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

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'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 (LD) 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 that promote the rapid growth of bread wheat, durum wheat, barley, oat, various *Brassica* species, chickpea, pea, grass pea, 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 (SSD). In addition, 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.

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 SB that involves extending the photoperiod using supplementary lighting and temperature control, enabling rapid generation advancement in glasshouses with sodium vapor lamps (SVL) or growth chambers fitted with a mixture of metal halide and light-emitting diode (LED) lighting[1]. By adopting a 22-h photoperiod and a controlled temperature regime, generation times were substantially 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, *B. distachyon*, and the model legume, *Medicago truncatula*, in comparison to those of plants grown in a 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 that adult plant phenotyping for traits such as flowering time, plant height and disease resistance in wheat; leaf sheath glaucousness in barley; and pod shattering in canola could be performed under SB conditions[1].

The use of an extended photoperiod to hasten plant growth is not novel. Sysoeva et al.[2] provide an extensive review of the literature surrounding this subject published within the past 90 years, which

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Box 1 | Speed breeding setup

This box provides information about setting up SB in an existing plant growth chamber or CER. Here, we outline the core 'recipe' for programming 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 that reasonably covers the 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 be taken before commencement of the SB experiment.

  In addition to controlling the light quality, we recommend a PPFD of ~450–500 μmol/m^2/s 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 μmol/m^2/s has been demonstrated to be effective for a range of crop species[1].
- *Photoperiod*. We recommend a photoperiod of 22 h with 2 h of darkness in a 24-h 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 also provided an example in which an 18-h 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, whereas a fall in temperature during the dark period can aid in stress recovery. At UQ, a 12-h 22 °C/17 °C temperature cycling regime with 2 h of darkness occurring within 12 h of 17 °C has proven successful (SB II)[1]. By contrast, a temperature cycling regime of 22 °C/17 °C for 22 h of light and 2 h of dark, respectively, is used at JIC (SB 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.

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*), fava bean (*Vicia faba*), lupin (*Lupinus angustifolius*)[5] and clover (*Trifolium subterraneum*)[6].

Here, we provide a standardized 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 SSD, to generate large populations. Because 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 that is suitable for small-scale research projects and trialing 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 (Box 1) and in temperature-controlled glasshouses using supplementary LED lighting, which provides significant cost savings over traditional SVLs (Equipment). The procedures have been tested in the United Kingdom 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). We provide supporting data to demonstrate the suitability of these setups (Anticipated results) to substantially 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*), grass pea (*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 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 3 years to complete. Although only one or two seeds are needed from each plant to begin the next generation, plant researchers and breeders seek to maximize 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 (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 programs').

Development of the approach

The SB concept was inspired by NASA's efforts to grow crops in space, using an enclosed chamber and an extended photoperiod[8]. In recognizing 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 Ian Delacy and 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 approach 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 2-h 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 and genetic structuring, as well as for molecular studies such as those on 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 for increasing generation turnover is 'shuttle breeding', introduced by 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 the 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 use of intense light. Such a method is well established and documented for rice[18] and has also been demonstrated for pea (Supplementary Fig. 1) and canola[19]. Embryo rescue—in which immature seed is harvested and induced to germinate on culture medium, with or without the addition of plant growth regulators (PGRs), to negate the waiting time for the seed to mature—is another common feature in many rapid-cycling methods. Bermejo et al.[20] used PGRs in embryo culture media to promote germination of immature lentil seed to achieve four generations annually. Mobini et al.[21] sprayed lentil and fava bean plants with PGRs to promote early flowering and applied embryo rescue with PGR-enriched agar media to achieve up to 8 and 6.8 generations per year, respectively. Castello et al.[22] reported three to four generations per year in subterranean clover (*T. subterraneum*), also with PGRs in the culture medium. Application of PGRs is not required for SB,

which may be desirable, considering the additional time and effort required for handling these and working out the logistics of their application at specific times. In addition, if a species-specific protocol is not available, extensive testing would be needed to optimize such applications. There are also examples of the use of embryo rescue without PGR to shorten generation time. Zheng et al.[23] and Yao et al.[24] reported up to eight generations per year for wheat, and Zheng et al.[23] reported up to nine generations per year for barley. Ochatt et al.[25] and Mobini and Warkentin[5] reported up to 6.9 and 5.3 generations of pea per year, respectively, and Roumet and Morin[26] reported five cycles per year in soybean (*Glycine max* L.), all with embryo rescue without PGRs. Other methods of reducing generation time have involved combining embryo rescue with other techniques. In addition to hastening flowering through stress, Liu et al.[27] used embryo rescue to achieve shorter generation times in oat (*Avena sativa*) and triticale (*Triticosecale*) and Ribalta et al.[28] in pea. Yao et al.[19] reported seven generations per year in canola when combining stress and embryo rescue. Ribalta et al.[29] used the PGR flurprimidol to reduce plant growth and induce early maturation in pea, followed by embryo rescue to achieve more than five generations per year. Without embryo rescue, SB conditions are capable of producing six generations per year for spring wheat, barley, chickpea and pea, and four generations per year for canola[1]. Testing is needed for any plant species before implementation, but this approach is promising for other cereal, pulse and legume crops. Seed of wheat and barley produced under SB conditions can be harvested prematurely at 2 weeks post anthesis, followed by a short period of drying and chilling to achieve high and uniform germination rates and healthy plants[1]. Approaches involving embryo rescue are important and useful for breeding and research programs if the required infrastructure is available[30], particularly for species that are recalcitrant to other parameters used to accelerate generation advancement, such as temperature or photoperiod manipulation[31–33]. In comparison, the SB approach outlined here is less labor intensive, especially with large populations, and laboratory facilities are not required, making the procedures more accessible.

Plant growth can also be promoted by increasing the CO_2 concentration. For example, for C_3 plants such as 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, in which haploid (n) embryos are rescued and undergo chromosome doubling ($2n$), 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, it requires specialist skills, it restricts recombination to a single round of meiosis, and it has a variable success rate that may be genotype dependant[36]. The approach can also be labor 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 that uses 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 trialed for the SB photoperiod (22 h/2 h (light/dark)) and temperature (22 °C/17 °C) regime, and successfully reproduced the accelerated development of one rapid-cycling variety each of wheat and pea (Supplementary Tables 1 and 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 LD plants, time to flowering is often accelerated under extended photoperiods because the critical daylength is generally exceeded. This is also the case with day-neutral plants, in which flowering will occur regardless of the photoperiod. By contrast, short-day (SD) plants require the photoperiod to be less than the critical day length to flower[39], which could be at odds with SB conditions. However, there are exceptions, and some species show a facultative response in which, although flowering is promoted by a particular photoperiod, flowering will still occur in the opposite photoperiod. Furthermore, the time difference between being classified as an SD or an LD plant can be a matter of minutes[40]. These

factors highlight both a limitation of SB and a point of flexibility. In cases in which the photoperiod response is unknown or complex in nature, experimentation with light and temperature parameters is required to optimize an SB strategy, for example, by using the benchtop growth cabinet. For instance, applying extended light before 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.), an SD species, in which a 16-h LD photoperiod was used to initiate strong vegetative growth, after which plants were transferred to an 8-h SD photoperiod to induce flowering[41]. The overall effect was a shorter lifecycle and the ability to produce eight generations per year rather than two in the field. The need for vernalization, such as in winter wheat, creates a situation similar to the above. Young plants require chilling for a number of weeks to trigger the transition to flowering. Once the vernalization 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 straightforward and easier to implement for LD and day-neutral species that do not require vernalization. Experimentation and optimization 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. Although 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, in which dormant and nondormant genotypes must be differentiated, phenotyping of 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.[18] 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 1 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 is needed to minimize operating costs. Research activities often do not require the high plant numbers needed in breeding, so growth chambers are common. The cost of these starts at tens of thousands of dollars, making them inaccessible for many projects and a barrier for implementing SB. In addition, the energy needed to provide extended supplementary lighting is significant. A cost–benefit analysis should be carried out to determine feasibility, although there are areas in which cost savings can be made. Supplemental LED lighting provides more efficient power usage and reduced heat as compared with 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 et al.[1]. Investing in solar panels is another strategy to offset the increased energy costs, depending on availability and location.

The investment in SB must 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 into a crop improvement program needs careful consideration and may require significant re-design or restructure to the overall program. Before 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 the following.

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, the plant species and energy resources. For example, LED lighting may be preferred, owing 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 responses 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 by no means represents the final conditions that may be optimal for another situation. The procedures outlined here have been successful for the species trialed, 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/red ratios, red/far-red ratios and the proportional level of UV light that can 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, a cultivar of that species and the desired phenotype, should be determined before 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 (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 in 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 photoperiods. Care should therefore be exercised in selection of the germplasm to be grown under SB, and appropriate modifications should 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 setup 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 must be determined. Conversely, growth of 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 an 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

Biological materials
- 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.

Reagents
Soil ▲CRITICAL Soil mixtures should be chosen based on the crop of interest. Soil mixtures that have previously been shown to work for certain crops in SB conditions are provided in Table 1.
- UQ Compost Mix (designed by K. Hayes; Central Glasshouse Services, UQ; composition outlined in Supplementary Table 4)
- JIC Cereal Compost Mix (prepared by Horticulture Services at the JIC; composition outlined in Supplementary Table 5)
- JIC Peat and Sand Mix (prepared by Horticulture Services at the JIC; composition outlined in Supplementary Table 6)

Table 1 | Soil mixes that have been demonstrated to be compatible with speed breeding using our approach

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

For details on the soil media composition, see Supplementary Tables 4, 5, and 6.

Nutrients

- 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)

Equipment

Benchtop growth cabinet: hardware ▲ CRITICAL This section provides an overview of the equipment required for constructing a small benchtop cabinet for SB, which can 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 accommodates eight 1-L square pots. To construct your low-cost growth cabinet, the components listed below are required.

- 12-V, 50-A DC power supply, 600 W (Amazon, cat. no. B072M7P7QJ)
- 12–5 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)

Benchtop growth cabinet: cabinet structure

- Aluminum composite panel (757 × 307 × 3 mm, quantity = 6; Cut Plastics, cat. no. CP027-03)
- Aluminum composite panel (757 × 357 × 3 mm; Cut Plastics, cat. no. CP027-03)
- Aluminum composite panel (757 × 107 × 3 mm; Cut Plastics, cat. no. CP027-03)
- Aluminum composite panel (757 × 757 × 3 mm; Cut Plastics, cat. no. CP027-03)
- PVC foam board (757 × 157 × 3 mm, quantity = 2; Cut Plastics, cat. no. CP015-03)
- PVC foam board (757 × 141 × 3 mm; Cut Plastics, cat. no. CP015-03)
- PVC foam board (757 × 307 × 3 mm, quantity = 2; Cut Plastics, cat. no. CP015-03)
- Perspex clear acrylic sheet (757 × 307 × 3 mm; Cut Plastics, cat. no. CP001-03)
- OpenBeam (1,000 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)

Benchtop growth cabinet: 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, SeeedStudio (Mouser, cat. no. 713-103020004)

Benchtop growth cabinet: 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, SeeedStudio (quantity = 4; CPC, cat. no. MK00330)
- 12-V cooling fan (50 mm; Amazon, cat. no. B00HPKC5MO)
- Software: Arduino IDE (v.1.8.5, https://www.arduino.cc/en/Main/Software)

LED-supplemented glasshouse setup ▲ **CRITICAL** This section provides an overview 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 it with a temperature-control system and programmable lights. Controllable blinds are also an option, if blocking out high irradiance on very sunny days is required.
- LED lamps: Although 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 and UQ was supplied by the same company, Heliospectra. Details of both setups are provided, along with the results of experiments carried out at both locations. The lighting system configuration, and the make and model of the lights for both locations are provided in the 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 compositions are 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
- Photosynthetically active radiation (PAR) meter: The PAR is measured in either photosynthetic photon flux density (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 (AsenseTek, Taiwan) at JIC and UQ, respectively.
- Energy meter: This allows measurement of 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 used a clamp-on Current Transformer meter (Panoramic Power, Centrica Business Solutions) 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 that 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 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

fertilizer spread through the soil and a buildup of salts occurring in the stored compost, as the slow-release fertilizer 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 vigor and seed set. In the JIC glasshouses and growth chambers, we have successfully used Vitafeed Balanced 1-1-1 from Vitax for wheat growing in high-density trays. ▲ **CRITICAL** Owing to the rapid growth of plants under SB, fertilizer application and swift amelioration of nutrient deficiencies are of utmost importance. Appropriate slow-release fertilizer 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 fertilizer applications may be required for rapid access of nutrients to the leaves, although some level of calcium deficiency is common. See Supplementary Fig. 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 the Troubleshooting section 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 (SeeedStudio).

Benchtop growth cabinet: cabinet structure

Assemble the beam profile, using the joining plates. Position the panels, boards and sheets before fully assembling each side.

Benchtop growth cabinet: 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 must be drilled into one of the 757 × 307 × 3-mm aluminum composite panels, to fit the E27 lamp holders. The lamp holders are then inserted and wired in parallel.

Benchtop growth cabinet: 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, aluminum 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.

Benchtop growth cabinet: software installation and setup

The SB cabinet is controlled by three main subsystems: the Arduino microcontroller that monitors and controls the environment according to a desired optimum; 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-h range. The circuit diagram for making the connections is provided in Supplementary Fig. 3, and a photograph of the assembled cabinet is provided in Supplementary Fig. 4. The cabinet has an available area of 0.225 m^2. For the lamps we have used, the spectrum is provided in Supplementary Fig. 5, with the light levels in PPFD being on an average of ~120 $\mu mol/m^2/s$ at 16 cm above the base where the pots are kept, and ~320 $\mu mol/m^2/s$ and 220 $\mu mol/m^2/s$ 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

Table 2 | LED-supplemented glasshouse setups for speed breeding at JIC and UQ

		John Innes Centre (JIC), UK	University of Queensland (UQ), Australia
LED lamp make and model		LX602C LED Grow Lights from Heliospectra. More information can be found at https://www.heliospectra.com/led-grow-lights/elixia/	E602G LED Grow Lights from Heliospectra. More information can be found at https://www.heliospectra.com/led-grow-lights/eos/
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	Eight 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 Fig. 9	Supplementary Fig. 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
Lighting regime and PPFD levels		Two similar compartments within the same glasshouse were set up with two different photoperiod regimes: (i) 22 h of light, followed by 2 h of darkness (ii) 16 h of light, followed by 8 h of darkness. The PPFD values and spectrum at various distances from the lights are provided in Supplementary Table 14 and Supplementary Fig. 11	Photoperiod of 22 h, followed by 2 h of darkness. The PPFD values and spectrum at various distances from the lights are provided in Supplementary Table 15 and Supplementary Fig. 12
Temperature regime		20 °C is the maximum temperature to be operative during the photoperiod (16 or 22 h, depending on the photoperiod regime; see above). 15 °C is the minimum temperature to be operative during the dark period (8 or 2 h, depending on photoperiod regime; see above)	22 °C is the maximum temperature to be operative for 12 h during the photoperiod. 17 °C is the minimum temperature to be operative during the dark period (12 h)
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), which is a glasshouse-specific business management system	*Heating and cooling*: 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 is automatically controlled using a business management system running on an Innotech system using Magellan Builder. The temperatures are controlled to ±1 °C

fire if operated without being grounded. Ensure that 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 vary 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

Table 3 | Speed breeding approaches that have been demonstrated for different species, along with pointers for locating the associated data

Species	Demonstrated SB conditions and associated data		
	This protocol	Watson et al.[1]	Other
Spring wheat (*T. aestivum*)	JIC-GH-LED[a] (Supplementary Tables 16–24) UQ-GH-LED[b] (Supplementary Tables 10 and 11)	UQ-GH-SVL[c] (Supplementary Tables 11, 15, 21, 28, 30 and 31) CER-JIC[d] (Supplementary Tables 2, 5–8, 19, 27 and 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.[11]
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 and 38)	
Canola (*Brassica napus*)	JIC-GH-LED (Supplementary Tables 31–35)	UQ-GH-SVL (Supplementary Tables 13, 17, 23, 25, 30 and 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)	
Grass pea (*L. sativus*)	JIC-GH-LED (Supplementary Tables 38–40)		
Medicago truncatula		CER-JIC (Supplementary Table 9)	
Brachypodium distachyon	JIC-GH-LED (Supplementary Tables 41 and 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 and 30)	
Peanut (*A. hypogaea*)			O'Connor et al.[10]
Amaranth (*Amaranthus* spp.)			Stetter et al.[41]

[a]JIC-GH-LED: LED-supplemented glasshouse setup, JIC, UK (described in this protocol, see Equipment setup, LED-supplemented glasshouse).
[b]UQ-GH-LED: LED-supplemented glasshouse setup, UQ, Australia (described in this protocol, see Equipment setup, LED-supplemented glasshouse).
[c]UQ-GH-SVL: SVL-supplemented glasshouse setup, UQ, Australia (described in Box 1 as SB II[1]).
[d]CER-JIC: controlled environment room, JIC, UK (described in Box 1 as SB I[1]).

feedback, or use controllable blinds to regulate photoperiod. Provision of a short dark period is recommended for optimal plant health. We highly recommend setting up a temperature monitoring and control system.

Procedure

Preparation of seed for sowing

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

(A) **Germination with pretreatment to break seed dormancy** ● **Timing 5–7 d**

▲ **CRITICAL** The requirements for germination pretreatments are specific to each species, and accessions of that species, and should be determined on an individual basis.

(i) Place dormant seed on moistened filter paper in a Petri dish to imbibe for 24 h and then chill at 4 °C for ~3 d (longer times may be required, depending on the level of dormancy) in the dark. In a large-scale scenario, directly sow seeds in high-density trays and place the trays in a cold (~4–5 °C) room.

(ii) Leave the seeds at room temperature (~20–25 °C) for 1–3 d to germinate in the dark before transferring to soil. In the large-scale scenario, trays can now be moved to the growing environment in the glasshouse (see Troubleshooting section for tips on handling seed-germination issues).

? TROUBLESHOOTING

(iii) Grow the plants under the desired SB conditions (Box 1).

(B) **Germination without pretreatment to break seed dormancy ● Timing 3–5 d**

(i) If pretreatment 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 in soil in the glasshouse/growth chamber. Note that for some crop species, such as pea or grass pea, you must scarify the 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 transplant them into soil 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.

? TROUBLESHOOTING

(ii) Grow the plants under the desired SB conditions (Box 1).

Monitoring of key growth stages and growth parameters, and phenotyping ● Timing variable

▲ **CRITICAL** Timing depends on crop, cultivar/genotype and SB setup used. Refer to Table 3 for guidance timelines in the associated Supplementary Tables.

2 To enable comparison to normal development, monitor the key growth stages of the plants. For many crops, for example, cereal crops[44], canola[45], quinoa[46] and legumes[47], defined growth stages have been published. Take note of the heading times and earliest time point to harvest viable seeds. We also advise monitoring of 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 the Troubleshooting section.

Experiments performed using a LED-supplemented glasshouse setup at the JIC involved an 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).

? TROUBLESHOOTING

Harvesting of the seed ● Timing variable

▲ **CRITICAL** Timing depends on crop, cultivar/genotype and SB setup used. Refer to Table 3 for guidance timelines in the associated Supplementary Tables.

3 Shortened generation times can also be achieved in some species by harvesting premature seed. 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.

For experiments performed using the LED-supplemented glasshouse setup (at the JIC), 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.

? TROUBLESHOOTING

Monitoring of energy use ● Timing: a few hours

4 At the end of one cycle, review the energy costs for your SB system. This is particularly useful in evaluating the generation time versus cost trade-off when multiple conditions have been tested

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.[48] Hickey et al.[14]
Leaf rust (*Puccinia recondita* f. sp. *tritici*, 'brown rust') (*Puccinia triticina*, 'black rust')	Spring wheat (*T. aestivum*)	Pretorius et al.[48] Riaz et al.[14]
Yellow spot/tan spot (*Pyrenophora tritici-repentis*)	Spring wheat (*T. aestivum*)	Dinglasan et al.[12]
Leaf rust (*Puccinia hordei*) Net form net blotch (*Pyrenophora teres* f. sp. *teres*) Spot form net blotch (*Pyrenophora teres* f. sp. *maculata*) Spot blotch (*Bipolaris sorokiniana*)	Barley (*H. vulgare*)	Hickey et al.[15]
Stem rust (*Puccinia graminis* f. sp. *tritici*)	Spring wheat (*T. aestivum*)	Riaz and Hickey[49]
Crown rot (*Fusarium pseudograminearum*)	Durum wheat (*T. durum*)	Alahmad et al.[11]
Preharvest sprouting	Spring wheat (*T. aestivum*)	Hickey et al.[9]
Pod shattering	Canola (*B. napus*)	Watson et al.[1]

concurrently (e.g., different day lengths). For the LED-supplemented glasshouse setup at JIC, there were two rooms set up concurrently with 16- and 22-h 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 SVL.

Troubleshooting

Troubleshooting advice can be found in Table 5.

Table 5 | Troubleshooting table

Step	Problem	Possible reason	Solution
1A(ii), 1B(i), 3	Seeds do not germinate	Seeds were harvested too early and are not viable.	Harvest the seeds slightly later.
		Seeds are dormant	Store the seeds for a few additional days or weeks before trying again. Alternatively, cold-stratify the seeds at 4–5 °C for several days and/or treat with a low concentration (~0.5 p.p.m.) of gibberellic acid (GA_3) by dipping the seeds into the solution or spraying
2	Plants exhibit tip-burn necrosis. The leaves curl inward or outward, and may have small, circular depressions or 'bubbles' (Supplementary Fig. 2)	Calcium deficiency; this is common in accelerated growth	Apply a liquid fertilizer containing calcium as a foliar spray early in growth to control any developing deficiency. This may be a 1% (wt/vol) calcium nitrate solution applied two to three times per week or as part of another broad-spectrum fertilizer. Acidic soil can interfere with calcium uptake; adding dolomite to the soil can reduce acidity if the base soil mix tends toward a lower pH
	Initial curling and dying of young leaf tips, extending down the leaf blade. Young leaves may also not emerge properly and may form loops or twists. Later, spike tops can wither, turn white and fail to produce grain. Spikes may also become twisted into curls (Supplementary Fig. 13)	Copper deficiency; this is common in accelerated growth	Apply a liquid fertilizer containing copper as a foliar spray early in growth to control any developing deficiency. Alkaline or waterlogged soil can affect copper uptake; do not overwater or add excessive amounts of dolomite when ameliorating calcium deficiency as described above
	Young leaves appear striped, with interveinal yellowing (Supplementary Fig. 14)	Iron deficiency	Apply a liquid fertilizer containing iron as a foliar spray early in growth to control any developing deficiency

Table continued

Table 5 (continued)

Step	Problem	Possible reason	Solution
	Plants are weak and spindly or suffering from chlorosis	These are possible symptoms of a range of nutrient deficiencies	Apply a liquid fertilizer with a broad range of nutrients to the soil and as a foliar spray
	Plants did not cycle much faster than those in the glasshouse with no supplemental lights and/or those in field conditions, even though they are LD or day-neutral plants	The optimal 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 determine if it is a genotype- or species-specific issue
	LD or day-neutral plants do not flower	Vernalization is needed	Depending on the species, vernalize the plants for up to 8 weeks at 4–10 °C

Timing

Step 1A, germination with pretreatment: 5–7 d
Step 1B, germination without pretreatment: 3–5 d
Step 2, monitoring of key growth stages and growth parameters, and phenotyping: variable, depending on crop, cultivar/genotype and SB setup used; refer to Table 3 for guidance timelines in the associated Supplementary Tables
Step 3, harvesting of the seed: variable, depending on crop, cultivar/genotype, and SB setup used; refer to Table 3 for guidance timelines in the associated Supplementary Tables
Step 4, monitoring of energy use: a few hours

Anticipated results

As demonstrated in our previous study, under SB conditions with a 22-h 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 the 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 benchtop cabinet

The self-made, benchtop SB cabinet will facilitate identification of conditions that enable rapid cycling of wheat and pea, and by extension, the other crops listed (Supplementary Fig. 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 and 2). These data are comparable to data from our previous study[1] in which 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) 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-h 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 (Fig. 1), with minor reductions in seed set (refer to Table 3 to view the related data for the crop of interest) and spike size (Supplementary Fig. 6) or pod size (Supplementary Figs. 7 and 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

Table 6 | Mean days to anthesis under speed breeding using LED-supplemented glasshouses at JIC, UK

Species	Associated data	Photoperiod	Mean days to flowering[a]
Spring wheat (*T. aestivum*)	Supplementary Tables 10, 11 and 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[b]
		16 h	53.7 ± 1.0[b]
Spring barley (*H. vulgare*)	Supplementary Tables 12, 13 and 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[c]
		16 h	45.0 ± 0.0
Brassica rapa	Supplementary Tables 31–35	22 h	36.5 ± 2.5[c]
		16 h	41.0 ± 3.7
Brassica oleracea	Supplementary Tables 31–35	22 h	49.2 ± 1.8[c]
		16 h	61.2 ± 2.3
Pea (*P. sativum*)	Supplementary Tables 36 and 37	22 h	32.2 ± 5.3[d]
		16 h	42.9 ± 5.3
Grass pea (*L. sativus*)	Supplementary Tables 38–40	22 h	31[c] ±
		16 h	ND
Brachypodium distachyon	Supplementary Tables 41 and 42	22 h	31.5 ± 5.2
		16 h	44.0 ± 5.2
Quinoa (*C. quinoa*)	Supplementary Tables 43–45	22 h	54.6[e] ± 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

All plants had a temperature cycle regime of 22 h at 22 °C and 2 h at 17 °C to coincide with the light and dark period, respectively.
[a]Days to flowering/anthesis (GS65, Zadoks scale) from sowing[44].
[b]Days to 50% ear emergence from sowing (GS55, Zadoks scale).
[c]Days to first flower opening from sowing.
[d]Days to the first flower bud from sowing.
[e]Days to anthesis (growth stage 6 according to BBCH scale[46]).
ND, not determined.

surface areas. Occasionally, micronutrient deficiencies manifest themselves because of the rapid growth and change in soil pH; some of these issues (in particular for wheat and barley) are highlighted in the Troubleshooting section. Despite efforts to optimize 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 (heat or drought stress) resilience 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 programs

In breeding programs, SSD is often an important step in cultivar development that requires high-density plantings. The SB approach provided for glasshouses is 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 can be expected at various sowing densities in SB. Under the UQ-GH-LED (LED-supplemented glasshouse setup, UQ, Australia (Equipment setup, 'LED-supplemented glasshouse')) approach, at a density of 1,000 plants/m^2, up to six 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, owing to the greater competition and low soil volume. Despite this, even at the highest

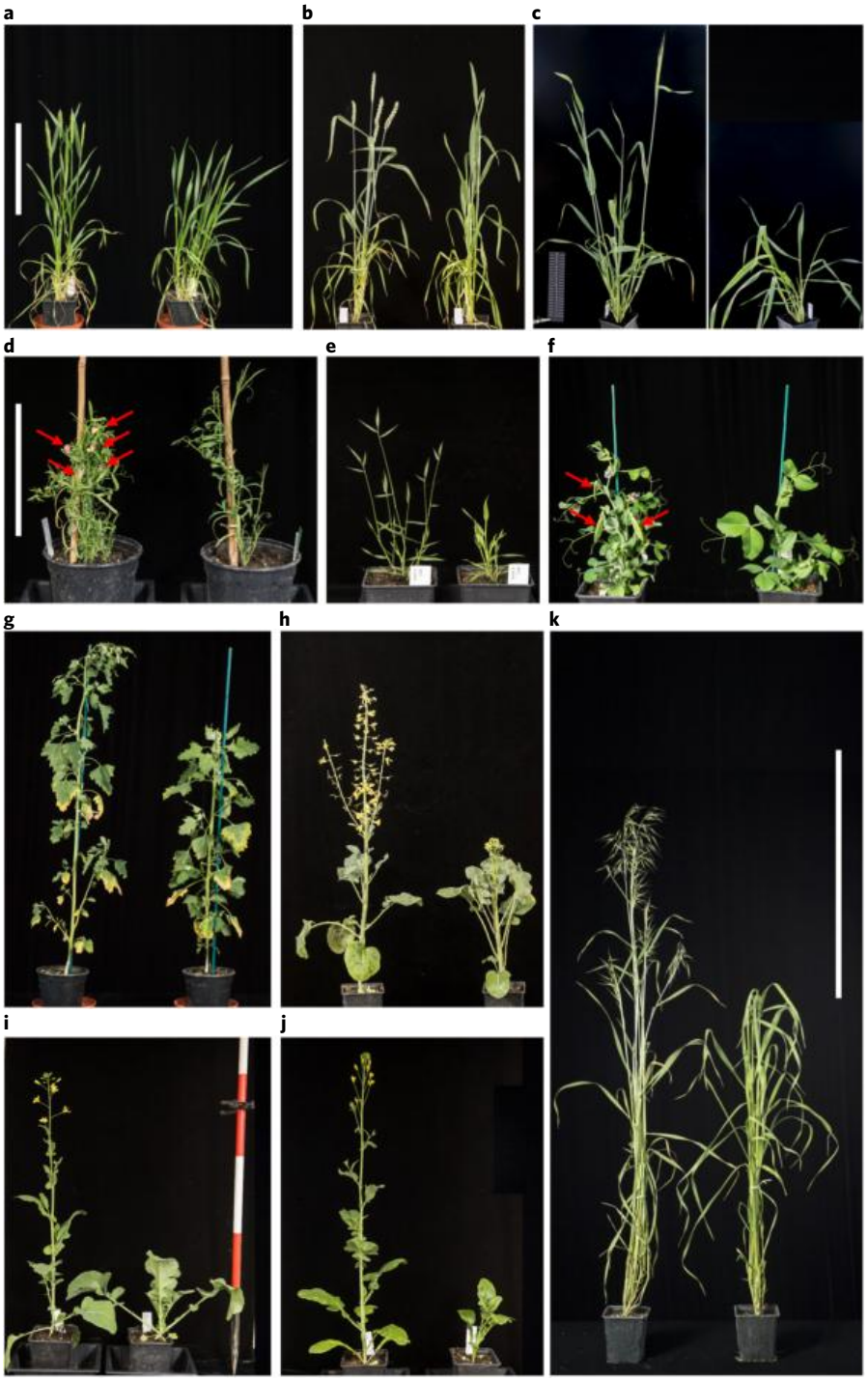

Fig. 1 | Accelerated plant growth and development under speed breeding compared to standard long-day conditions. a–k, Plants on the left are grown under SB (22-h photoperiod conditions), and plants on the right are grown under standard long-day (16-h photoperiod) conditions in LED-supplemented glasshouses at the John Innes Centre, UK. **a**, Winter-growth-habit wheat (*T. aestivum* cv. Crusoe) at 112 d after sowing (DAS), including 12 d of growth under 16-h photoperiod conditions followed by 56 d of vernalization at 6 °C with an 8-h photoperiod; **b**, spring wheat (*T. aestivum* cv. Cadenza) at 57 DAS; **c**, spring barley (*H. vulgare* cv. Manchuria) at 35 DAS (scale bar, 20 cm; applies to **a**–**c**); **d**, grass pea (*L. sativus* cv. Mahateora) at 35 DAS (red arrows indicate positions of flowers); **e**, *B. distachyon* (accession Bd21) at 34 DAS; **f**, pea (*P. sativum* accession JI 2822) at 34 DAS (red arrows indicate pea pods) (scale bar, 20 cm; applies to **d**–**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 (scale bar, 60 cm; applies to **g**–**k**). All plants were sown in October or November 2017, except for the quinoa, which was sown in February 2018.

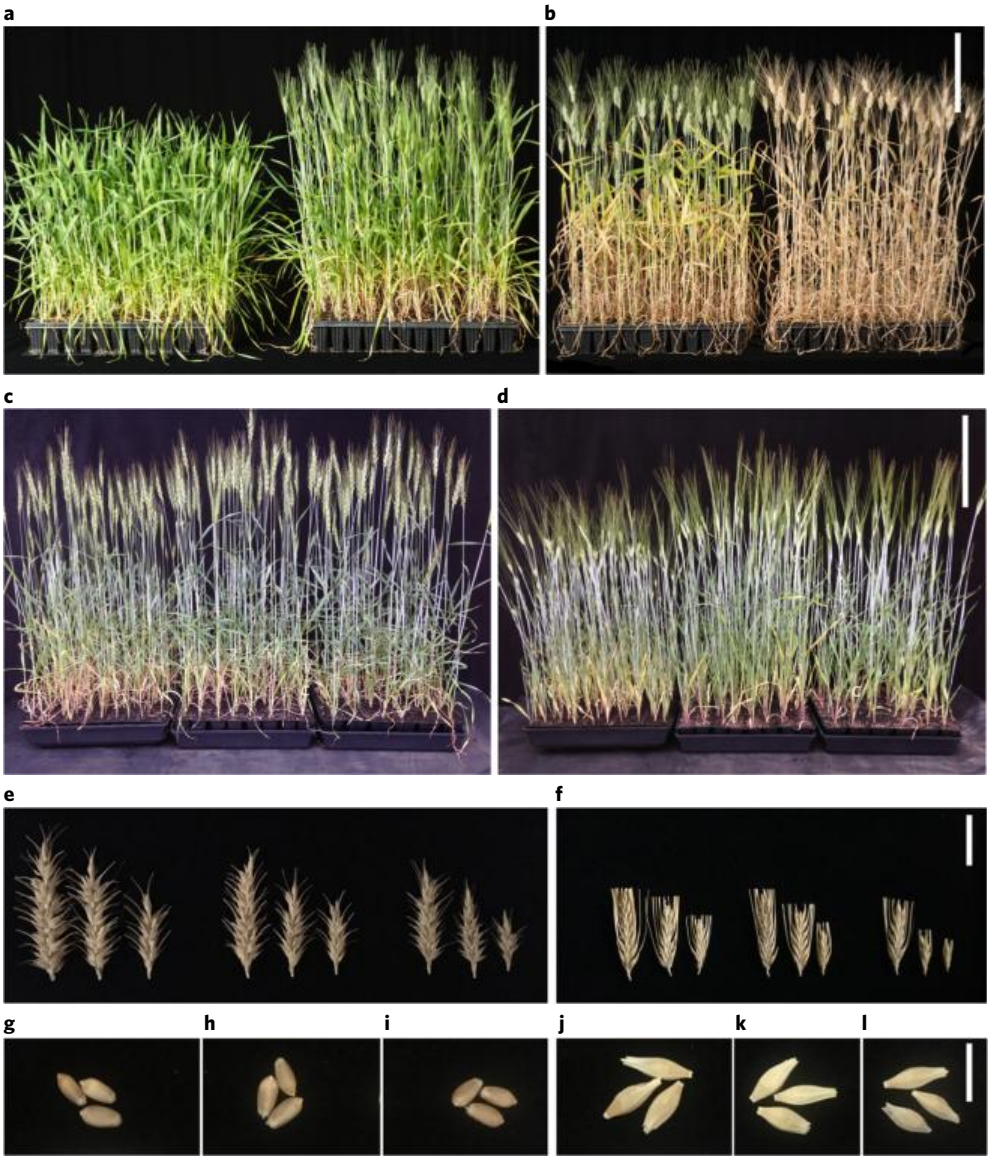

Fig. 2 | Single-seed descent sowing densities of spring wheat (bread and durum) and barley. All plants were grown under an LED-supplemented glasshouse setup at the JIC, UK, or the UQ, Australia. **a**,**b**, Durum wheat (*T. durum* cv. Kronos) grown under the LED-supplemented glasshouse setup, JIC, in 96-cell trays: 43 d after sowing, under a 16-h photoperiod (**a**, left); 43 d after sowing, under 22-h photoperiod (**a**, right); 79 d under a 16-h photoperiod (**b**, left); 79 d under a 22-h photoperiod (**b**, right). Scale bar, 20 cm (applies to **a**,**b**). **c**, Spring wheat (*T. aestivum* cv. Suntop) grown under an LED-supplemented glasshouse setup, at the UQ, at 37 d after sowing: plants in a 30-cell tray (left); plants in a 64-cell tray (center); plants in a 100-cell tray (right). **d**, Barley (*H. vulgare* cv. Commander) grown under an LED-supplemented glasshouse setup, at the UQ, at 34 d after sowing: plants in a 30-cell tray (left); plants in a 64-cell tray (center); plants in a 100-cell tray (right). Scale bar, 20 cm (applies to **c**,**d**). **e**, Mature spikes of spring wheat (*T. aestivum* cv. Suntop) grown under LED-supplemented glasshouse setup, at the UQ: spikes from plants in a 30-cell tray (**e**, left); spikes from plants in a 64-cell tray (**e**, center); spikes from plants in a 100-cell tray (**e**, right). **f**, Mature spikes of barley (*H. vulgare* cv. Commander) grown under an LED-supplemented glasshouse setup, at the UQ: spikes from plants in a 30-cell tray (**f**, left); spikes from plants in a 64-cell tray (**f**, center); spikes from plants in a 100-cell tray (**f**, right). Scale bar, 3 cm (applies to **e**,**f**). **g**–**i**, Mature seeds of spring wheat (*T. aestivum* cv. Suntop) grown under an LED-supplemented glasshouse setup, at the UQ: seeds from plants in a 30-cell tray (**g**); seeds from plants in a 64-cell tray (**h**); seeds from plants in a 100-cell tray (**i**). **j**–**l**, Mature seeds of barley (*H. vulgare* cv. Commander) grown under an LED-supplemented glasshouse setup, at the UQ: seeds from plants in a 30-cell tray (**j**); seeds from plants in a 64-cell tray (**k**); seeds from plants in a 100-cell tray (**l**). Scale bar, 1 cm (applies to **g**–**l**).

sowing density shown here, all plants produced a spike with at least enough seeds 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 h. Under the JIC-GH-LED (LED-supplemented glasshouse

Table 7 | Mean days to reproductive stages of single-seed descent sowing densities under speed breeding using the JIC-GH-LED or UQ-GH-LED approach

Species	Approach	Sowing density	Photoperiod	Mean days to reproductive stage
Spring wheat (*T. aestivum*)	JIC-GH-LED	96-cell (560 plants/m^2)	22 h	45.0 ± 0.0[a]
		96-cell (560 plants/m^2)	16 h	58.0 ± 0.0[a]
	UQ-GH-LED	30-cell (300 plants/m^2)	22 h	31.3 ± 0.7[b]
		64-cell (640 plants/m^2)	22 h	30.0 ± 0.0[b]
		100-cell (1,000 plants/m^2)	22 h	31.0 ± 0.0[b]
Tetraploid wheat (*T. durum*)	JIC-GH-LED	96-cell (560 plants/m^2)	22 h	42.0 ± 0.0[a]
		96-cell (560 plants/m^2)	16 h	50.0 ± 0.0[a]
Spring barley (*H. vulgare*)	UQ-GH-LED	30-cell (300 plants/m^2)	22 h	27.3 ± 1.2[c]
		64-cell (640 plants/m^2)	22 h	24.7 ± 0.3[c]
		100-cell (1,000 plants/m^2)	22 h	24.0 ± 0.6[c]

JIC-GH-LED is the LED-supplemented glasshouse setup at JIC, UK (Equipment setup, 'LED-supplemented glasshouse'). It uses a temperature cycle regime of 22 h at 22 °C and 2 h at 17 °C to coincide with light and dark times, respectively. UQ-GH-LED is the LED-supplemented glasshouse setup at UQ, Australia (Equipment setup, 'LED-supplemented glasshouse'). It uses a temperature cycle regime of 12 h at 22 °C and 12 h at 17 °C.
[a]Days to 50% ear emergence from sowing (GS55, Zadoks scale)[44].
[b]Days to mid-anthesis (GS65, Zadoks scale) from sowing.
[c]Days to awn peep (GS49, Zadoks scale) from sowing.

setup, JIC, UK (Equipment setup, 'LED-supplemented glasshouse')) approach, spring and durum wheat were >10 d faster in development with an additional 6 h 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 or UQ. 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.

Reporting Summary

Further information on experimental design is available in the Nature Research Reporting Summary linked to this article.

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 customized SB growth cabinet is provided at the public GitHub link: https://github.com/PhenoTIPI/SpeedSeed3/wiki.

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Author contributions

S.G. and A.W. drafted the manuscript and oversaw many of the experiments. L.T.H. and B.B.H.W. contributed to the design of experiments and manuscript writing. A.W. designed and implemented the SSD approach for wheat and barley in the LED-supplemented glasshouse at UQ. S.G., O.E.G.-N., R.H.R.-G., L.Y. and M.M.-S. designed, constructed, programmed and tested the benchtop growth cabinet. J.C. performed the energy consumption calculations for the LED-supplemented glasshouse at JIC. For the LED-supplemented glasshouse at JIC, J.S. performed the experiments for wheat, including the SSDs; R.W. for brassicas; R.E.M. for oats; S.H. for additional wheat cultivars; P.G. for barley; T.R. for pea; A.H. for quinoa; A. Sarkar for grass pea; and A. Steed for *Brachypodium*. J.L., L.P., C.D., M.J.M., W.H., A.O., C.M., C.U., B.H., M.T., P.N., B.B.H.W. and L.T.H. contributed intellectually to the experiments and/or the writing of the manuscript. All authors reviewed and approved the final manuscript before submission.

Competing interests

The authors declare no competing interests.

Additional information

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Randomization	no randomization was performed. For SSD experiments, to prevent edge effects, only plants growing in the middle of the tray were used for measurements.
Blinding	Not relevant in this study.

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