrapa Trigo, Passo Fundo, Brazil (Trigo BR18 Terena)
-cv. BRS179	Embrapa Trigo, Passo Fundo, Brazil (BRS197)
Spring durum wheat (*Triticum durum*)	
-cv. Kronos	https://www.seedstor.ac.uk/ (W10282)
Winter bread wheat (*Triticum aestivum*)	
-cv. Trinity	commercial variety, KWS UK Ltd.
-cv. Crusoe	commercial variety, Limagrain (UK) Ltd.
Spring barley (*Hordeum vulgare*)	
-cv. Nigrate	https://npgsweb.ars-grin.gov/ (entry number CIho 2444)
-cv. Manchuria	https://npgsweb.ars-grin.gov/ (entry number CIho 2330)
-cv. Golden Promise	https://www.seedstor.ac.uk/ (entry number B4015)
-cv. Baronesse	commercial variety, Nordsaat Saatzucht GmbH, Germany
-cv. Commander	commercial variety, University of Adelaide, Australia
Brachypodium distachyon	
- accession Bd21	https://npgsweb.ars-grin.gov/ (entry number W6 36678)
- accession Bd21-3	https://npgsweb.ars-grin.gov/ (entry number W6 39233)
- accession Bd3-1	https://npgsweb.ars-grin.gov/ (entry number W6 46203)
Pea (*Pisum sativum*)	
- Line JI 2822	https://www.seedstor.ac.uk/ (entry number JI2822)
- cv. Princess	https://www.seedstor.ac.uk/ (entry number JI2623)
- cv. Cameor	https://www.seedstor.ac.uk/ (entry number JI3253)
Grasspea (*Lathyrus sativus*)	
- cv. Mahateora	Released cultivar in India. Can be ordered through ICARDA or available on request from Dr. Cathie Martin at the John Innes Centre
Brassica napus	
- line RV31	Available on request from Rachel Wells, John Innes Centre, UK
Brassica rapa	
- line R-0-18	Available on request from Rachel Wells, John Innes Centre, UK
Brassica oleracea	
- line DH1012	Available on request from Rachel Wells, John Innes Centre, UK
Quinoa (*Chenopodium quinoa*)	
- accession QQ-74	https://npgsweb.ars-grin.gov/ (PI 614886)
- cv. Titicaca	commercial variety (bred by Sven-Erik Jacobsen, UK)
Oat (*A. strigosa*)	
- accession S75	Institute of Grasslands and Environmental Research, Aberystwyth, Wales, UK

Supplementary Table 8 | **Tray dimensions for single seed descent demonstration**. Specifications of the plastic cell trays used for comparison of different plant densities under speed breeding in a glasshouse with LED supplementary lighting in Queensland, Australia.

Tray type	**Volume of individual cell (mL)**	**Cell dimension (Length x Height x Width, mm)**	**Extrapolated density (plants per m^2)**
30-cell tray	100	50 x 60 x 50	300
64-cell tray	60	35 x 50 x 40	640
100-cell tray	18	25 x 45 x 30	1000

Supplementary Table 9 | **Energy consumption calculations for two kinds of lighting for SB purposes in a glasshouse in JIC.** Energy consumption information for LED-Supplemented versus Sodium Vapour Lamp-supplemented glasshouses at the John Innes Centre, UK. The values indicated are for the same glasshouses, with the Sodium Vapour Lamps being tested in December 2016, and the LED Lamps being tested in December 2017. Values indicated are the average per metre square in a 30-day cycle.

	LED lamps (25 x 600 W fittings)		**Sodium Vapour Lamps (40 x 440 W fittings)**	
	22 h	**16 h**	**22 h**	**16 h**
Lighting energy requirements (kWh/m^2)	4.97	3.61	5.83	4.24

Supplementary Table 10 | Growth rate of spring bread wheat (cv. Suntop) in SSD trays in the LED-supplemented glasshouse setup at UQ. Development stages of spring wheat (*T. aestivum* cv. Suntop) under speed breeding at three plant densities in a glasshouse with LED supplementary lighting in Queensland, Australia. Sown on 3 February, 2018. Values are expressed as mean days after sowing[1] ± standard deviation based on three replicates.

Developmental stage	30-cell tray (100 mL)	64-cell tray (60 mL)	100-cell tray (18 mL)
1st leaf (GS11)[2]	5.0 ± 0.0	5.0 ± 0.0	5.0 ± 0.0
3rd leaf (GS13)	10.7 ± 0.5	11.0 ± 0.0	11.0 ± 0.0
Elongation (GS39)	22.0 ± 0.0	21.3 ± 0.5	21.7 ± 0.5
Anthesis (GS65)	31.3 ± 1.2	30.0 ± 0.0	31.0 ± 0.0

[1] Seeds were pre-germinated prior to sowing.

[2] GS = growth stage from Zadoks et al. (1974).

Supplementary Table 11 | Characteristics of harvested spring bread wheat (cv. Suntop) grown in SSD trays in the LED-supplemented glasshouse setup at UQ. Plant height, spike number per plant, seed number per plant, spike weight per plant, single seed weight and germination percentage of immature and mature seed of spring wheat (*T. aestivum* cv. Suntop) under speed breeding at three plant densities in a glasshouse with LED supplementary lighting in Queensland, Australia. Sown on 3 February 2018. Values expressed as mean days after sowing[1] ± standard deviation based on three replicates.

Trait	30-cell tray (100 mL)	64-cell tray (60 mL)	100-cell tray (18 mL)
Plant height (cm)	65.5 ± 1.4	62.4 ± 2.4	58.1 ± 2.9
Spike number per plant	1.6 ± 0.0	1.0 ± 0.0	1.0 ± 0.0
Seed number per spike	22.7 ± 2.1	18.6 ± 2.3	12.3 ± 1.4
Spike weight per plant (g)	2.5 ± 0.2	1.2 ± 0.2	0.7 ± 0.0
Single seed weight (mg)	43.3 ± 0.9	47.9 ± 2.1	39.6 ± 0.7
Immature[1] germination[2] (%)	93.9 ± 5.4	77.2 ± 14.2	87.9 ± 1.4
Mature[3] germination (%)	99.1 ± 1.6	100.0 ± 0.0	100.0 ± 0.0

[1] Harvested 14 days post-anthesis (all plants in the case of trays).

[2] After 5 days at 35°C, seeds underwent 1 day of imbibition at room temperature followed by 4 days at 4°C after which they were moved to room temperature for germination.

[3] Maturity was when all green colouration had been lost from the peduncle.

Supplementary Table 12 | **Growth rate of spring barley (cv. Commander) in SSD trays in the LED-supplemented glasshouse setup at UQ.** Development stages of spring barley (*H. vulgare* cv. Commander) under speed breeding at three plant densities in a glasshouse with LED supplementary lighting in Queensland, Australia. Sown on 3 February, 2018. Values expressed as mean days after sowing[1] ± standard deviation based on three replicates.

Developmental stage	30-cell tray (100 mL)	64-cell tray (60 mL)	100-cell tray (18 mL)
1st leaf (GS11)[2]	7.7 ± 0.5	7.3 ± 0.5	7.0 ± 0.0
3rd leaf (GS13)	14.0 ± 0.0	13.3 ± 0.5	13.7 ± 0.5
Elongation (GS39)	22.0 ± 0.0	19.7 ± 0.5	20.3 ± 0.5
Awn peep (GS49)	27.3 ± 2.1	24.7 ± 0.5	24.0 ± 1.0

[1] Seeds were pre-germinated prior to sowing.

[2] GS, growth stage from Zadoks et al. (1974).

Supplementary Table 13 | **Characteristics of harvested spring barley (cv. Commander) grown in SSD trays in the LED-supplemented glasshouse setup at UQ.** Plant height, spike number per plant, seed number per plant, spike weight per plant, single seed weight and germination percentage of immature and mature seed of spring barley (*H. vulgare* cv. Commander) under speed breeding at three plant densities in a glasshouse with LED supplementary lighting in Queensland, Australia. Sown on 3 February, 2018. Values expressed as mean days after sowing[1] ± standard deviation based on three replicates.

Trait	30-cell tray (100 mL)	64-cell tray (60 mL)	100-cell tray (18 mL)
Plant height (cm)	53.1 ± 1.0	51.9 ± 3.3	47.5 ± 4.0
Spike number per plant	2.5 ± 0.3	2.4 ± 0.2	1.7 ± 0.5
Seed number per spike	9.8 ± 0.9	10.0 ± 0.5	6.2 ± 1.7
Spike weight per plant (g)	1.2 ± 0.0	1.1 ± 0.0	0.4 ± 0.0
Single seed weight (mg)	44.8 ± 3.5	41.1 ± 4.0	40.0 ± 7.8
Immature[1] germination[2] (%)	46.2 ± 27.9	37.6 ± 13.5	32.4 ± 19.9
Mature[3] germination (%)	97.8 ± 3.8	98.8 ± 2.1	95.6 ± 1.7

[1] Harvested 21 days post-awn peep (all plants in the case of trays).

[2] After 5 days at 35°C, seeds underwent 1 day of imbibition at room temperature followed by 4 days at 4°C after which they were moved to room temperature for germination. Seeds were bulked for germination testing.

[3] Maturity was when all green colouration had been lost from the peduncle.

Supplementary Table 14 | **PPFD measurements for the LED-supplemented glasshouse setup at JIC.** Photosynthetic photon flux density (PPFD; µmol m^{-2} s^{-1}) measured at a central location in the LED-supplemented glasshouses (GH) at John Innes Centre, UK, using the UPRTek MK350S spectrometer and associated uSpectrum software (UPRTek, Taiwan). Values are the mean of five measurements ± the standard deviation taken in a metre square area under a light fixture.

Position	Day (12 noon) - Sunny	Day (12 noon) – Overcast	Night
Pot height[1]	320.4 ± 9.6	311.3 ± 33.6	222.7 ± 15.9
Sensor height[2]	341.5 ± 14.6	334.7 ± 28.0	244.4 ± 19.8

[1] Pot height was 228 cm from the light fixture.

[2] Sensor height was taken as 100 cm from the bench level, or 144 cm from light fixture.

Supplementary Table 15 | **PPFD measurements for the LED-supplemented glasshouse setup at UQ.** Photosynthetic photon flux density (PPFD; µmol m^{-2} s^{-1}) measured under a Heliospectra E602G light using the Spectrum Genius Essence Lighting Passport light sensor and associated Spectrum Genius Agricultural Lighting app (AsenseTek Inc., Taiwan). Values indicated are the mean ± standard deviation based on five measurements at the corner and centre of a 1 m^2 area under a light fixture at a central location in the glasshouse.

Position	Day (12 pm) - Sunny	Day (12 pm) – Overcast	Night
Bench height[1]	956.5 ± 185.0	356.8 ± 16.5	253.9 ± 12.7
Spike height[2]	972.6 ± 126.4	753.4 ± 92.6	701.7 ± 56.8

[1] Bench height was 155 cm from the light fixture.

[2] Spike height was taken as 95 cm from the light fixture, representing the approximate height of an adult wheat plant.

Supplementary Table 16 | Growth rate of spring wheat (cvs. Fielder and Cadenza) in the LED-supplemented glasshouse setup at JIC. Days to key growth stages of wheat (*Triticum aestivum* cvs. Fielder and Cadenza) grown under 22 and 16-hour photoperiods in LED-supplemented glasshouses at John Innes Centre, UK. Seeds were sown on 14 November 2017 directly into 100 mL of JIC Peat and Sand Mix and seedlings were transferred to 1 L pots of JIC Cereal Compost Mix 23 days later. Values indicated are mean days after sowing (DAS)[1] ± standard deviation based on six replicates.

	***T. aestivum* cv. Fielder**		***T. aestivum* cv. Cadenza**	
Development stage[1,2]	**22 h**	**16 h**	**22 h**	**16 h**
3rd leaf	10.0 ± 0.0	15.5 ± 1.2	9.0 ± 0.0	14.0 ± 0.0
GS31[3]	31.1 ± 0.5	38.8 ± 1.3	23.7 ± 0.5	33.7 ± 1.2
Flag leaf	31.4 ± 0.5	42.0 ± 0.0	27.3 ± 0.5	52.5 ± 1.8
Head (GS51)	42.1 ± 0.3	55.1 ± 1.9	42.0 ± 0.0	57.0 ± 0.6
Anthesis	49.2± 1.5	64.9 ± 1.8	49.5 ± 1.5	65.3 ± 2.4
Mature seed harvest	96.5 ± 0.0	104.0 ± 0.0	92.5 ± 0.0	111.0 ± 0.0
Height (cm)	83.6 ± 2.7	93.8 ± 4.3	73.7 ± 0.7	78.8 ± 3.5

[1] DAS refers to the number of days (post-transfer of germinated seedlings) to reach the indicated developmental growth stages.

[2] All measurements are with respect to the main tiller.

[3] Growth stages measured according to Zadok's scale.

NOTE: Plants were phenotyped every 3-5 days. This may cause whatever differences there might be between replicates or varieties to even out at the time of measurement.

Supplementary Table 17 | **Seed germination rates of harvested spring wheat (cvs. Fielder and Cadenza) grown in the LED-supplemented glasshouse setup at JIC.** Seed viability demonstrated through germination percentages of 30 seeds harvested at physiological maturity from wheat (*Triticum aestivum* cvs. Fielder and Cadenza) grown under 22 and 16-hour photoperiods in LED-Supplemented glasshouses at John Innes Centre, UK. Values indicated are mean ± standard deviation based on 3 replicates of 30 seeds under each photoperiod condition. All seeds were kept at 4°C for 2 days prior the germination.

Cultivar	**Fielder**		**Cadenza**	
	16 h	**22 h**	**16 h**	**22 h**
Germination Percentage (%)	100.0 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0

Supplementary Table 18 | **Growth rate of Brazilian spring wheat (cvs. BRS179 and BR18) in the LED-supplemented glasshouse setup at JIC.** Measurement of key growth stages and growth and development parameters for Brazilian spring wheat (*T. aestivum* cvs. BRS179 and BR18) grown under 22 and 16-hour photoperiods in LED-supplemented glasshouses at John Innes Centre, UK. Seeds were sown on 15 November 2017 directly into 100 mL of JIC Peat and Sand Mix and seedlings were transferred to 1 L pots of JIC Cereal Compost Mix 23 days later. Values indicated are mean ± standard deviation based on 9-10 replicates.

***T. aestivum* cultivar**	**Mean days to anthesis**		**Mean plant height (cm)**		**Mean tiller number**		**Harvest window[1,2]**		**Mean grain yield (g)**	
	22 h[1]	**16 h[1]**	**22 h**	**16 h**	**22 h**	**16 h**	**22 h**	**16 h**	**22 h**	**16 h**
BRS179	50.0 ± 0.0	64.9 ± 4.7	102.2 ± 7.2	89.8 ± 6.9	6.2 ± 0.4	7.8 ± 1.5	87.0	119.0	8.2 ± 0.6	14.0 ± 2.4
BR18	43.0 ± 0.0	55.4 ± 0.5	75.3 ± 7.4	79.4± 4.3	6.9 ± 1.6	7.9 ± 0.8	87.0	119.0	8.9 ± 1.7	11.6 ± 2.3

[1]Days calculated from date of sowing.

[2]Material was harvested at physiological maturity. Plants were dried at 30°C for 7 days prior to weighing.

Supplementary Table 19 | Seed germination rates of harvested Brazilian spring wheat (cvs. BRS179 and BR18) grown in the LED-supplemented glasshouse setup at JIC. Seed viability demonstrated through germination percentages of seed harvested at physiological maturity from two cultivars of rapid-cycling Brazilian wheat (*T. aestivum* cvs. BRS179 and BR18) grown under 22 and 16-hour photoperiods in LED-Supplemented glasshouses at John Innes Centre, UK. All plants were harvested when the ears on individual plants were drying and most ears had turned brown. Plants were dried at 35°C for 7 days post-harvest. Germination tests were conducted with five replicate Petri dishes with 29-34 seeds per dish. After wetting, seeds were kept for 24 hours at 4°C then moved to 22°C. Germination was assessed after 72 hours at 22°C. Values indicated are mean ± standard deviation.

	BRS179		**BR18**	
	22 h	**16 h**	**22 h**	**16 h**
Germination percentage (%)	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0

Supplementary Table 20 | Growth rate of spring durum (cv. Kronos) and bread wheat (cvs. Paragon and Cadenza) in the LED-supplemented glasshouse setup at JIC. Days to key growth stages and measurement of key growth parameters of spring growth habit durum wheat (*T. durum* cv. Kronos) and bread wheat (*T. aestivum* cvs. Paragon and Cadenza) grown under 22 and 16-hour photoperiods in LED-supplemented glasshouses at John Innes Centre, UK. Seeds were germinated for 5 days before pricking out on 3 November 2017 into 100 mL of JIC Peat and Sand Mix. The seedlings were then grown under a 16 h photoperiod for three weeks after which they were transferred to 1 L pots of JIC Cereal Compost Mix and kept under the respective photoperiod treatments. Values indicated are mean ± standard deviation based on six replicates.

	Kronos		**Paragon**		**Cadenza**	
Variable	**22 h**	**16 h**	**22 h**	**16 h**	**22 h**	**16 h**
Days to GS31[1]	30.0 ± 1.2	37.0 ± 0.6	31.0 ± 0.6	39.0 ± 0.7	34.7 ± 1.2	42.0 ± 0.9
Days to GS55	46.0 ± 1.9	53.7 ± 1.0	48.2 ± 0.4	61.8 ± 0.8	50.5 ± 0.6	62.8 ± 0.8
Early harvest	64.0 ± 0.0	72.0 ± 0.0	66.0 ± 0.0	80.0 ± 0.0	69.0 ± 0.0	81.0 ± 0.0
Days to GS90 (late harvest)	94.0 ± 0.0	112.0 ± 0.0	94.0 ± 0.0	112.0 ± 0.0	98.0 ± 0.0	116.0 ± 0.0
Height	68.0 ± 4.4	68.9 ± 1.9	85.1 ± 3.2	86.4 ± 2.6	82.6 ± 2.2	83.9 ± 3.0
Tiller No.	5.8 ± 1.9	6.8 ± 1.0	5.5 ± 0.6	4.6 ± 0.6	5.0 ± 0.6	4.8 ± 0.8

[1]Days calculated from the time seeds were put into germination.

Supplementary Table 21 | Spike characteristics of harvested spring durum (cv. Kronos) and bread wheat (cvs. Paragon and Cadenza) grown in the LED-supplemented glasshouse setup at JIC.

Measurement of key characteristics of spikes harvested early (Spike_1) and at maturity (Spike_2) of spring growth habit durum wheat (*T. durum* cv. Kronos) and bread wheat (*T. aestivum* cvs. Paragon and Cadenza) grown under 22 and 16-hour photoperiods in LED-supplemented glasshouses at John Innes Centre, UK. Seeds were germinated for 5 days before being pricked out on 3 November 2017 into 100 mL of JIC Peat and Sand Mix. The seedlings were then grown under a 16 h photoperiod for three weeks after which they were transferred to 1 L pots of JIC Cereal Compost Mix and kept under the respective photoperiod treatments. Values indicated are mean ± standard deviation based on six replicates.

	Kronos		**Paragon**		**Cadenza**	
Variable[1]	**22 h**	**16 h**	**22 h**	**16 h**	**22 h**	**16 h**
Seeds per Spike_1	27.8 ± 7.9	29.8 ± 4.3	63.7 ± 7.8	63.8 ± 4.2	64.0 ± 2.7	65.5 ± 10.0
Seeds per Spike_2	30.4 ± 4.5	33.8 ± 5.9	59.0 ± 7.6	63.8 ± 6.8	58.0 ± 6.8	68.3 ± 7.8
Yield per Spike_1 (g)	0.4 ± 0.2	0.3 ± 0.1	1.4 ± 0.2	1.1 ± 0.1	1.0 ± 0.2	0.6 ± 0.2
Yield per Spike_2 (g)	1.9 ± 0.3	2.1 ± 0.4	2.9 ± 0.4	3.3 ± 0.5	3.0 ± 0.3	3.6 ± 0.6
TGW_1[2] (g)	12.6 ± 5.5	8.3 ± 2.1	21.4 ± 1.7	16.5 ± 2.2	15.2 ± 2.2	8.8 ± 2.1
TGW_2 (g)	62.2 ± 5.0	62.9 ± 3.1	49.1 ± 3.6	51.2 ± 2.9	51.9 ± 4.4	52.2 ± 3.4

[1] The suffixes "_1" and "_2" indicate early and late harvest (GS90), respectively.

[2] TGW – Thousand Grain Weight.

Supplementary Table 22 | **Seed germination rates of harvested spring durum (cv. Kronos) and bread wheat (cvs. Paragon and Cadenza) grown in the LED-supplemented glasshouse setup at JIC.** Seed viability demonstrated through germination percentages of 20 seeds harvested at 18 days post-heading (Early Harvest) and at maturity (Late Harvest) from spring growth habit durum wheat (*Triticum durum* cv. Kronos*)* and bread wheat *(Triticum aestivum* cvs*.* Cadenza, Paragon,) grown under 22 and 16-hour photoperiods in LED-Supplemented glasshouses at John Innes Centre, UK. Values indicated are percentage mean ± standard deviation based on 4 replicates of 20 seeds under each photoperiod condition. All seeds were sown at 4°C for 3 days and scored for germination after a further 3 days at room temperature.

Cultivar	**Early Harvest (germination %)**		**Late Harvest (germination %)**	
	16 h	**22 h**	**16 h**	**22 h**
Kronos	88.8 ± 0.1	97.5 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
Paragon	98.8 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
Cadenza	98.8 ± 0.0	97.5 ± 0.1	100.0 ± 0.0	100.0 ± 0.0

Supplementary Table 23 | **Growth rate of spring durum (cv. Kronos) and bread wheat (cv. Cadenza) in SSD trays in the LED-supplemented glasshouse setup at JIC.** Days to key growth stages and measurement of key growth parameters of spring growth habit tetraploid wheat (*Triticum durum* cv. Kronos) and hexaploid wheat (*Triticum aestivum* cv. Cadenza) grown under 22 and 16-hour photoperiods in LED-supplemented glasshouses at John Innes Centre, UK in 96-well trays (SSD system). Seeds were germinated for 5 days before transferring on 7 November 2017 into 96-well trays (each cell containing 75 mL of JIC Cereal Compost Mix). The seedlings were grown under a 16-hour photoperiod for 10 days after which the trays were transferred to the respective photoperiod treatments. Values indicated for the growth stages are a visual mean value across the tray. When indicated as mean ± standard deviation, values are based on 25 sampled spikes across the tray (excluding edge plants).

	SSD Cadenza		**SSD Kronos**	
	22 h	**16 h**	**22 h**	**16 h**
Days to GS31	32.0	40.0	28.0	34.0
Days to GS55	45.0	58.0	42.0	50.0
Harvest at 14 PA	63.0	76.0	60.0	68.0
Days to GS90	90.0	112.0	79.0	97.0
Seeds per Spike_1[1]	32.1 ± 3.4	27.5 ± 6.1	18.4 ± 4.5	15.4 ± 5.01
Seeds per Spike_2	30.8 ± 3.8	30.7 ± 5.1	18.8 ± 5.3	17.4 ± 3.9
Yield per Spike_1 (g)	0.44 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.1 ± 0.1
Yield per Spike_2 (g)	1.4 ± 0.2	1.7 ± 0.4	0.9 ± 0.2	1.0 ± 0.2
TGW_1[2] **(g)**	13.5 ± 1.8	9.0 ± 2.9	13.6 ± 2.6	8.9 ± 1.3
TGW_2 (g)	45.3 ± 4.7	54.0 ± 8.1	50.2 ± 7.4	58.7 ± 5.2

[1] The suffixes "_1" and "_2" indicate early and late harvest (GS90), respectively.

[2] TGW – Thousand Grain Weight.

Supplementary Table 24 | **Seed germination rates of harvested spring durum (cv. Kronos) and bread wheat (cv. Cadenza) grown in SSD trays in the LED-supplemented glasshouse setup at JIC.** Seed viability demonstrated through germination percentages of 20 seeds harvested at 18 days post-heading (Early Harvest) and at maturity (Late Harvest) from durum wheat (*Triticum durum* cv. Kronos*)* and bread wheat *(Triticum aestivum* cv. Cadenza) grown as SSD under 22 and 16-hour photoperiods in LED-Supplemented glasshouses at John Innes Centre, UK. Values indicated are mean ± standard deviation based on 5 replicates of 20 seeds under each photoperiod condition. All seeds were sown at 4°C for 3 days and scored for germination after a further 3 days at room temperature.

T. aestivum **cultivar**	**Early Harvest (%)**		**Late Harvest (%)**	
	16 h	**22 h**	**16 h**	**22 h**
Kronos	100.0 ± 0.0	97.6 ± 0.1	100.0 ± 0.0	100.0 ± 0.0
Cadenza	93.1 ± 0.1	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0

Supplementary Table 25 | **Growth rate of winter bread wheat (cvs. Crusoe and KWS Trinity) in the LED-supplemented glasshouse setup at JIC.** Days to key growth stages and measurement of key growth parameters of winter growth habit bread wheat (*T. aestivum* cvs. Crusoe and KWS Trinity) grown under 22 and 16-hour photoperiods in LED-supplemented glasshouses at John Innes Centre, UK. Seeds were germinated for five days before being pricked out on 20 September, 2017 into 100 mL of JIC Peat and Sand Mix. The seedlings were grown under a 16-hour photoperiod for 12 days after which they were vernalised for 8 weeks (8-hour photoperiod, 6 °C). Seedlings were then transferred to 1 L pots of JIC Cereal Compost Mix and kept under the respective photoperiod treatments. Values indicated are mean ± standard deviation based on six replicates.

	Crusoe		**KWS Trinity**	
Variable	**22 h**	**16 h**	**22 h**	**16 h**
Days to GS31[1,2]	90.2 ± 0.5	94.0 ± 0.0	87.2 ± 0.8	92.3 ± 0.8
Days to GS55	106.6 ± 1.5	114.0 ± 0.7	104.2 ± 0.8	116.7 ± 0.8
Height	58.5 ± 4.3	57.1 ± 2.1	68.1 ± 3.4	55.5 ± 2.3
Tiller No.	6.2 ± 0.5	6.6 ± 0.6	4.7 ± 0.5	5.2 ± 0.4
Days to GS90	159.0 ± 0.0	168.0 ± 0.0	154.0 ± 0.0	170.0 ± 0.0

[1] Days were counted from the time germinated seeds were sown. Germination took five days (including three days of cold stratification at 4 °C to break dormancy).

[2] All measurements are made with respect to the first tiller, and in accordance with the Zadoks scale (Zadoks et al., 1974).

Supplementary Table 26 | Spike characteristics of harvested winter bread wheat (cvs. Crusoe and KWS Trinity) grown in the LED-supplemented glasshouse setup at JIC. Measurement of key characteristics of spikes harvested early (Spike_1) and at maturity (Spike_2) from winter growth habit bread wheat (*T. aestivum* cvs. Crusoe and KWS Trinity) grown under 22 and 16-hour photoperiods in LED-supplemented glasshouses at John Innes Centre, UK. Seeds were germinated for five days before being pricked out on 20 September, 2017 into 100 mL of JIC Peat and Sand Mix. Seedlings were then grown under a 16-hour photoperiod for 12 days after which they were vernalised for 8 weeks (8-hour photoperiod, 6 °C). Seedlings were then transferred to 1 L pots of JIC Cereal Compost Mix and kept under the respective photoperiod treatments. Values indicated are mean ± standard deviation based on six replicates.

	Crusoe		**KWS Trinity**	
Variable[1]	**22 h**	**16 h**	**22 h**	**16 h**
Seeds per Spike_1	52.2 ± 11.3	65.0 ± 2.4	72.5 ± 5.8	73.5 ± 6.9
Seeds per Spike_2	44.2 ± 3.8	54.2 ± 6.7	62.5 ± 3.9	62.3 ± 7.0
Yield per Spike_1 (g)	0.8 ± 0.2	0.8 ± 0.1	1.1 ± 0.2	0.8 ± 0.1
Yield per Spike_2 (g)	2.4 ± 0.1	2.5 ± 0.4	3.3 ± 0.2	3.3 ± 0.4
TGW_1[2] **(g)**	16.0 ± 3.8	11.6 ± 1.1	15.1 ± 2.4	10.3 ± 1.6
TGW_2 (g)	53.8 ± 4.5	46.1 ± 2.7	52.1 ± 3.8	52.8 ± 3.2

[1] The suffixes "_1" and "_2" indicate early and late harvest (GS90), respectively.

[2] TGW – Thousand Grain Weight.

Supplementary Table 27 | **Seed germination rates of harvested winter bread wheat (cvs. Crusoe and KWS Trinity) grown in the LED-supplemented glasshouse setup at JIC.** Seed viability demonstrated through germination percentages of 20 seeds harvested at 18 days post-heading (Early Harvest) and at maturity (Late Harvest) from winter growth habit bread wheat *(Triticum aestivum* cvs. KWS Trinity and Crusoe) grown under 22 and 16-hour photoperiods in LED-Supplemented glasshouses at John Innes Centre, UK. Values indicated are percentage mean ± standard deviation based on 4 replicates of 20 seeds under each photoperiod condition. All seeds were sown at 4°C for 3 days and scored for germination after a further 3 days at room temperature.

Cultivar	**Early Harvest (germination %)**		**Late Harvest (germination %)**	
	16 h	**22 h**	**16 h**	**22 h**
Crusoe	97.5 ± 0.01	85.0 ± 0.1	100.0 ± 0.0	100.0 ± 0.0
KWS Trinity	87.5 ± 0.1	95.0 ± 0.1	100.0 ± 0.0	100.0 ± 0.0

Supplementary Table 28 | Growth rate of spring barley (cvs. Golden Promise, Manchuria, Nigrate and Baronesse) in the LED-supplemented glasshouse setup at JIC. Days to key growth stages of barley (*Hordeum vulgare* cvs. Golden Promise, Manchuria, Nigrate and Baronesse) grown under 22 and 16-hour photoperiods in LED-supplemented glasshouses at John Innes Centre, UK. Seeds were directly sown on 25 October, 2017 in 1 L pots of JIC Cereal Compost Mix. Values indicated are mean days after sowing (DAS)[1] ± standard deviation based on five replicates.

Development stage[1]	***H. vulgare* cv. Golden Promise**		***H. vulgare* cv. Manchuria**		***H. vulgare* cv. Nigrate**		***H. vulgare* cv. Baronesse**	
	22 h	**16 h**	**22 h**	**16 h**	**22 h**	**16 h**	**22 h**	**16 h**
1st leaf[2]	5.0 ± 0.0	5.0 ± 0.0	5.0 ± 0.0	5.0 ± 0.0	5.0 ± 0.0	5.0 ± 0.0	5.0 ± 0.0	5.0 ± 0.0
3rd leaf	12.0 ± 0.0	12.0 ± 0.0	12.0 ± 0.0	19.0 ± 0.0	12.0 ± 0.0	19.0 ± 0.0	12.0 ± 0.0	12.0 ± 0.0
1st node	20.0 ± 0.0	20.0 ± 0.0	17.0 ± 0.0	20.0 ± 0.0	20.0 ± 0.0	24.0 ± 0.0	20.0 ± 0.0	20.0 ± 0.0
Flag leaf	29.8 ± 3.8	38.0 ± 0.0	24.0 ± 0.0	34.0 ± 0.0	48.0 ± 4.2	59.8 ± 4.6	28.6 ± 0.9	39.2 ± 1.6
Emergence of awns	36.4 ± 2.2	42.8 ± 1.6	27.0 ± 0.0	38.0 ± 0.0	58.4± 10.1	64.4 ± 4.0	31.6 ± 0.9	41.0 ± 0.0
Grain milk	55.6 ± 2.2	63.8 ± 1.6	48.0 ± 0.0	59.0 ± 0.0	79.6 ± 11.8	84.0 ± 2.1	52.0 ± 2.2	63.8 ± 1.1
Early Harvest (viable seed collection)	ND[3]	70.8 ± 1.6	ND[3]	64.0 ± 0.0	ND[3]	84.0 ± 0.0	ND[3]	71.2 ± 1.6
Mature Seed Harvest	71.0 ± 0.0	82.0 ± 0.0	63.0 ± 0.0	77.0 ± 0.0	85.0 ± 0.0	97.0 ± 0.0	71.0 ± 0.0	82.0 ± 0.0

[1] DAS refers to the number of days (post seed sowing) to reach the indicated developmental growth stages. Seeds were sown directly in Cereal mix contained in 1 L pots.

[2] All measurements are with respect to the main tiller.

[3] Not determined.

Supplementary Table 29 | **Characteristics of harvested spring barley (cvs. Golden Promise, Manchuria, Nigrate and Baronesse) grown in the LED-supplemented glasshouse setup at JIC.** Number of spikes per plant, grains per spike, 100-grain weight per plant of spikes and seeds harvested at physiological maturity from barley (*Hordeum vulgare* cvs. Golden Promise, Manchuria, Nigrate and Baronesse) grown under 22 and 16-hour photoperiods in LED-supplemented glasshouses at John Innes Centre, UK. Seeds were sown on 25 October 2017 directly into 1 L pots of JIC Cereal Compost Mix. Values indicated are mean ± standard deviation based on five replicates.

***H. vulgare* cultivar**	**Spikes per plant**		**Grain per spike**		**100-grain weight (g)**	
	22 h	**16 h**	**22 h**	**16 h**	**22 h**	**16 h**
Golden Promise	14.6 ± 2.6	24.4 ± 3.6	22.2 ± 1.3	24.4 ± 1.5	4.5 ± 0.6	3.9 ± 0.4
Manchuria	8.0 ± 1.6	8.4 ± 1.8	32.6 ± 2.6	52.0 ± 2.6	4.1 ± 0.3	4.1 ± 0.6
Nigrate	12.8 ± 4.3	9.0 ± 2.1	53.6 ± 2.9	62.0 ± 4.7	2.5 ± 0.1	2.8 ± 0.1
Baronesse	14.4 ± 2.5	20.6 ± 5.3	19.4 ± 1.5	22.8 ± 0.8	5.2 ± 0.6	5.2 ± 0.5

Supplementary Table 30 | **Seed germination rates of harvested spring barley (cvs. Golden Promise, Manchuria, Nigrate and Baronesse) grown in the LED-supplemented glasshouse setup at JIC.** Seed viability demonstrated through germination percentages of 60 seeds harvested early (14 days post anthesis) and at maturity from barley (*Hordeum vulgare* cvs. Golden Promise, Manchuria, Nigrate and Baronesse) grown under 22 and 16-hour photoperiods in LED-supplemented glasshouses at John Innes Centre, UK. Seeds were sown on 25 October 2017 directly into 1 L pots of JIC Cereal Compost Mix. Values indicated are mean ± standard deviation based on five replicates.

***H. vulgare* cultivar**	**Germination percentage (%)**		
	16 h Early	**16 h Mature**	**22 h Mature**
Golden Promise	58.7 ± 21.5	86.0 ± 4.5	97.0 ± 1.8
Manchuria	85.0 ± 4.6	84.7 ± 8.9	88.3 ± 8.4
Nigrate	95.7 ± 2.3	96.9 ± 2.7	93.0 ± 7.4
Baronesse	95.7 ± 5.2	96.0 ± 3.4	90.0 ± 2.9

Supplementary Table 31 | **Growth rate of *Brassica rapa* (line R-0-18), *B. napus* (line RV31) and *B. olerecea* (line DH1012) in the LED-supplemented glasshouse setup at JIC.** Days to key growth stages and measurement of key growth parameters of *Brassica rapa* (line R-0-18), *B. napus* (line RV31) and *B. olerecea* (line DH1012) grown under 22 and 16-hour photoperiods in LED-supplemented glasshouses at John Innes Centre, UK. Seeds were sown on 14 November 2017 in 100 mL of Levington® Advance F2 Seed and Modular Compost (ICL Specialty Fertilizers) and grown in a 16-hour photoperiod for seven days, and thereafter transferred to 1 L pots of JIC Cereal Compost Mix and placed in the respective photoperiod conditions. Values indicated are mean ± standard deviation based on 12 replicates.

	***B. rapa* (R-0-18)**		***B. oleracea* (DH1012)**		***B. napus* (RV31)**	
	22 h	**16 h**	**22 h**	**16 h**	**22 h**	**16 h**
Days till first flower opens	36.5 ± 2.5	41.0 ± 3.7	49.2 ± 1.8	61.2 ± 2.3	34.5 ± 0.7	45.0 ± 0.0
Flowering duration	20.5 ± 2.5	66.0 ± 3.7	41.8 ± 1.8	85.8 ± 2.3	22.5 ± 0.7	62.0 ± 0.0
Days till drying off, first pods on main raceme can be harvested[1]	91	112	128	169	91	109
Time to harvest[1]	112	120	155	189	113	123

[1] Batch treated.

Supplementary Table 32 | **Characteristics of harvested *Brassica rapa* (line R-0-18), *B. napus* (line RV31) and *B. olerecea* (line DH1012) grown in the LED-supplemented glasshouse setup at JIC.** Measurement of key parameters of mature plants of *B. rapa* (line R-0-18), *B. napus* (line RV31) and *B. oleracea* (line DH1012) grown under 22 and 16-hour photoperiods in LED-supplemented glasshouses at John Innes Centre, UK. Seeds were sown on 14 November 2017 in 100 mL of Levington® Advance F2 Seed and Modular Compost (ICL Specialty Fertilizers) and grown in a 16-hour photoperiod for seven days, and thereafter transferred to 1 L pots of JIC Cereal Compost Mix and placed in the respective photoperiod conditions. Values indicated are mean ± standard deviation based on 12 replicates.

	***B. rapa* (R-0-18)**		***B. oleracea* (1012)**		***B. napus* (RV31)**	
	22 h	**16 h**	**22 h**	**16 h**	**22 h**	**16 h**
Number of branches bearing fertile pods	5.2 ± 1.2	3.8 ± 0.7	5.5 ± 0.8	6.0 ± 1.3	5.5 ± 0.8	6.0 ± 1.3
Number of later branches not producing fertile pods	1 ± 0.7	0.3 ± 0.9	0.8 ± 0.9	0.1 ± 0.3	0.8 ± 0.9	0.1 ± 0.3
Number of non-branching nodes	3.0 ± 1.0	5.8 ± 1.3	3.2 ± 0.7	6.3 ± 1.5	3.2 ± 0.7	6.3 ± 1.5
Plant height (m)	1.4 ± 0.1	1.4 ± 0.2	1.1 ± 0.2	1.6 ± 0.1	1.1 ± 0.2	1.6 ± 0.1

Supplementary Table 33 | **Characteristics of pods harvested from *Brassica rapa* (line R-0-18), *B. napus* (line RV31) and *B. olererecea* (line DH1012) grown in the LED-supplemented glasshouse setup at JIC.** Measurement of key post-harvest parameters of mature plants of *Brassica rapa* (line R-0-18), *Brassica napus* (line RV31) and *Brassica oleracea* (line DH1012) grown under 22 and 16-hour photoperiods in LED-supplemented glasshouses at John Innes Centre, UK. Seeds were sown on 14 November 2017 in 100 mL of Levington® Advance F2 Seed and Modular Compost (ICL Specialty Fertilizers) and grown in a 16-hour photoperiod for seven days, and thereafter transferred to 1 L pots of JIC Cereal Compost Mix and placed in the respective photoperiod conditions. Values indicated are mean ± standard deviation based on 12 replicates.

	***B. rapa* (R-0-18)**		***B. oleracea (1012)***		***B. napus* (RV31)**	
	22 h	**16 h**	**22 h**	**16 h**	**22 h**	**16 h**
Length of beak (remains of stigma) (mm)	20.9 ± 5.0	34.0 ± 3.6	2.4 ± 0.6	2.9 ± 0.7	7.9 ± 1.8	11.7 ± 2.0
pod valve length (mm)	35.2 ± 7.8	47.8 ± 4.2	30.5 ± 5.8	42.6 ± 6.0	43.5 ± 12.7	59.7 ± 8.5
Total pod length (valve plus beak) (mm)	56.0 ± 11.4	81.8 ± 6.4	32.9 ± 6.0	45.5 ± 6.2	51.4 ± 13.7	71.4 ± 9.4

Supplementary Table 34 | **Seed characteristics of harvested *Brassica rapa* (line R-0-18), *B. napus* (line RV31) and *B. olerecea* (line DH1012) grown in the LED-supplemented glasshouse setup at JIC.** Measurement of key post-harvest seed parameters harvested from earliest set pods of mature plants of *B. rapa* (line R-0-18), *B. napus* (line RV31) and *B. oleracea* (line DH1012) grown under 22 and 16-hour photoperiods in LED-supplemented glasshouses at John Innes Centre, UK. Seeds were sown on 14 November 2017 in 100 mL of Levington® Advance F2 Seed and Modular Compost (ICL Specialty Fertilizers) and grown in a 16-hour photoperiod for seven days, and thereafter transferred to 1 L pots of JIC Cereal Compost Mix and placed in the respective photoperiod conditions. Values indicated are mean ± standard deviation based on 12 replicates.

	***B. rapa* (line R-0-18)**		***B. oleracea* (line DH1012)**		***B. napus* (line RV31)**	
	22 h	**16 h**	**22 h**	**16 h**	**22 h**	**16 h**
Seeds per pod	10.3 ± 3.1	32.3 ± 3.5	3.9 ± 1.0	7.3 ± 2.0	8.3 ± 3.9	24.0 ± 2.9
Thousand grain weight (g)	3.0 ± 0.5	4.5 ± 0.4	2.7 ± 0.4	3.7 ± 0.2	3.9 ± 0.5	5.1 ± 0.7
Area (mm^2)	3.1 ± 0.2	3.5 ± 0.2	3.3 ± 0.2	3.8 ± 0.2	4.4 ± 0.3	4.8 ± 0.4

Supplementary Table 35 | **Seed germination rates of harvested *Brassica rapa* (line R-0-18), *B. napus* (line RV31) and *B. olerecea* (line DH1012) grown in the LED-supplemented glasshouse setup at JIC.** Seed viability demonstrated through germination percentages of seed harvested at physiological maturity from earliest set pods of plants of *B. rapa* (line R-0-18), *B. napus* (line RV31) and *B. oleracea* (line DH1012) grown under 22 and 16-hour photoperiods in LED-supplemented glasshouses at John Innes Centre, UK. Seeds were sown on 14 November 2017 in 100 mL of Levington® Advance F2 Seed and Modular Compost (ICL Specialty Fertilizers) and grown in a 16-hour photoperiod for seven days, and thereafter transferred to 1 L pots of JIC Cereal Compost Mix and placed in the respective photoperiod conditions. Values indicated are mean ± standard deviation based on three replicates (10 seeds per replicate).

	***B. rapa* (R-0-18)**		***B. oleracea* (DH1012)**		***B. napus* (RV31)**	
	22 h	**16 h**	**22 h**	**16 h**	**22 h**	**16 h**
Germination percentage (%)	100 ± 0.0	100 ± 0.0	96.7 ± 5.8	96.7 ± 5.8	100 ± 0.0	96.7 ± 5.8

Supplementary Table 36 | Growth rate of pea (accessions JI 2822 and cultivars Cameor and Princess) in the LED-supplemented glasshouse setup at JIC. Days to key growth stages, and measurement of key growth and development parameters for three genotypes of pea (*Pisum sativum* accession JI 2822, JI 3253 (cv. Cameor) and JI 2623 (cv. Princess)), grown under 22 and 16-hour photoperiods in LED-supplemented glasshouses at John Innes Centre, UK. Seeds were scarified and sown on 14 November 2017 in 100 mL of JIC Peat and Sand Mix and seedlings were transferred to 1 L pots of JIC Cereal Compost Mix 23 days later. Values indicated are mean ± standard deviation based on five replicates.

	JI 2822		JI 3253 (Cameor)		JI 2623 (Princess)	
	22 h	**16 h**	**22 h**	**16 h**	**22 h**	**16 h**
1st Flower bud node	6.4 ± 0.9	6.4 ± 0.6	9.6 ± 0.6	10.2 ± 0.8	17.0 ± 1.0	16.6 ± 0.9
1st open flower DAS[1]	28.4 ± 0.6	38.6 ± 1.1	30.0 ± 1.0	41.2 ± 1.6	38.2 ± 0.8	48.8 ± 1.3
No. pods	6.8 ± 1.1	10.4 ± 1.5	6.4 ± 1.3	8.0 ± 0.7	6.2 ± 2.2	10.0 ± 2.6
No. side shoots	3.6 ± 0.6	2.6 ± 0.9	3.6 ± 1.3	1.8 ± 0.8	1.8 ± 2.1	1.0 ± 1.2
Final node No.	11.8 ± 0.5	12.4 ± 0.9	14.8 ± 0.5	15.6 ± 0.9	21.2 ± 1.5	22.2 ± 1.1
Final height (mm)	276.0 ± 4.2	397.0 ± 28.0	561.0 ± 11.9	723.0 ± 67.0	845.0 ± 58.1	1120.0 ± 94.6
Seed harvest (DAS)[2]	61.0 ± 0.0	84.0 ± 0.0	61.0 ± 0.0	86.8 ± 3.8	68.0 ± 0.0	91.0 ± 0.0
No. of seeds	23.6 ± 2.7	36.4 ± 6.1	32.4 ± 8.5	40.4 ± 7.5	21.4 ± 3.1	41.8 ± 6.4

[1] DAS = Days After Sowing. Days counted from sowing date

[2] All plants were kept under water stress for 7 days before harvesting. Seeds were not harvested at physiological maturity (early harvest).

[3] All seeds were dried at 30°C for 7 days.

Supplementary Table 37 | **Seed germination rates of harvested pea (accessions JI 2822 and cultivars Cameor and Princess) grown in the LED-supplemented glasshouse setup at JIC.** Seed viability tests by monitoring germination of seed harvested early from for three genotypes of pea (*Pisum sativum* accession JI 2822, JI 3253 (cv. Cameor) and JI 2623 (cv. Princess)), grown under 22 and 16-hour photoperiods in LED-Supplemented glasshouses at John Innes Centre, UK. Plants were deprived of water for 7 days prior to harvesting of seed, and harvested pods were dried at 30°C in for 7 days. Values indicated are mean ± standard deviation based on five replicates.

	JI 2822		**JI 3253 (Cameor)**		**JI 2623 (Princess)**	
	22 h	**16 h**	**22 h**	**16 h**	**22 h**	**16 h**
Germination percentage (%)	95.0 ± 7.1	100.0 ± 0.0	98.0 ± 2.7	99.0 ± 2.2	94.0 ± 10.8	97.0 ± 4.5

Supplementary Table 38 | **Growth rate of grasspea (cv. Mahateora) in the LED-supplemented glasshouse setup at JIC.** Days to key growth stages and measurement of key growth parameters of grasspea (*Lathyrus sativus* cv. Mahateora) grown under 22 and 16-hour photoperiods in LED-supplemented glasshouses at John Innes Centre, UK. Seeds were sown on 14 November 2017 directly into 100 mL of JIC Peat and Sand Mix and seedlings were transferred to 1 L pots of JIC Cereal Compost Mix 23 days later. Values indicated are mean ± standard deviation based on 10 replicates.

	***L. sativus* cv. Mahateora**	
	22 h	**16 h**
First flower opening	31 days	Not determined
Early harvest[1,2]	80 days	129 days
Mature harvest[1]	173 days	173 days

[1] All replicates were treated as a batch for harvesting

[2] For early harvest, a few pods that were ready to be harvested were sampled from two replicates of each variety for each photoperiod treatment.

Supplementary Table 39 | **Seed characteristics of harvested grasspea (cv. Mahateora) grown in the LED-supplemented glasshouse setup at JIC.** Average weight of seeds per plant, harvested at physiological maturity, from grasspea (*L. sativus* cv. Mahateora) grown under 22 and 16-hour photoperiods in LED-supplemented glasshouses at John Innes Centre, UK. Seeds were sown on 14 November, 2017 directly into 100 mL of JIC Peat and Sand Mix and seedlings were transferred to 1 L pots of JIC Cereal Compost Mix 23 days later. Values indicated are mean ± standard deviation based on 10 replicates.

	***L. sativus* cv. Mahateora**	
	22 h	**16 h**
No. of seeds per plant	36.3 ± 16.9	49.3 ± 25.0
Seed weight per plant (g)	3.5 ± 1.7	3.8 ± 2.2

Supplementary Table 40 | **Seed germination rates of harvested grasspea (cv. Mahateora) grown in the LED-supplemented glasshouse setup at JIC.** Seed viability demonstrated through germination tests of seed harvested early from relatively mature pods of grasspea (*L. sativus* cv. Mahateora) grown under 22 and 16-hour photoperiods in LED-supplemented glasshouses at John Innes Centre, UK. Seeds were sown on 14 November 2017 directly into 100 mL of JIC Peat and Sand Mix and seedlings were transferred to 1 L pots of JIC Cereal Compost Mix 23 days later. Pods were sampled from plants kept under each photoperiod treatment.

	***L. sativus* cv. Mahateora**	
	22 h	**16 h**
No. of seeds from pods sampled early	19	18
No. of seeds sampled for germination tests	15	15
Germination percentage (%)	100.0 ± 0.0	100.0 ± 0.0

Supplementary Table 41 | **Growth rate of *Brachypodium distachyon* (accessions Bd21, Bd21-3 and Bd3-1) in the LED-supplemented glasshouse setup at JIC.** Measurement of key growth stages and growth and development parameters for *B. distachyon* (accessions Bd21, Bd21-3 and Bd3-1) grown under 22 and 16-hour photoperiods in LED-supplemented glasshouses at John Innes Centre, UK. Seeds were sown on 15 November, 2017 directly into 100 mL of 50% JIC Cereal Mix/50% JIC Peat and Sand Compost, and seedlings were transferred to 600 mL pots of the same soil mix 23 days later. Values indicated are mean ± standard deviation based on 9-10 replicates.

***B. distachyon* accession**	**Mean days to heading[1]**		**Mean final plant height (cm)**		**Mean grain weight per plant (g)**		**Harvest window[1,2]**	
	22 h	**16 h**	**22 h**	**16 h**	**22 h**	**16 h**	**22 h**	**16 h**
Bd21	27.0 ± 0.0	40.7 ± 0.9	30.1 ± 1.6	41.9 ± 2.4	1.1 ± 0.2	1.2 ± 0.3	83	98-119
Bd21-3	27.0 ± 0.0	42.0 ± 2.5	35.1 ± 4.1	54.7 ± 4.4	1.1 ± 0.2	1.2 ± 0.4	83	98-119
Bd3-1	29.4 ± 2.2	45.4 ± 2.6	47.7 ± 5.1	58.0 ± 4.0	ND	ND	83	98-119

[1]Days calculated from date of sowing.

[2]Material was harvested when the ears on individual plants were drying and most ears had turned brown. Plants were dried at 30°C for 7 days prior to weighing.

Supplementary Table 42 | **Seed germination rates of harvested *Brachypodium distachyon* (accessions Bd21 and Bd21-3) grown in the LED-supplemented glasshouse setup at JIC.** Seed viability demonstrated through germination percentages of seed harvested at physiological maturity from for two accessions of *B. distachyon* grown under 22 and 16-hour photoperiods in LED-Supplemented glasshouses at John Innes Centre, UK. All plants were harvested when the ears on individual plants were drying and most ears had turned brown. Plants were dried at 30°C for 7 days post-harvest. Germination tests were conducted with five replicate Petri dishes with 18-23 seeds per dish. After wetting, seeds were kept for 24 hours at 4°C and then moved to 22°C. Germination was assessed after 72 hours at 22°C. Values indicated are mean ± standard deviation.

	Bd21		**Bd21-3**	
	22 h	**16 h**	**22 h**	**16 h**
Germination percentage (%)	96.0 ± 2.3	82.4 ± 4.9	81.8 ± 18.9	83.8 ± 8.1

Supplementary Table 43 | **Growth rate of *C. quinoa* (accession QQ-74 and cv. Titicaca) in the LED-supplemented glasshouse setup at JIC.** Days to key growth stages of quinoa (*Chenopodium quinoa* accession QQ-74 and cv. Titicaca) grown under 22 and 16-hour photoperiods in LED-supplemented glasshouses at John Innes Centre, UK. Seeds were germinated for 4 days and then transferred directly into 1 L pots containing Peat and Sand Mix on 9 February 2018. Values indicated are mean days after transfer of germinated seedlings[1] ± standard deviation based on 3-5 replicates.

Development stage[2,3]	***C. quinoa* QQ-74**		***C. quinoa* Titicaca**	
	22 h	**16 h**	**22 h**	**16 h**
Inflorescence emergence	45.2 ± 4.4	57.0 ± 0.0	43.6 ± 3.6	43.6 ± 3.6
Anthesis[2]	55.0 ± 4.6	64.3 ± 6.1	54.2 ± 3.8	57.8 ± 1.1
Fruit development[2] **(early harvest point)**	78.6 ± 3.6	87.0 ± 3.5	78.6 ± 3.6	81.8 ± 4.4
Ripe/mature fruit[2]	106.5 ± 7.8	103.8 ± 5.5	104.8 ± 2.5	101.0 ± 0.0
Senescence	113.2 ± 8.3	123.7 ± 4.0	113.2 ± 8.3	124.6 ± 3.1

[1] Germination for all samples required 4 days. Seeds were germinated by application of GA_3.

[2] Three 16 h QQ-74 plants were followed due to pest-related death of two plants from weeks 7-8. All other measurements refer to 5 plants.

[3]All measurements are with respect to the primary inflorescence, using the BBCH Code System.

NOTE: Plants were phenotyped every 2-8 days. This may cause whatever differences there might be between replicates or varieties to even out at the time of measurement, causing a net zero standard deviation.

Supplementary Table 44 | Seed characteristics of harvested *C. quinoa* (accession QQ-74 and cv. Titicaca) grown in the LED-supplemented glasshouse setup at JIC. One-thousand seed weight (g) of quinoa (*Chenopodium quinoa* accession QQ-74 and cv. Titicaca) grown under 22 and 16-hour photoperiods in LED-supplemented glasshouses at John Innes Centre, UK (sown on 9 February 2018). Early harvest was carried out at the fruit development grain stage (~25 days and ~26 days post-anthesis in 22 and 16 h rooms, respectively). Seed was later harvested at physiological maturity once at least half of the inflorescence had senesced (~61 and ~65 days post-anthesis in 22h and 16h rooms, respectively). Inflorescences were dried at 30°C for 3-5 days prior to weighing. Values are expressed as mean ± SD, based on three replicates.

	C. quinoa			
	accession QQ-74		**cv. Titicaca**	
	22 h	**16 h**	**22 h**	**16 h**
Early harvest (1000 grain weight, g)	1.9 ± 0.1[2]	3.1 ± 0.0[1,2]	2.1 ± 0.1[2]	2.8 ± 0.0[2]
Mature harvest (1000 grain weight, g)	2.4 ± 0.1[2,3]	3.7 ± 0.1[1,3]	1.6 ± 0.2[2,3]	2.7 ± 0.0[3]

[1] Based on two replicates as one replicate did not seem to have produced viable seed.

[2] Based on extrapolated 200-seed weights

[3] Based on extrapolated 100-seed weights

Supplementary Table 45 | **Seed germination rates of harvested *C. quinoa* (accession QQ-74 and cv. Titicaca) grown in the LED-supplemented glasshouse setup at JIC.** Germination percentage of 30 seeds of quinoa (*Chenopodium quinoa* accession QQ-74 and cv. Titicaca) grown under 22 and 16-hour photoperiods in LED-Supplemented glasshouses at John Innes Centre, UK (sown on 9 February 2018). Early harvest was carried out at the fruit development grain stage (80 and 87 days post-anthesis in 22 and 16 h rooms, respectively). Seed was later harvested at physiological maturity once at least half of the inflorescence had senesced (108-119 and 126 days post-anthesis in 22h and 16h rooms, respectively). Inflorescences were dried at 30°C for 3-5 days prior to weighing. Values are expressed as mean ± SD, based on three replicates.

	C. quinoa			
	accession QQ-74		**cv. Titicaca**	
	22 h	**16 h**	**22 h**	**16 h**
Early harvest (germination %)	98.8 ± 1.9	100.0 ± 0.0	82.2 ± 1.9	80.0 ± 6.2
Mature harvest (germination %)	100.0 ± 0.0	99.0 ± 0.6	100.0 ± 0.0	95.0 ± 0.6

Supplementary Table 46 | **Growth rate of *Avena Strigosa* (accession S75) grown in the LED-supplemented glasshouse setup at JIC.** Days to key growth stages, and measurement of key growth and development parameters for oat (*Avena strigosa* accession S75) grown under 22 and 16-hour photoperiods in LED-Supplemented glasshouses at John Innes Centre, UK. Seeds were sown on 14 November 2017 directly into 100 mL of JIC Peat and Sand Mix and seedlings were transferred to 1 L pots of JIC Cereal Compost Mix 23 days later. Values indicated are mean ± standard deviation based on seven replicates.

	22 h	**16 h**
Days to 2nd leaf emergence[1]	9.7 ± 0.8	15.0 ± 0.0
Days to flowering	52.0 ± 0.0	66.0 ± 0.0
Days to harvest	100.0 ± 0.0	114.0 ± 0.0
No. of tillers	12.6 ± 1.1	8.6 ± 1.4
Total seed weight (g)	8.9 ± 1.1	13.9 ± 3.1

[1] Days counted from sowing date.

[2] All plants were kept under water stress 14 days before harvesting.

[3] All seeds were dried at 25°C in the oven for 15 days.

Supplementary Table 47 | **Characteristics of mature plants of *Avena Strigosa* (accession S75) grown in the LED-supplemented glasshouse setup at JIC.** Plant height measured at different time points as an indicator of growth progress for oat (*Avena Strigosa* accession S75) grown under 22 and 16-hour photoperiods in LED-supplemented glasshouses at John Innes Centre, UK. Seeds were sown on 14 November 2017 directly into 100 mL of JIC Peat and Sand Mix
and seedlings were transferred to 1 L pots of JIC Cereal Compost Mix 23 days later. Values indicated are mean ± standard deviation based on seven replicates.

Plant Height	**22 h**	**16 h**
Plant Height: Day 8[1]	11.0 ± 1.0	5.0 ± 0.7
Plant Height: Day 10	17.1 ± 0.9	10.8 ± 0.9
Plant Height: Day 15	30.6 ± 0.9	17.5 ± 0.9
Plant Height: Day 22	46.6 ± 1.8	35.4 ± 2.0
Plant Height: Day 25	48.1 ± 2.3	38.4 ± 1.9
Plant Height: Day 30	56.0 ± 1.8	48.8 ± 1.5
Plant Height: Day 36	79.23 ± 4.8	60.0 ± 2.7
Plant Height: Day 52	127.4 ± 15.4	116.7 ± 10.6

[1] Days counted from sowing date.

Supplementary Table 48 | **Seed germination rates of harvested *Avena Strigosa* (accession S75) grown in the LED-supplemented glasshouse setup at JIC.** Seed viability demonstrated through germination percentages of 30 seeds harvested at physiological maturity from oat (*Avena Strigosa* accession S75) grown under 22 and 16-hour photoperiods in LED-Supplemented glasshouses at John Innes Centre, UK. Plants were not watered for 2 weeks prior to harvesting seed. Values indicated are mean ± standard deviation based on 3 replicates of 30 seeds under each photoperiod condition.

***A. strigosa* accession S75**	**22 h**	**16 h**
Germination percentage (%)	100.0 ± 0.0	100.0 ± 0.0

Supplementary Table 49 | **FP Media composition**

	Stock solution	**Volume to add for 1L of solution**
Calcium Chloride ($CaCl_2.2H_2O$)	40g/L	2.5mL
Magnesium Sulphate ($MgS0_4.7H20$)	40g/L	3.0mL
Potassium phosphate monobasic (KH_2PO_4)	30g/L	3.33mL
Di-sodium hydrogen phosphate dodecahydrate ($Na_2HPO_4.12H_2O$)	45g/L	3.33mL
Ferric Citrate	2.5g/L	2.0mL
Gibson's Trace *In 500 mL of distilled water, add:* *1.43g Boric acid (H_3BO_3),* *1.015g of Manganese Sulphate ($MnSO_4.4H_2O$),* *0.11g of Zinc Sulphate ($ZnSO_4.7H_2O$),* *0.04g of Copper Sulphate ($CuSO_4.5H_2O$), 0.04g of Molybdic acid (H_2MoO_4)*	-	1.0mL