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

Item Type	Article
Authors	Ghosh, Sreya; Watson, Amy; Gonzalez-Navarro, Oscar E.; Ramirez-Gonzalez, Ricardo H.; Yanes, Luis; Mendoza-Suárez, Marcela; Simmonds, James; Wells, Rachel; Rayner, Tracey; Green, Phon; Hafeez, Amber; Hayta, Sadiye; Melton, Rachel E.; Steed, Andrew; Sarkar, Abhimanyu; Carter, Jeremy; Perkins, Lionel; Lord, John; Tester, Mark A.; Osbourn, Anne; Moscou, Matthew J.; Nicholson, Paul; Harwood, Wendy; Martin, Cathie; Domoney, Claire; Uauy, Cristobal; Hazard, Brittany; Wulff, Brande B. H.; Hickey, Lee T.
Citation	Ghosh S, Watson A, Gonzalez-Navarro OE, Ramirez-Gonzalez RH, Yanes L, et al. (2018) Speed breeding in growth chambers and glasshouses for crop breeding and model plant research. Nature Protocols. Available: http://dx.doi.org/10.1038/s41596-018-0072-z.
Eprint version	Post-print
DOI	10.1038/s41596-018-0072-z
Publisher	Springer Nature
Journal	Nature Protocols
Rights	Archived with thanks to Nature Protocols
Download date	09/08/2022 08:59:46
Link to Item	http://hdl.handle.net/10754/629896

1 # Speed breeding in growth chambers and glasshouses for
2 # crop breeding and model plant research
3

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10 **1. Abstract**

11 To meet the challenge of feeding a growing population, breeders and scientists are continuously
12 looking for ways to increase genetic gain in crop breeding. One way this can be achieved is through
13 'speed breeding' (SB), which shortens the breeding cycle and accelerates research studies through
14 rapid generation advancement. The SB method can be carried out in a number of ways, one of which
15 involves extending the duration of a plant's daily exposure to light (photoperiod) combined with early
16 seed harvest in order to cycle quickly from seed to seed, thereby reducing the generation times for
17 some long-day (LD) or day-neutral crops. Here we present glasshouse and growth chamber-based SB
18 protocols with supporting data from experimentation with several crop species. These protocols
19 describe the growing conditions, including soil media composition, lighting, temperature and spacing,
20 which promote rapid growth of spring and winter bread wheat, durum wheat, barley, oat, various
21 members of the *Brassica* family, chickpea, pea, grasspea, quinoa and the model grass *Brachypodium*
22 *distachyon*. Points of flexibility within the protocols are highlighted, including how plant density can
23 be increased to efficiently scale-up plant numbers for single seed descent (SSD) purposes. Conversely,
24 instructions on how to perform SB on a small-scale by creating a benchtop SB growth cabinet that
25 enables optimization of parameters at a low cost are provided. We also outline the procedure for
26 harvesting and germinating premature wheat, barley and pea seed to reduce generation time. Finally,
27 we provide troubleshooting suggestions to avoid potential pitfalls.
28

29 **Key words:** Speed breeding, Rapid generation advancement, Photoperiod, Glasshouse, Growth
30 chamber, Growth cabinet, Wheat, Barley, Pea, *Brachypodium*, Quinoa, Oat, Brassica.

2. 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 protocols for 'speed breeding' (SB), which involve 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 protocol 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 protocol 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

2

66   observed that the protocols are flexible and can be tailored to fit a wide range of breeding or

67   research objectives and crop species. By sharing these protocols, we aim to provide a pathway for

68   accelerating crop research and breeding challenges.

69

70   **Overview of the procedure**

71   In this protocol, we describe how to implement SB in temperature-controlled glasshouses using

72   supplementary LED lighting, which provides significant cost savings over traditional SVLs. The

73   protocols have been tested in the UK and Australia, with lights from the same company, but with

74   slightly different models. We also outline compatible soil mixes for various crops when growing

75   them under these lighting regimes, along with advice for early harvest to reduce generation time

76   further. We provide supporting data to demonstrate the suitability of these setups to significantly

77   decrease the number of days to flowering and overall generation advancement for spring wheat,

78   barley, canola, chickpea, pea, *B. distachyon*, *M. truncatula,* oat (*Avena strigosa*), grasspea (*Lathyrus*

79   *sativus*) and quinoa (*Chenopodium quinoa*). We also include the design, step-by-step construction

80   protocol, and operation of a small growth cabinet, which allows control over the light quality,

81   intensity and photoperiod to help optimize the SB recipe for different crops and cultivars before

82   implementing a large-scale glasshouse experiment.

83

84   Crop breeding programs commonly use SSD for several generations, on large numbers of

85   segregating plants, to generate homozygous lines with fixed traits[7]. A glasshouse is often preferred

86   for SSD because plant populations can be grown year-round. This process involves both a large

87   investment in time as well as space within the glasshouse. Following the crossing of two

88   homozygous lines, six generations of self-pollination are required to produce progeny that are 98.4%

89   homozygous, which, at a rate of two generations per year, would take three years to complete.

90   While only one or two seeds are needed from each plant to begin the next generation, plant

91   researchers and breeders seek to maximise the number of plants within a restricted space. Plant

92   density can be scaled-up under SB to enable concurrent rapid cycling of large plant populations,

93   which is ideal for SSD programs. To demonstrate this, we evaluated spring wheat and barley sown at

94   different plant densities in a glasshouse fitted with LED supplementary lighting. By comparing the

95   physiological, morphological and yield parameters, we illustrate the normal development of these

96   plants and highlight how this SB approach can save time and resources for SSD programs.

97

98 **Development of the protocols**

99 The SB concept was inspired by the efforts of NASA to grow crops in space, using an enclosed

100 chamber and extended photoperiod[8]. In recognising the opportunity to more rapidly produce adult

101 wheat and barley plants and allow faster selection and population development, SB became the

102 norm in cereal research activities at the University of Queensland (UQ), Australia, thanks to Dr Ian

103 Delacy and Dr Mark Dieters. The original protocol was first described and implemented for wheat[9]

104 and peanut (*Arachis hypogaea*)[10]. Variations of this protocol have been demonstrated to be an

105 efficient system for rapid screening of wheat germplasm for adult plant resistance to various

106 diseases[11-14] and also for pyramiding multiple disease resistance in barley[15]. The protocol has also

107 been adapted for high-density plant production systems for SSD programs. The current SB protocol

108 described in this paper was developed from the initial implementation described for wheat to

109 include a two-hour dark period that improved plant health[1]. This change was made following

110 experiments in a controlled environment chamber at the John Innes Centre (JIC), UK, and was

111 demonstrated to be suitable for accelerating research activities involving adult plant phenotyping,

112 genetic structuring, and molecular studies like gene transformation in wheat and barley. It was

113 further demonstrated to be suitable for rapid generation advancement for durum wheat (*T. durum*),

114 pea, the model grass, *B. distachyon* and the model legume, *M. truncatula*, and could be scaled up in

115 the SB glasshouse system at UQ, to be made suitable for rapid generation advancement of wheat,

116 barley, canola and chickpea.

117

118 **Comparison with other approaches**

119 Perhaps the most well-known strategy to increase generation turnover is 'shuttle breeding',

120 introduced by Dr Norman Borlaug in the 1950s at the international Centre for Maize and Wheat

121 Improvement (CIMMYT), which enabled growing two generations per year by sowing wheat

122 populations at field locations differing in altitude, latitude, and climate in Mexico[16]. There is also a

123 long history of extensive efforts to accelerate plant growth of many species by manipulating

124 photoperiod under artificial conditions, as briefly outlined above.

125 Supplementary lighting is not the only basis for rapid generation advance in plants. A common

126 approach involves exerting physiological stress to trigger flowering and earlier setting of seed. This

127 involves restricting plant growth area (by growing plants at high densities) or nutrient and water

128 access[17], accompanied by thinning of the plant canopy. Such a method is well-established and

129 documented for rice[18] and has also been demonstrated for pea (Supplementary Figure 1). Embryo

130 rescue is another common feature in many rapid cycling methods where immature seed is harvested

131 and induced to germinate on culture media, with or without the addition of plant growth regulators

4

132 (PGR), to negate the waiting time for seed to mature. Bermejo et al. (2016)[19] used PGR in embryo

133 culture media to promote germination of immature lentil seed to achieve 4 generations annually.

134 Mobini et al. (2015)[20] sprayed lentil and faba bean plants with PGR to promote early flowering and

135 applied embryo rescue with PGR-enriched agar media to achieve up to 8 and 6.8 generations per

136 year, respectively. Application of PGR is not required for SB, which may be desirable considering the

137 additional time and effort required for handling these and working out the logistics of their

138 application at specific times. In addition, if a species-specific protocol is not available, extensive

139 testing would be needed to optimise such applications. There are also examples of embryo rescue

140 without PGR to shorten generation time. Zheng et al. (2013)[21] and Yao et al. (2017)[22] reported up to

141 8 generations per year for wheat and Zheng et al. (2013) [21] reported up to 9 generations per year for

142 barley. Both Ochatt et al. (2002)[23] and Mobini and Warkentin (2016)[5] reported up to 6.9 and 5.3

143 generations of pea per year respectively, and Roumet and Morin (1997)[24] reported 5 cycles per year

144 in soybean (*Glycine max* L.), all with embryo rescue without PGRs. On the other hand, SB conditions

145 without embryo rescue is capable of producing 6 generations per year for spring wheat, barley,

146 chickpea and pea, and 4 generations per year for canola[1]. Testing is needed for any plant species

147 prior to implementation, but this approach is promising for other cereal, pulse and legume crops.

148 Seed of wheat and barley produced under SB conditions can be harvested prematurely at two weeks

149 post-anthesis, followed by a short period of drying and chilling to achieve high and uniform

150 germination rates and healthy plants[1]. Protocols involving embryo rescue are important and useful

151 for breeding and research programs if the required infrastructure is available[25], particularly for

152 species that are recalcitrant to other parameters used to accelerate generation advancement such

153 as temperature or photoperiod manipulation[26-28]. In comparison, the SB protocols outlined here are

154 less labour intensive, especially with large populations, and laboratory facilities are not required,

155 making the protocols more accessible.

156

157 Plant growth can also be promoted by increasing the CO_2 concentration. For example, for C_3 plants

158 like rice and wheat, photosynthetic efficiency increases with increasing CO_2 levels, leading to an

159 increase in biomass and early flowering. In fact, there are documented methods for rapid generation

160 advance in rice that combine restricted root growth and canopy thinning with high CO_2

161 concentration, followed by early harvest and embryo rescue to cut down generation times of many

162 rice varieties[29].

163

164 Doubled haploid (DH) technology, where haploid (*n*) embryos are rescued and undergo chromosome

165 doubling (2*n*), is extensively and routinely used in the breeding of several crop species, thus reducing

166   the number of generations required to achieve homozygous lines from six or more to just two

167   generations[30]. Despite this, DH technology has some disadvantages: it can be expensive, requires

168   specialist skills, restricts recombination to a single round of meiosis, and has a variable success rate

169   that may be genotype-dependant[31]. The approach can also be labour intensive for large populations,

170   especially those requiring removal of the embryos from the seed coat. Notably, there is the potential

171   for SB to further accelerate the production of DH lines by speeding up the crossing, plant

172   regeneration and seed multiplication steps.

173

174   We have presented a design for building a low-cost benchtop growth cabinet to trial SB. Compared

175   to other published protocols for self-made growth chambers[32,33], our design makes use of a more

176   widely available control system using a Raspberry Pi and compatible sensors, with codes for the user

177   interface (UI) freely available from GitHub (https://github.com/PhenoTIPI/SpeedSeed3/wiki). The

178   cabinet was trialled for the 22-hour SB lighting, temperature and photoperiod regime (22 °C/17 °C

179   (22 hours/2 hours)), and successfully reproduced the accelerated development of one rapid-cycling

180   variety of each of wheat and pea (Supplementary Tables 1, 2). The component costs for constructing

181   such a cabinet are provided in Supplementary Table 3).

182

183   **Limitations of the approach**

184   Different plant species can have markedly different responses when exposed to extended

185   photoperiods. For long-day (LD) plants, time to flowering is often accelerated under extended

186   photoperiods since the critical day length is generally exceeded. This is also the case with day-

187   neutral plants, where flowering will occur regardless of the photoperiod. In contrast, short-day (SD)

188   plants require the photoperiod to be below the critical daylength to flower[34], which could be at odds

189   with SB conditions. However, there are exceptions and some species show a facultative response

190   where, although flowering is promoted by a particular photoperiod, flowering will still occur in the

191   opposite photoperiod. Furthermore, the time difference between being a SD or LD plant can be a

192   matter of minutes[35]. These factors highlight both a limitation of SB and a point of flexibility. In cases

193   where the photoperiod response is unknown or complex in nature, experimentation of light and

194   temperature parameters is required to optimise a SB strategy, for example, by using the benchtop

195   growth cabinet. For instance, applying extended light prior to and following a shortened

196   photoperiod to induce flowering, could hasten initial vegetative growth and accelerate maturity,

197   respectively, thus producing an overall shorter generation time. Such an approach has been

198   successfully applied to amaranth (*Amaranthus* spp. L), a SD species, where a 16-hour LD photoperiod

199   was used to initiate strong vegetative growth after which plants were transferred to an 8-hour SD

200   photoperiod to induce flowering[36]. The overall effect was a shorter lifecycle and ability to produce

201   eight generations per year rather than two in the field. The need for vernalisation, such as in winter

202   wheat, creates a situation similar to above. Young plants require chilling for a number of weeks to

203   trigger the transition to flowering. Once the vernalisation requirement is met in winter wheat,

204   exposing the plants to extended photoperiod is likely to accelerate growth[37,38]. Overall, the 'SB

205   recipe' is more straight forward and easier to implement for LD and day neutral species which do

206   not require vernalisation. Experimentation and optimisation of parameters are highly recommended

207   for each species.

208

209   The SB protocols presented here take place in an enclosed, artificial environment, which differs

210   significantly from the field where eventual crop production may occur. While this is acceptable for

211   many activities, such as crossing, SSD and screening for some simple traits[1], other activities, such as

212   selection for adaptation in the target environment must still occur in the field. Nevertheless,

213   programs alternating between SB and the field save time overall. The ability to shorten generation

214   time further through early harvest of immature seed can interfere with the phenotyping of some

215   seed traits. For this reason, in spring wheat breeding programs where dormant and non-dormant

216   genotypes need differentiating, phenotyping grain dormancy under SB conditions is limited to only

217   four generations per year[9].

218

219   The initial investment to build a glasshouse or purchase a growth chamber with appropriate

220   supplementary lighting and temperature control capabilities is substantial if these facilities are not

221   already available. However, depending on the budget of the research or breeding program, the

222   benefits may outweigh the costs. For instance, an economic analysis performed by Collard et al.

223   (2017)[39] compared the rapid generation advance (i.e., no phenotypic selection at each generation)

224   with the pedigree-based breeding method (i.e., with phenotypic selection at each generation) for

225   rice and determined that rapid generation (achieved through restricted soil access and canopy

226   thinning) was more cost-effective and advantages would be realized after one year even if new

227   facilities were constructed. Nevertheless, most breeding programs have pre-existing glasshouse

228   facilities that can be converted for SB applications, but careful selection of energy efficient lighting

229   and temperature control systems are needed to minimise operating costs. Research activities often

230   do not require the high plant numbers needed in breeding, so growth chambers are common. The

231   cost of these start at tens of thousands of dollars, making them inaccessible for many projects and a

232   barrier for implementing SB. In addition, the energy to provide extended supplementary lighting is

233   significant. A cost-benefit analysis should be carried out to determine feasibility although there are

234   areas where cost-savings can be made. Supplemental LED lighting provides more efficient power

235   usage and reduced heat than other lighting types, such as SVLs. An estimate of the maintenance and

236   energy costs associated with LED lighting is provided in the supplementary material of Watson and

237   Ghosh et al. (2018)[1]. Investing in solar panels is another strategy to offset the increased energy

238   costs, depending on availability and location.

239

240   The investment in SB needs to be weighed in terms of the potential benefits to variety development

241   and research output. As with most technologies, determining the optimal way to integrate SB in a

242   crop improvement program needs careful consideration and may require significant re-design or

243   restructure to the overall program. Prior to implementing such changes, computer simulations are a

244   good way to evaluate the different breeding programs incorporating SB.

245

246   **Experimental Design**

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

248

249   a)   **Lighting requirements:** Many lighting sources are appropriate for SB, including SVLs and LEDs[1].

250   Even incandescent lighting has been shown to accelerate flowering in clover[6]. However,

251   selection should be based on the space available, plant species and energy resources. For

252   example, LED lighting may be preferred due to its energy efficiency although simple

253   incandescent lighting may be suitable within a smaller area, with sufficient cooling to

254   counteract the higher heat output. Plant species may also differ in their response to the

255   different spectra of wavelengths emitted by different lighting sources so this should be carefully

256   considered. The lighting setup for glasshouses and growth chambers detailed in this protocol

257   can act as a starting point but is by no means the final conditions that may be optimum for

258   another situation. The protocols outlined here have been successful for the species trialled but

259   a modified approach may be more suitable for another crop. We recommend mining existing

260   literature and studies on suitable light spectra (particularly with regard to blue to red ratios, red

261   to far-red ratios, and the proportional level of UV light that may be introduced into the system)

262   for the crop and trait of interest.

263   b)   **Initial light calibrations:** Requirements in terms of light quality and intensity for a particular

264   species, cultivar of that species, and desired phenotype, should be determined prior to

265   application on a large scale or use within an experiment. Several 'dummy' or 'test' growth

266   cycles are recommended to initially assess the rate of growth and quality of the plants so that

267   alterations can be made to enable optimal outcomes. For this purpose, we recommend starting

268   with the benchtop growth cabinet option – the costs of which are low enough to build several

269   and trial, in parallel, different light-combinations, photoperiods and temperatures to determine

270   the optimal conditions to implement on a larger scale, such as a glasshouse, for your crop and

271   trait.

272 c) **Germplasm:** As detailed above, not all plant species (or indeed cultivars within a species) are

273   amenable to extended photoperiod. Care should therefore be exercised in selection of the

274   germplasm to be grown under SB and appropriate modifications implemented to ensure

275   optimal conditions for each species.

276 d) **End-use requirements:** The intended end-use of the resultant plants can affect all aspects of

277   the initial set-up of the SB protocol, such as glasshouse space and sowing density. For example,

278   within an SSD program large numbers of plants are grown within a defined space, so an

279   appropriate sowing density needs to be determined. Conversely, a small number of plants

280   needed for a research experiment under variable lighting parameters is more appropriate for a

281   small growth chamber experiment with flexible settings.

282 e) **Control conditions**: Before beginning a SB experiment, it is important to have replicates of your

283   germplasm growing under the conditions you would normally use in your breeding program or

284   institute. This will allow you to directly compare plant growth parameters (including generation

285   time), operational costs (e.g. electricity) and plant quality. For popular varieties grown for many

286   generations in the field or glasshouses, the control data may be readily available.

287

288 **3.   Materials**

289 **Reagents**

290   **a)   Soil**

291 Soil mixtures which have previously been shown to work for certain crops in SB conditions are

292 provided in Table 1. Details of the soil mixture composition can be found in Supplementary

293 Tables 4, 5 and 6.

294

295   **Table 1 | List of soil mixes that have been demonstrated to be compatible for speed**

296   **breeding using our protocols.**

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

297

298

299   **b)   Nutrient feed**

300 Depending on the size of the pots and the type of soil, the plants may need a nutrient feed.

301 If the pots are small (~100 ml), a single or fortnightly application of a liquid nutrient feed

302 should be considered to prevent the plant leaves from turning yellow prematurely with

303 concomitant reduced vigour and seed set. In the JIC glasshouses and growth chambers, we

304 have successfully used Solufeed 1-1-1 from Vitax

305 (http://www.vitaxgrower.co.uk/product/vitafeeds/) for wheat growing in high density trays.

10

306   Critical: Due to the rapid growth of plants under SB, fertiliser application and swift

307   amelioration of nutrient deficiencies are of utmost importance. Appropriate slow-release

308   fertiliser within the soil media is recommended for growth to maturity, and maintenance of

309   soil pH is important to avoid restriction of nutrient absorption; e.g. a pH that is too acidic can

310   inhibit calcium uptake. Foliar fertiliser applications may be required for rapid access of

311   nutrients to the leaves although some level of calcium deficiency is common. See

312   Supplementary Figure 2 for common symptoms of calcium deficiency. In our experience, for

313   wheat, barley and *Brachypodium*, symptoms are more common at early growth stages

314   during the period of prolific vegetative growth and are relieved at later growth stages. See

315   Troubleshooting (Section 6) for specific suggestions on calcium applications.

316

317   **Equipment**

318 The sections below describe the equipment needed for different SB purposes:

319 **Section a:** Provides information to set up SB in an existing plant growth chamber or controlled

320 environment room (CER). This section outlines the core "recipe" for programing an existing growth

321 room to set up SB conditions.

322 **Section b:** Provides details for the design and construction of a small benchtop cabinet for SB, which

323 may be used for small-scale pilot trials before investing in a larger system, such as a glasshouse. The

324 cabinet has a footprint of 0.225 m^2 and comfortably accomodates eight 1 L square pots.

325 **Section c:** Provides details for setting up SB in a glasshouse using LED lamps for supplementary

326 lighting. Its efficacy is demonstrated for a range of crop species, along with some examples of how

327 single-seed descent for wheat and barley can be carried out. The LED supplemental lighting within

328 glasshouses at JIC (UK) and UQ (Australia), were supplied by the same company, Heliospectra

329 (Göteborg, Sweden). Details of both setups are provided, along with the results of experiments

330 carried out at both locations.

331   **Section a) Speed breeding setup**

332 i)   ***Lights***: We have shown in our previous studies[1], that any light that produces a spectrum which

333   reasonably covers the photosynthetically active radiation (PAR) region (400-700 nm), with

334   particular focus on the blue, red and far-red ranges, is suitable to use for SB. The referenced

335   study has several examples of these spectra, and similar examples of possible SB spectra are

336   provided here. An appropriate spectral range can be achieved through LEDs, or a combination of

337   LEDs and other lighting sources (e.g. halogen lamps), or in the case of a glasshouse, by simply

338   supplementing the ambient lighting with LEDs or SVLs. We highly recommend that

339   measurements of the light spectrum are taken prior to commencement of the SB experiment.

11

340   In addition to controlling the light quality, we recommend a photosynthetic photon flux density

341   (PPFD) of approximately 450-500 µmol·m^{-2}·s^{-1} at plant canopy height. Slightly lower or higher

342   PPFD levels are also suitable. Crops species vary in their response to high irradiance. However,

343   the suggested level of 450-500 µmol·m^{-2}·s^{-1} has been demonstrated to be effective for a range of

344   crop species[1].

345   ii) ***Photoperiod***: We recommend a photoperiod of 22 hours with 2 hours of darkness in a 24-hour

346   diurnal cycle. Continuous light is another option, but our experience has shown that the dark

347   period slightly improves plant health. Gradually increasing light intensity to mimic dawn and dusk

348   states should be done, if possible, but is not vital. In our previous paper, we have also provided

349   an example where an 18-hour photoperiod was sufficient to achieve faster generation times for

350   wheat, barley, oat and triticale[1].

351   iii) ***Temperature***: The optimal temperature regime (maximum and minimum temperatures) should

352   be applied for each crop. A higher temperature should be maintained during the photoperiod,

353   while a fall in temperature during the dark period can aid in stress recovery. At UQ, a 12 hour 22

354   °C / 17 °C temperature cycling regime with the 2 hours of darkness occurring within the 12 hours

355   of 17 °C has proven successful (Speed breeding II)[1]. In contrast, a temperature cycling regime of

356   22 °C / 17 °C for 22 hours light and 2 hours dark, respectively, is used at JIC (Speed breeding I)[1]. In

357   both scenarios, the generation times of all crops were successfully accelerated and comparable.

358   In the controlled environment chamber in which this was demonstrated, the temperature was

359   ramped up and down similarly to the lights, but this was subsequently found to not be of

360   particular importance.

361   *iv) **Humidity***: Most controlled environment chambers have limited control over humidity but a

362   reasonable range of 60-70% is ideal. For crops that are more adapted to drier conditions, a lower

363   humidity level may be advisable.

364

365 **Section b) Benchtop growth cabinet**

366 To construct your low cost growth cabinet the following components are required.

367 **Hardware**

368 • 12 V, 50 A DC power supply 600 W (Amazon, cat. no. B072M7P7QJ)

369 • 12 V to 5 V, 3 A DC/DC converter module (Amazon, cat. no. B00G890MIC)

370 • USB extension cable – 30 cm (Amazon, cat. no. B002M8RVKA)

371 • Ethernet extension cable – 30 cm (Amazon, cat. no. B077V421QH)

372 • Arduino UNO (Amazon, cat. no. B00CGU1VOG)

373 • Raspberry Pi 3 model B (CPC, cat. no. 2525225)

374 • Raspberry Pi display 7 inch touchscreen (CPC, cat. no. 2473872)

375 • Arduino base shield v2 – SeeedStudio (CPC, cat. no. SC13822)

376 **Cabinet structure**

377 • Aluminium composite panel, 757 X 307 X 3 mm, quantity = 6 (Cut Plastics, cat. no. CP027-03)

378 • Aluminium composite panel, 757 X 357 X 3 mm (Cut Plastics, cat. no. CP027-03)

379 • Aluminium composite panel, 757 X 107 X 3 mm (Cut Plastics, cat. no. CP027-03)

380 • Aluminium composite panel, 757 X 757 X 3 mm (Cut Plastics, cat. no. CP027-03)

381 • PVC foam board, 757 X 157 X 3 mm, quantity = 2 (Cut Plastics, cat. no. CP015-03)

382 • PVC foam board, 757 X 141 X 3 mm (Cut Plastics, cat. no. CP015-03)

383 • PVC foam board, 757 X 307 X 3 mm, quantity = 2 (Cut Plastics, cat. no. CP015-03)

384 • Perspex clear acrylic sheet, 757 X 307 X 3 mm (Cut Plastics, cat. no. CP001-03)

385 • OpenBeam, 1000 mm, quantity = 4 (Technobots Online, cat. no. 4451-900)

386 • OpenBeam, 750 mm, quantity = 13 (Technobots Online, cat. no. 4451-750)

387 • OpenBeam, 300 mm, quantity = 10 (Technobots Online, cat. no. 4451-300)

388 • Corner bracket – MakerBeam, quantity = 4 (Technobots Online, cat. no. 4446-013)

389 • L-joining plate – OpenBeam, quantity = 36 (Technobots Online, cat. no. 4450-003)

390 • T-joining plate – OpenBeam, quantity = 2 (Technobots Online, cat. no. 4450-004)

391 **Lighting system**

392 • Full spectrum grow light LED bulb, quantity = 16 (Amazon, cat. no. 071J3BC1W)

393 • E27 lamp holder, quantity = 16 (Sinolec Components, cat. no. E27-SD04-2)

394 • Solid state relay – grove SeeedStudio (Mouser, cat. no. 713-103020004)

395 **Temperature and humidity control system**

396 • 12 V, 10 A thermoelectric cooler, quantity = 3 (Amazon, cat. no. B01M2ZBBVM)

397 • Temperature and humidity sensor pro–grove SeeedStudio (CPC, cat. no. MK00343)

398 • Relay – grove SeeedStudio, quantity = 4 (CPC, cat. no. MK00330)

399   • 12 V cooling fan, 50 mm (Amazon, cat. no. B00HPKC5MO)

400   Software

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

402

403 **Section c) LED-supplemented glasshouse setup**

404

405   i. **Glasshouse**: A well-located glasshouse with the required space and sufficient ambient

406   lighting. We recommend fitting a temperature control system and programmable lights.

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

408   required.

409   ii. **LED lamps**: While any kind of lighting system can be used to supplement the ambient

410   lighting in the glasshouse, we recommend LED lamps above all because of the significant

411   savings these provide in terms of maintenance and energy consumption. The glasshouse-

412   based SB experiments detailed in our previous paper[1] were based on SVLs, but we have

413   obtained similar results with LED-lighting at both UQ and JIC. The lighting system

414   configuration, make and model of the lights for both locations are provided in Equipment

415   setup.

416 iii. **SSD trays**: For demonstration, at UQ, three seedling tray types with increasing sowing

417   densities were used. The dimensions and volumes are given in Supplementary Table 7. The

418   soil media composition is given in Supplementary Table 4.

419

420   Caution: Energy tariffs can vary according to the time of day, depending on peak energy

421   usage patterns in the location. Substantial savings can be achieved by programming the dark

422   period to coincide with the energy tariff imposed during peak electricity consumption.

423

424   Additional equipment needed:

425   i. **PAR meter**: The PAR is measured in either PPFD or Lux. Any off-the-shelf PAR meter can be

426   used, as long as it provides PPFD levels and relative wavelength composition. We used the

427   MK350S Spectrometer from UPRtek and the Spectrum Genius Essence Lighting Passport light

428   sensor from AsenseTek Inc. (Taiwan) at JIC and UQ, respectively.

429   ii. **Energy meter**: This allows measuring the energy consumption for lighting and temperature

430   maintenance thereby providing insight into SB operational costs. Any off-the-shelf energy

431   meter can be used for this purpose. To obtain energy consumption data for both the lights

432   employed and the Controlled Environment Rooms (CERs) at JIC, we utilised a clamp-on

14

433   Current Transformer meter with the capacity to store and download data. The instrument

434   provided half hourly readings and as such was highly accurate in determining energy costs

435

436

437   **Equipment setup**

438   In this section, we provide detailed protocols for the SB setups discussed in the previous section.

439   **a) Benchtop growth cabinet**

440   •   **Hardware:** Connect the display to the Raspberry Pi using the provided cables as instructed

441   by the manufacturer. The Arduino connects to the Raspberry Pi via USB ports. Sensors and

442   relay modules are connected using the Grove system (SeedStudio).

443   •   **Cabinet structure:** Assemble the beam profile using the joining plates. Slide the panels,

444   boards and sheets before fully assembling each side.

445   •   **Lighting system:** The photoperiod with the full-spectrum LED light bulbs is controlled by a

446   solid-state relay connected to the Arduino microcontroller. Sixteen 57 mm diameter holes

447   need to be drilled in one of the 757 x 307 x 3 mm aluminium composite panels, to fit the E27

448   lamp holders. The lamp holders are then inserted and wired in parallel.

449   •   **Temperature and humidity system:** Pre-assembled thermoelectric cooling modules are used

450   to simplify the construction of the benchtop growth cabinet. These are composed of fans,

451   aluminium heat sinks, and Peltier elements. The cooling modules are controlled by relays

452   connected to the Arduino. Airflow is used to control the humidity, *i.e.* the humidity sensor

453   will trigger the 12 V fan to circulate air from outside the cabinet in order to reduce the

454   humidity inside.

455   •   **Software installation and setup:** The speed breeding cabinet is controlled by three main

456   subsystems: The arduino micro controller that monitors and controls the environment

457   according to a desired optimal; a python daemon that stores the current conditions and

458   reads the expected conditions from a MongoDB database and; a graphical interface written

459   in ReactJS that allows the users to set up the expected conditions in a 24-hour range.

460   The circuit diagram for making the connections are provided in Supplementary Figure 3 and a

461   photograph of the assembled cabinet is provided in Supplementary Figure 4. The cabinet has an

462   available area of 0.225 m^2 . For the lamps we have used, the spectrum is provided in

463   Supplementary Figure 5, with the light levels in PPFD (Photosynthetic Photon Flux Density) being

464   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

465   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

466   the top of the cabinet where the lights are situated. The energy consumption of the mini cabinet

467   is 6.24 kWh per day.

15

468

469   *NOTE:* A step-by-step guide for constructing the cabinet and installing the software is

470   available at https://github.com/PhenoTIPI/SpeedSeed3/wiki, along with troubleshooting

471   tips.

472

473   Caution: The construction of the cabinet requires the use of sharp cutting and drilling tools

474   that may cause physical injury if handled improperly. Many steps involve electrical

475   components, which can cause fire if operated without being earthed. Ensure all necessary

476   safety steps are followed and use personal protective equipment when constructing the

477   cabinet.

478   **b) LED-supplemented glasshouse**

479   Table 2 provides the lighting arrangement in two glasshouse configurations. Both setups have

480   been demonstrated to successfully support SB for the species listed.

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

	John Innes Centre, United Kingdom		University of Queensland, 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	*No. of lights in the given area*	8 Heliospectra E602G lights
	Distance between lights and bench	244 cm	*Distance between lights and bench*	155 cm
	Distance between lights and plant canopy/ sensor	144 cm (LICOR sensor, kept approximately at plant canopy height)	*Distance between lights and plant canopy/ sensor*	95 cm from approximately the spike-height of a tall, adult wheat plant.

16

	Approximate distance of canopy from bench surface	100 cm	*Approximate distance of canopy from bench surface*	60 cm
	Schematic	Supplementary Figure 6	*Schematic*	Supplementary Figure 7
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 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 8 and Supplementary Figure 8.
			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 9 and Supplementary Figure 9.	

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).	

17

Heating/Cooling system	*Heating*: gas-fired central heating	*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.
	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.	*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 $^{\circ}$C.

482

483   Critical: Weather and ambient light varies by location and season, especially at higher

484   latitudes. Thus, for the glasshouse setups listed here, the light spectrum is determined not just

485   by the presence of the LED lights but also by the ambient light. To ensure reproducibility,

486   consider setting up your experiment in a way that mitigates these environmental variables.

487   For example, use programmable lights that allow intensity modification based on sensor

488   feedback, or controllable blinds to regulate photoperiod. Provision of a short dark-period is

489   recommended for optimum plant health. We highly recommend setting up a temperature

490   monitoring and control system.

491

492   A summary of the crops for which we have successfully demonstrated a shortening of

493   generation time using SB, including information on which specific SB setups were used, and

494   where the reader can find more information on the key growth stages and other growth

495   parameters of the crop grown under those conditions is provided in Table 3.

496

497

498

499

500

501   **Table 3 | A list of speed breeding protocols that have been demonstrated for different**

502   **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 10 - 18) UQ-GH-LED[2] (Supplementary Tables 19 and 20)	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 21 - 23)		
Durum wheat *T. durum*	JIC-GH-LED (Supplementary Tables 14 - 18)		Alahmad et al., 2018
Spring barley *H. vulgare*	JIC-GH-LED (Supplementary Tables 24 - 26) UQ-GH-LED (Supplementary Tables 27 and 28)	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 29 - 33)	UQ-GH-SVL (Supplementary Tables 13, 17, 23, 25, 30, 39)	
Brassica rapa	JIC-GH-LED (Supplementary Tables 29 - 33)		
Brassica oleracea	JIC-GH-LED (Supplementary Tables 29 - 33)		
Pea	JIC-GH-LED	CER-JIC	

19

P. sativum	(Supplementary Tables 34 and 35)	(Supplementary Table 10)	
Grasspea *L. sativus*	JIC-GH-LED (Supplementary Tables 36 - 38)		
Medicago		CER-JIC (Supplementary Table 9)	
Brachypodium distachyon	JIC-GH-LED (Supplementary Tables 39, 40)	CER-JIC (Supplementary Table 4)	
Quinoa *C. quinoa*	JIC-GH-LED (Supplementary Tables 41 - 43)		
Oat *A. strigosa*	JIC-GH-LED (Supplementary Tables 44 - 46)		
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[36]

503   [1] **JIC-GH-LED**: LED-Supplemented Glasshouse setup, JIC, UK (described in this paper,

504   Equipment Setup Section c).

505   [2] **UQ-GH-LED**: LED-Supplemented Glasshouse setup, UQ, Australia (described in this paper,

506   Equipment Setup Section c).

507   [3] **UQ-GH-SVL**: SVL-Supplemented Glasshouse setup, UQ, Australia (described in Methods

508   section: Speed Breeding II[1]).

509   [4] **CER-JIC**: Controlled Environment Room, JIC, UK (described in Methods section: Speed

510   Breeding I[1]).

20

511 **4. Procedure**

512   a) **Preparing seed for sowing:**

513   To increase germination efficiency some seeds may need a pre-treatment either by cold

514   stratification (prolonged imbibition in the cold) or scarification (physical or chemical

515   weakening of the seed coat). The requirements for germination pre-treatments are specific

516   for each species, and accessions of that species, and should be determined on an individual

517   basis. Dormant spring wheat and barley seed can be imbibed on moistened filter paper in a

518   Petri dish for 24 hours and then chilled at 4 °C for approximately three days (longer times

519   may be required depending on the level of dormancy). The seeds can then be left at room

520   temperature for one to three days to germinate prior to transferring to soil. If pre-treatment

521   is not required, the seed can be germinated in a Petri dish on moistened filter paper before

522   transferring to soil. In a large-scale scenario, seeds can be directly sown into high density

523   trays and placed in a cold-room, then trays can be moved to the growing environment in the

524   glasshouse. If a pre-treatment is not required, seed may be sown directly into soil in the

525   glasshouse/growth chamber.

526

527   Caution: If seeds germinate in a Petri dish and become too well established (i.e. develop

528   green leaves) before transplanting to soil, the shift to SB conditions, especially the presence

529   of intense light, can shock the plants, resulting in a strong hypersensitive response and

530   possibly death. Take care to prick them out early, or if they are already established, transfer

531   them to soil and place a mesh over the plants to reduce light intensity while they adapt to the

532   new environmental conditions.

533

534   b) **Monitoring key growth stages, growth parameters, and phenotyping:**

535   To enable comparison to normal development, monitor the key growth stages of the plants.

536   For many crops, defined growth stages have been published; for example, cereal crops[40],

537   canola[41], quinoa[42] and legumes[43]. Take note of the heading times and earliest time point to

538   harvest viable seeds. We also advise monitoring the height and general physiology of the

539   plants.

540

541   *NOTE:* Experiments performed in Section c, LED-supplemented glasshouse setup at the JIC,

542   UK, involved a SB glasshouse compartment as detailed above (i.e. 22 h day length), and a

543   twin compartment with a 16 h day length to measure the effect and value of increased day

544   length. Growth parameters and harvest times are provided for both lighting regimes where

545   available.

21

546   For wheat and barley, we have also previously demonstrated how SB conditions do not

547   interfere with the phenotyping of a number of key traits[1], and how variations of the SB

548   protocol can be used to rapidly screen wheat and barley for resistance to a number of major

549   diseases or disorders (Table 4).

550

551   **Table 4 | Protocols for phenotyping diseases and disorders under speed breeding**

552   **conditions.**

553

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[44] 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[44] 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)[45]
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]

554

555

556   **c) Seed harvesting:**

557   Shortened generation times can also be achieved in some species by harvesting premature

558   seed. This usually involves waiting until the seeds have set in the plant (indicated by filled

559   seed in spikes for wheat, or filled pods for legumes), then either increasing the temperature

560   or withholding water from the plant to hasten seed ripening and drying. After a week of this

561   stress application, seeds may be harvested.

562

563   *NOTE:* For experiments performed in the third protocol setup (Section c, LED-supplemented

564   glasshouse) at the JIC, UK, early harvest times are provided for both lighting regimes where

565   available. If not indicated, the harvest time outlined is for harvest at physiological maturity.

566

567   Caution: Freshly harvested seed may display dormancy. See troubleshooting (Section 5) for

568   more details on how to overcome this issue.

569

570   **d) Monitoring energy use:**

571   At the end of one cycle, review the energy costs for your SB system. This is particularly

572   useful to evaluate the generation time vs cost trade-off where multiple conditions have

573   been tested concurrently (e.g. different day lengths). For the LED-Supplemented glasshouse

574   setup in JIC, there were two rooms set up concurrently with 16-hour and 22-hour

575   photoperiods. The energy calculations for running each of these setups per month is given in

576   Supplementary Table 47, along with a comparison of how much it would cost to run a similar

577   setup with Sodium Vapour Lamps.

23

578   **5. Troubleshooting**

579

580   **Table 5 | Suggested solutions to common issues under speed breeding.**

581

Problem	Possible Reason	Solution
Plants exhibit tip-burn necrosis. The leaves curl inward or outward, and may have small, circular depressions or "bubbles" (Supplementary Figure 2).	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% calcium nitrate 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.
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 10).	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.
Young leaves appear striped with interveinal yellowing (Supplementary Figure 11).	Iron deficiency.	Apply a liquid fertiliser containing iron as a foliar spray early in growth to

24

		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.
Seeds do not germinate.	Seed harvested too early and are not viable.	Harvest seed slightly later.
	Seeds are dormant.	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.
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.	Make adjustments for temperature, light intensity, light quality and/or day length.
	The particular genotype may be recalcitrant to SB.	Try other genotypes to explore if it is a genotype- or species-specific issue.
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.

582

583

25

584 **6. Anticipated Results**

585 As demonstrated in our previous study, under SB conditions with a 22-hour photoperiod, it should

586 be possible to produce up to 6 generations per year in spring wheat and barley and up to 4 and 4.5

587 generations per year in canola and chickpea, respectively[1]. However, it is important to remember

588 that results are highly dependent on the crop species and can vary greatly between cultivars. The

589 light quality, duration of the photoperiod and temperature regime also impact the extent to which

590 the generation time is reduced. It should also be noted that ambient sunlight strength and duration

591 will vary with location and season, thus resulting in differences in rate of development. These

592 factors, in addition to basic growing conditions, such as soil type, can be manipulated to obtain the

593 optimal parameters for the crop of interest. The various protocols outlined above are designed to

594 facilitate this process.

595 The self-made, bench-top speed breeding cabinet will facilitate identification of conditions that

596 enable rapid-cycling of wheat and pea, and by extension, the other crops listed (Supplementary

597 Figure 4). We demonstrated the efficacy of this cabinet design by growing rapid-cycling varieties of

598 pea (*P. sativum* cv. JI 2822) and wheat (*T. aestivum* cv. USU Apogee) and showing the shortened

599 time from seed to seed, without compromising the viability of early harvested seed (Supplementary

600 Tables 1, 2). This is comparable with data from our previous study[1] where we evaluated the same

601 pea variety (JI 2822) under SB conditions using a commercial CER.

602 The time taken for reproductive development to occur for a range of crop species under the LED-

603 fitted, SB glasshouse (JIC, UK) is provided in Table 6. Two extended photoperiods are represented to

604 give an approximate expectation of the rapid development of these species under SB, and to give

605 the reader an idea of what a 6-hour difference in photoperiod can produce in a range of crops and

606 cultivars. The much slower rate of development under control or regular glasshouse conditions

607 without supplemental lighting was reported for some of these species in our previous study[1].

608 Plants grown under SB can be expected to look healthy (Figure 1) with minor reductions in seed set

609 (refer to Table 3 in order to view the related data for the crop of interest) and spike size

610 (Supplementary Figure 12) or pod size (Supplementary Figures 13 and 14). In some crop species, the

611 SB conditions can produce a slight reduction in height and/or internode length. In our experience,

612 while working on *M. truncatula* and *P. sativum*, we found the plants grown under SB produced

613 leaves with much smaller surface areas. Occasionally, micronutrient deficiencies manifest

614 themselves because of the rapid growth and change in soil pH – some of these issues (particularly

615 for wheat and barley) are highlighted in the Troubleshooting section. Despite efforts to optimise soil

616 composition, there may be a cultivar that responds very poorly to the long-photoperiod and high

617 irradiance.

618

26

619   We have previously demonstrated that wheat, barley and canola plants grown under SB are suitable

620   for crossing and phenotyping a range of adult plant traits[1]. That said, complex phenotypes such as

621   yield and abiotic stress resilience (heat or drought stress) are best evaluated in the field, particularly

622   for breeding objectives. We have also demonstrated how SB can be combined with transformation

623   of barley to speed up the process of obtaining transformed seeds[1].

624

625   **Table 6 | Mean days to anthesis[1] under speed breeding using LED-supplemented glasshouses at**

626   **JIC, UK.** All plants had a temperature cycle regime of 22 hours at 22 °C and 2 hours at 17 °C to

627   coincide with the light and dark period, respectively.

Species	Associated data	Photoperiod	Mean days to flowering[1]
Spring wheat *T. aestivum*	Supplementary Tables 10-18, 19, 20	22 h	49.6 ± 5.0
		16 h	62.5 ± 4.3
Winter wheat *T. aestivum*	Supplementary Tables 21 - 23	22 h	105.4 ± 1.7
		16 h	115.4 ± 1.9
Durum wheat *T. durum*	Supplementary Tables 14 - 18	22 h	46 ± 1.9[2]
		16 h	53.7 ± 1.0[2]
Spring barley *H. vulgare*	Supplementary Tables 24 - 28	22 h	38.4 ± 13.9
		16 h	46.6 ± 12.1
Canola *Brassica napus*	Supplementary Tables 29 - 33	22 h	34.5 ± 0.7[3]
		16 h	45.0 ± 0.0
Brassica rapa	Supplementary Tables 29 - 33	22 h	36.5 ± 2.5[3]
		16 h	41.0 ± 3.7
Brassica oleracea	Supplementary Tables 29 - 33	22 h	49.2 ± 1.8[3]
		16 h	61.2 ± 2.3
Pea *P. sativum*	Supplementary Tables 34, 35	22 h	32.2 ± 5.3[4]
		16 h	42.9 ± 5.3
Grasspea *L. sativus*	Supplementary Tables 36 - 38	22 h	31[3] ±
		16 h	ND
Brachypodium distachyon	Supplementary Tables 39, 40	22 h	31.5 ± 5.2
		16 h	44.0 ± 5.2
Quinoa *C. quinoa*	Supplementary Tables 41 - 43	22 h	54.6[5] ± 0.6
		16 h	61.1 ± 4.6
Oat	Supplementary Tables 44 - 46	22 h	52 ± 0.0

		16 h	66 ± 0.0
A. sativa			

628   [1]Days to flowering/anthesis (GS65) from sowing[40].

629   [2]Days to 50% ear emergence from sowing (GS55).

630   [3]Days to first flower opening from sowing.

631   [4]Days to the first flower bud from sowing.

632   [5]Days to anthesis (growth stage 6 according to BBCH scale[42]).

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634   In breeding programs, SSD is often an important step in cultivar development that requires high-

635   density plantings. The SB protocols provided for glasshouses are ideal for SSD programs, particularly

636   cereal crops. Increasing sowing density under SB can enable rapid cycling of many lines with healthy

637   plants and viable seed. Figure 2 shows an example of the plant condition, spike lengths and seed

638   sizes that could be expected at various sowing densities in SB. Under the UQ-GH-LED protocol, at a

639   density of 1000 plants/m^2, up to 6 generations of wheat and barley can be expected per year

640   (Supplementary Table D and E). At higher densities, plant height and seed numbers can be reduced

641   due to the greater competition and low soil volume. Despite this, even at the highest sowing density

642   shown here, all plants produced a spike with at least enough seed to perform SSD, and in most cases

643   many more. Large differences in the speed of development can be achieved by extending the

644   photoperiod from 16 to 22 hours. Under the JIC-GH-LED protocol, spring and durum wheat were

645   over ten days faster in development with an additional 6 hours of photoperiod. Table 7 provides the

646   approximate development times for several cereal crops at a range of sowing densities, appropriate

647   for intensive SSD. The SSD SB protocol was performed under two extended photoperiod and

648   temperature regimes at either JIC, UK, or UQ, Australia. These results demonstrate that plants can

649   be grown at high densities under SB conditions to produce plants suitable for effective and resource-

650   efficient generation turnover in SSD programs.

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653 **Table 7 | Mean days to reproductive stages[3-5] of single seed descent (SSD) sowing densities under**

654 **speed breeding using the JIC-GH-LED[1] or UQ-GH-LED[2] protocol.** JIC-GH-LED protocol used a

655 temperature cycle regime of 22 h at 22 °C and 2 h at 17 °C to coincide with light and dark times,

656 respectively. The UQ-GH-LED protocol used a temperature cycle regime of 12 h at 22 °C and 12 h at

657 17 °C.

Species	Protocol	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

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		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

658 [1] **JIC-GH-LED**: LED-Supplemented Glasshouse setup, JIC, UK (described in this paper, Equipment

659 Setup Section c).

660 [2] **UQ-GH-LED**: LED-Supplemented Glasshouse setup, UQ, Australia (described in this paper,

661 Equipment Setup Section c).

662 [3] Days to 50% ear emergence from sowing (GS55)[40].

663 [4] Days to mid-anthesis (GS65) from sowing.

664 [5] Days to awn-peep (GS49) from sowing.

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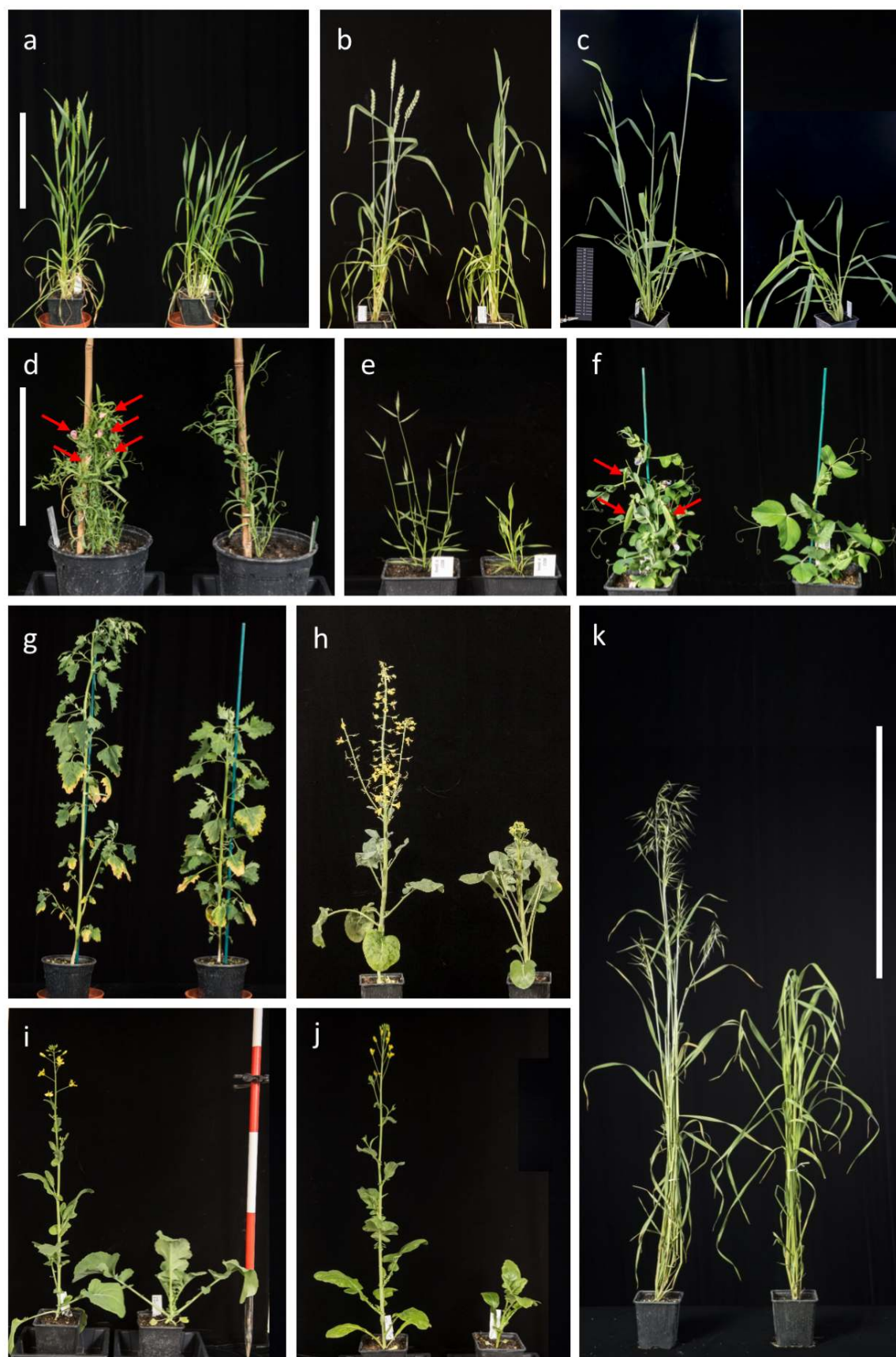
Figure 1. Accelerated plant growth and development under speed breeding (22 hour photoperiod conditions) (left) compared to standard long-day conditions (16 hour photoperiod) (right) in LED-

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670 **supplemented glasshouses at John Innes Centre, UK. a,** Winter growth-habit wheat (*T. aestivum* cv.

671 Crusoe) at 112 days after sowing (DAS), including 12 days of growth under 16-hour photoperiod

672 conditions followed by 56 days of vernalisation at 6 °C with 8 hour photoperiod; **b,** Spring wheat (*T.*

673 *aestivum* cv. Cadenza) at 57 DAS; **c,** Spring barley (*H. vulgare* cv. Manchuria) at 35 DAS; (scalebar is

674 20 cm for a, b, c) **d,** Grasspea (*L. sativus* cv. Mahateora) at 35 DAS (red arrows indicate position of

675 flowers); **e,** *B. distachyon* (accession Bd21) at 34 DAS; **f,** Pea (*P. sativum* accession JI 2822) at 34 DAS;

676 (scalebar is 20 cm for d, e, f) **g,** Quinoa (*C. quinoa* accession QQ74) at 58 DAS; **h,** *Brassica oleracea*

677 (line DH1012) at 108 DAS**; i,** *Brassica napus* (line RV31) at 87 DAS **; j,** *Brassica rapa* (line R-0-18 87) at

678 87 DAS**; k,** Diploid Oat (*A. strigosa* accession S75) at 52 DAS (scalebar is 60 cm for g, h, i, j). All plants

679 were sown in October or November 2017, except for the quinoa, which was sown in February 2018.

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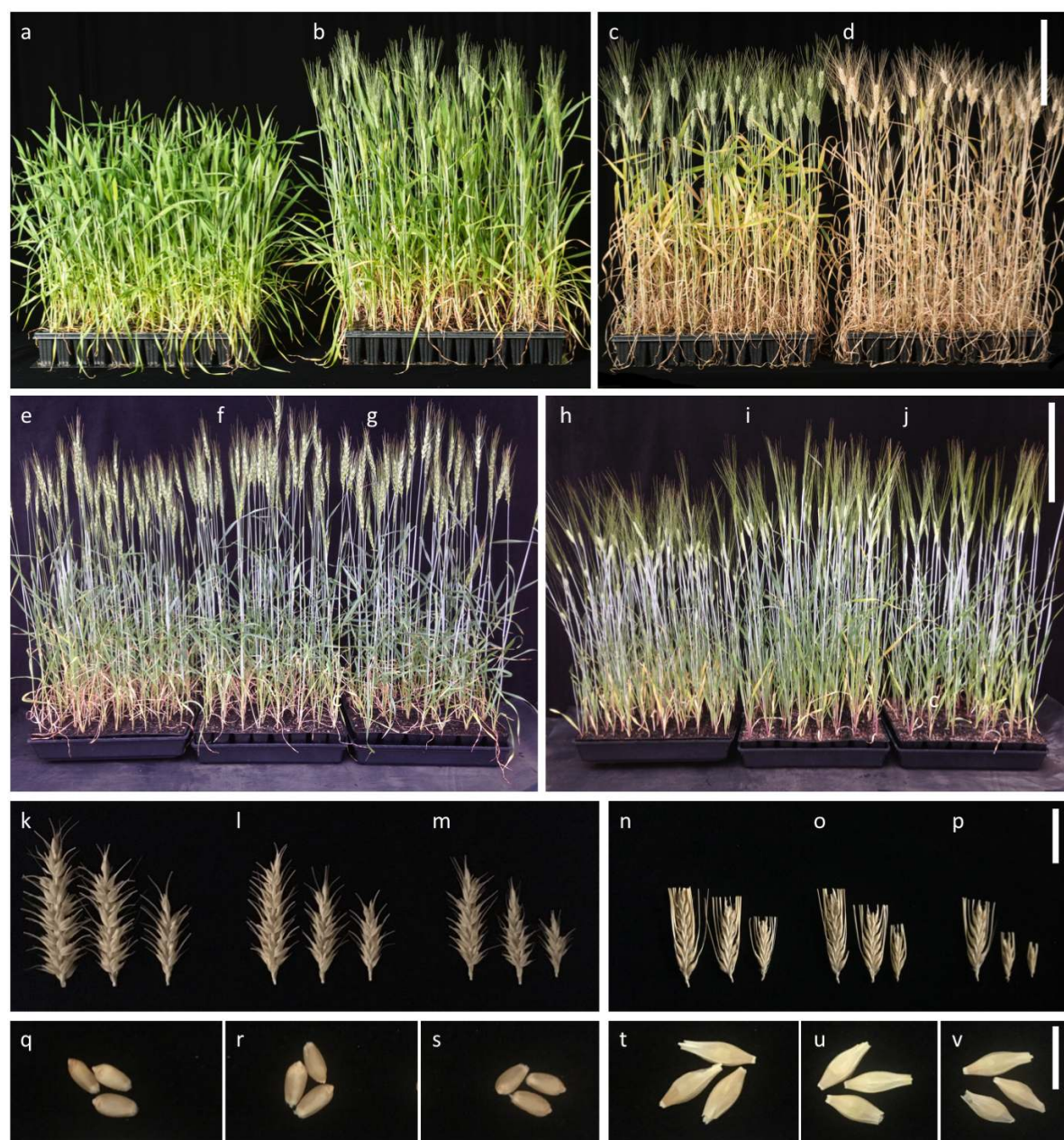
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Figure 2 | Single seed descent sowing densities of wheat (spring and durum) and barley under LED-Supplemented Glasshouse setup at JIC, UK and UQ, Australia. Durum wheat (*T. durum* cv. Kronos) grown under the LED-Supplemented Glasshouse setup, JIC, 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.*

693   *aestivum* cv. Suntop) grown under LED-Supplemented glasshouse setup, UQ, Australia: **k**, plants in a

694   30-cell tray; **l**, plants in a 64-cell tray; **m**, plants in a 100-cell. Mature spikes of barley (*H. vulgare* cv.

695   Commander) grown under LED-Supplemented glasshouse setup, UQ, Australia: **n**, plants in a 30-cell

696   tray; **o**, plants in a 64-cell tray; **p**, plants in a 100-cell. Scalebar is 3 cm. Mature seeds of spring wheat

697   (*T. aestivum* cv. Suntop) grown under LED-Supplemented glasshouse setup, UQ, Australia: **q**, plants in

698   a 30-cell tray; **r**, plants in a 64-cell tray; **s**, plants in a 100-cell. Mature seeds of barley (*H. vulgare* cv.

699   Commander) grown under LED-Supplemented glasshouse setup, UQ, Australia: **t**, plants in a 30-cell

700   tray; **u**, plants in a 64-cell tray; **v**, plants in a 100-cell. Scalebar is 1 cm.

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858   **Contributions –** SG and AW drafted the manuscript and oversaw many of the experiments. LTH and

859   BBHW contributed to design of experiments and manuscript writing. AW designed and implemented

860   the SSD protocol for wheat and barley in the LED-supplemented glasshouse at UQ. SG, OEGN, RHRG,

861   LY and MMS designed, constructed, programmed and tested the benchtop growth cabinet. JC

862   performed the energy consumption calculations for the LED-supplemented glasshouse at JIC. For the

863   LED-supplemented glasshouse at JIC, JS performed the experiments for wheat including the SSDs,

864   RW for brassicas, REM for oats, SH for additional wheat cultivars, PG for barley, TR for pea, AH for

865   quinoa, AS (Sarkar) for grasspea and AS (Steed) for *Brachypodium*. JL, LP, CD, MJM, WH, AO, CM, CU,

866   BH, MT, PN, BBHW and LTH contributed intellectually to the experiments and/or the writing of the

867   manuscript. All authors reviewed and approved the final manuscript prior to submission.

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869   **Competing Interests – All the authors declare that they have no competing financial interests.**

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