



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

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

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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*.

31 **2. Introduction**

32 To improve the productivity and stability of crops there is pressure to fast-track research and
33 increase the rate of variety development. The generation time of most plant species represents a
34 bottleneck in applied research programs and breeding, creating the need for technologies that
35 accelerate plant development and generation turnover. Recently we reported protocols for 'speed
36 breeding' (SB), which involve extending the photoperiod using supplementary lighting and
37 temperature control, enabling rapid generation advancement in glasshouses with sodium vapour
38 lamps (SVL) or growth chambers fitted with a mixture of metal halide and light-emitting diode (LED)
39 lighting¹. By adopting a 22-hour photoperiod and controlled temperature regime, generation times
40 were significantly reduced for spring bread wheat (*Triticum aestivum*), durum wheat (*T. durum*),
41 barley (*Hordeum vulgare*), chickpea (*Cicer arietinum*), pea (*Pisum sativum*), canola (*Brassica napus*),
42 the model grass, *Brachypodium distachyon* and the model legume, *Medicago truncatula*, in
43 comparison to the field or a glasshouse with no supplementary light. Under the rapid growth
44 conditions, plant development was normal, plants could be easily crossed (wheat and barley), and
45 seed germination rates were high. We also demonstrated that SB can be used to accelerate gene
46 transformation pipelines and adult plant phenotyping could be performed under SB conditions for
47 traits such as flowering time, plant height, and disease resistance in wheat, leaf sheath glaucousness
48 in barley, and pod shattering in canola¹.

49

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

58

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

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 traits⁷. 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 photoperiod⁸. 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 wheat⁹
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 diseases¹¹⁻¹⁴ and also for pyramiding multiple disease resistance in barley¹⁵. 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 health¹. 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 Mexico¹⁶. 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 access¹⁷, accompanied by thinning of the plant canopy. Such a method is well-established and
129 documented for rice¹⁸ 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

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 canola¹. 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 plants¹. Protocols involving embryo rescue are important and useful
151 for breeding and research programs if the required infrastructure is available²⁵, particularly for
152 species that are recalcitrant to other parameters used to accelerate generation advancement such
153 as temperature or photoperiod manipulation²⁶⁻²⁸. 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 CO₂ concentration. For example, for C₃ plants
158 like rice and wheat, photosynthetic efficiency increases with increasing CO₂ 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 CO₂
161 concentration, followed by early harvest and embryo rescue to cut down generation times of many
162 rice varieties²⁹.
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 generations³⁰. 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-dependant³¹. 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 flower³⁴, 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 minutes³⁵. 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 flowering³⁶. 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 traits¹, 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 year⁹.

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 LEDs¹.
250 Even incandescent lighting has been shown to accelerate flowering in clover⁶. 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 (<i>T. aestivum</i>)	JIC Cereal Compost Mix, UQ Compost Mix
Durum wheat (<i>T. durum</i>)	JIC Cereal Compost Mix, UQ Compost Mix
Barley (<i>H. vulgare</i>)	JIC Cereal Compost Mix, UQ Compost Mix
Pea (<i>P. sativum</i>)	JIC Cereal Compost Mix
Chickpea (<i>C. arietinum</i>)	UQ Compost Mix
<i>Brassica rapa</i>	JIC Cereal Compost Mix
<i>Brassica oleracea</i>	JIC Cereal Compost Mix
Canola (<i>Brassica napus</i>)	JIC Cereal Compost Mix, UQ Compost Mix
Quinoa (<i>C. quinoa</i>)	JIC Peat and Sand Mix
Oat (<i>A. strigosa</i>)	JIC Cereal Compost Mix
Grasspea (<i>L. sativus</i>)	JIC Cereal Compost Mix
<i>Brachypodium distachyon</i>	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.

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 programming 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 m² and comfortably accommodates 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 studies¹, 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.

340 In addition to controlling the light quality, we recommend a photosynthetic photon flux density
341 (PPFD) of approximately $450\text{-}500 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ 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 $450\text{-}500 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ has been demonstrated to be effective for a range of
344 crop species¹.

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 triticale¹.

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 SeedStudio (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 SeedStudio, 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 paper¹ 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

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 m². 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 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ at 16 cm above the base where the pots are kept, and
465 about 320 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ and 220 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ 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.

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	<i>No. of lights in the given area</i>	25 Heliospectra LX602C lights	<i>No. of lights in the given area</i>	8 Heliospectra E602G lights
	<i>Distance between lights and bench</i>	244 cm	<i>Distance between lights and bench</i>	155 cm
	<i>Distance between lights and plant canopy/sensor</i>	144 cm (LICOR sensor, kept approximately at plant canopy height)	<i>Distance between lights and plant canopy/sensor</i>	95 cm from approximately the spike-height of a tall, adult wheat plant.

	<i>Approximate distance of canopy from bench surface</i>	100 cm	<i>Approximate distance of canopy from bench surface</i>	60 cm
	<i>Schematic</i>	Supplementary Figure 6	<i>Schematic</i>	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	<p>Two similar compartments within the same glasshouse were set up with two different photoperiod regimes:</p> <ul style="list-style-type: none">i) 22 hours of light, followed by 2 hours of darknessii) 16 hours of light, followed by 8 hours of darkness <p>The PPFD values and spectrum at various distances from the lights are provided in Supplementary Table 9 and Supplementary Figure 9.</p> <p>The PPFD values and spectrum at various distances from the lights are provided in Supplementary Table 8 and Supplementary Figure 8.</p>		<p>Photoperiod of 22 hours, followed by 2 hours of darkness.</p> <p>The PPFD values and spectrum at various distances from the lights are provided in Supplementary Table 9 and Supplementary Figure 9.</p>	
Temperature Regime	<p>20 °C as the maximum temperature to be operative during the photoperiod (16 or 22 hours depending on the photoperiod regime, <i>see above</i>).</p> <p>15 °C as the minimum temperature to be operative during the dark period (8 or 2 hours depending on photoperiod regime, <i>see above</i>).</p>		<p>22 °C as the maximum temperature to be operative for 12 hours during the photoperiod.</p> <p>17 °C as the minimum temperature to be operative during the dark period (2 hours).</p>	

Heating/Cooling system	<p><i>Heating:</i> gas-fired central heating</p> <p><i>Cooling:</i> Cooling fans that go off when the temperature goes above a set-point.</p> <p><i>Temperature monitoring and control:</i> Glasshouse temperature monitoring is carried out through TomTech (TomTech UK Ltd) which is a glasshouse specific business management system.</p>	<p><i>Heating and cooling:</i> 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.</p> <p><i>Temperature monitoring and control:</i> Glasshouse temperature automatically controlled using a business management system running on an Innotech system using Magellan Builder (Brisbane, Australia). The temperatures are controlled to ± 1 °C.</p>
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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.

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Table 3 | A list of speed breeding protocols 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 and Ghosh et al., 2018	Other
Spring wheat <i>T. aestivum</i>	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 <i>T. aestivum</i>	JIC-GH-LED (Supplementary Tables 21 - 23)		
Durum wheat <i>T. durum</i>	JIC-GH-LED (Supplementary Tables 14 - 18)		Alahmad et al., 2018
Spring barley <i>H. vulgare</i>	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 <i>Brassica napus</i>	JIC-GH-LED (Supplementary Tables 29 - 33)	UQ-GH-SVL (Supplementary Tables 13, 17, 23, 25, 30, 39)	
<i>Brassica rapa</i>	JIC-GH-LED (Supplementary Tables 29 - 33)		
<i>Brassica oleracea</i>	JIC-GH-LED (Supplementary Tables 29 - 33)		
Pea	JIC-GH-LED	CER-JIC	

<i>P. sativum</i>	JIC-GH-LED (Supplementary Tables 34 and 35)	CER-JIC (Supplementary Table 10)	
Grasspea <i>L. sativus</i>	JIC-GH-LED (Supplementary Tables 36 - 38)		
Medicago		CER-JIC (Supplementary Table 9)	
<i>Brachypodium distachyon</i>	JIC-GH-LED (Supplementary Tables 39, 40)	CER-JIC (Supplementary Table 4)	
Quinoa <i>C. quinoa</i>	JIC-GH-LED (Supplementary Tables 41 - 43)		
Oat <i>A. strigosa</i>	JIC-GH-LED (Supplementary Tables 44 - 46)		
Chickpea <i>C. arietinum</i>		UQ-GH-SVL (Supplementary Tables 14, 18, 24, 26, 30)	
Peanut <i>A. hypogaea</i>			O'Connor et al., 2013 ¹⁰
Amaranth <i>Amaranthus</i> 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 II¹).
509 ⁴ **CER-JIC:** Controlled Environment Room, JIC, UK (described in Methods section: Speed
510 Breeding I¹).

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 crops⁴⁰,
537 canola⁴¹, quinoa⁴² and legumes⁴³. 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.

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 traits¹, 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](#)).

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

Disease / disorder	Species	Reference
Stripe rust (<i>Puccinia striiformis</i> f. sp. <i>tritici</i>)	Spring wheat (<i>T. aestivum</i>)	Pretorius <i>et al.</i> (2000). <i>Acta Phytopathologica et Entomologica Hungarica</i> , 35(1-4), 359-364 ⁴⁴ Hickey <i>et al.</i> (2012). <i>Plant Breeding</i> , 131(1), 54-61 ¹⁴
Leaf rust (<i>Puccinia recondita</i> f. sp. <i>tritici</i> , "brown rust") (<i>Puccinia triticina</i> , "black rust")	Spring wheat (<i>T. aestivum</i>)	Pretorius <i>et al.</i> (2000). <i>Acta Phytopathologica et Entomologica Hungarica</i> , 35(1-4), 359-364 ⁴⁴ Riaz <i>et al.</i> (2016). <i>Plant Methods</i> , 12, 17 ¹⁴
Yellow spot / Tan spot (<i>Pyrenophora tritici-repentis</i>)	Spring wheat (<i>T. aestivum</i>)	Dinglasan <i>et al.</i> (2016). <i>Euphytica</i> , 209(3), 693-707 ¹²
Leaf rust (<i>Puccinia hordei</i>) Net form net blotch (<i>Pyrenophora teres</i> f. sp. <i>teres</i>) Spot form net blotch (<i>Puccinia teres</i> f. sp. <i>maculata</i>) Spot blotch (<i>Cochliobolus sativus</i>)	Barley (<i>H. vulgare</i>)	Hickey <i>et al.</i> (2017). <i>Euphytica</i> , 213(3), 64 ¹⁵

Stem rust (<i>Puccinia graminis</i> f. sp. <i>tritici</i>)	Spring wheat (<i>T. aestivum</i>)	Riaz and Hickey (2017). <i>Wheat Rust Diseases: Methods and Protocols</i> (Vol. 1659, pp. 183-196) ⁴⁵
Crown rot (<i>Fusarium pseudograminearum</i>)	Durum wheat (<i>T. durum</i>)	Alahmad <i>et al.</i> (2018). <i>Plant Methods</i> , 14(1), 36 ¹¹
Pre-harvest sprouting	Spring wheat (<i>T. aestivum</i>)	Hickey <i>et al.</i> (2009). <i>Euphytica</i> 168, 303-310 ⁹
Pod shattering	Canola (<i>B. napus</i>)	Watson and Ghosh <i>et al.</i> (2018). <i>Nature Plants</i> , 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.

578 **5. Troubleshooting**

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

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

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

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, respectively¹. 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 study¹ 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 study¹.

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

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 traits¹. 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 seeds¹.

624
625 **Table 6 | Mean days to anthesis¹ 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 <i>T. aestivum</i>	Supplementary Tables 10-18, 19, 20	22 h	49.6 ± 5.0
		16 h	62.5 ± 4.3
Winter wheat <i>T. aestivum</i>	Supplementary Tables 21 - 23	22 h	105.4 ± 1.7
		16 h	115.4 ± 1.9
Durum wheat <i>T. durum</i>	Supplementary Tables 14 - 18	22 h	46 ± 1.9 ²
		16 h	53.7 ± 1.0 ²
Spring barley <i>H. vulgare</i>	Supplementary Tables 24 - 28	22 h	38.4 ± 13.9
		16 h	46.6 ± 12.1
Canola <i>Brassica napus</i>	Supplementary Tables 29 - 33	22 h	34.5 ± 0.7 ³
		16 h	45.0 ± 0.0
<i>Brassica rapa</i>	Supplementary Tables 29 - 33	22 h	36.5 ± 2.5 ³
		16 h	41.0 ± 3.7
<i>Brassica oleracea</i>	Supplementary Tables 29 - 33	22 h	49.2 ± 1.8 ³
		16 h	61.2 ± 2.3
Pea <i>P. sativum</i>	Supplementary Tables 34, 35	22 h	32.2 ± 5.3 ⁴
		16 h	42.9 ± 5.3
Grasspea <i>L. sativus</i>	Supplementary Tables 36 - 38	22 h	31 ³ ±
		16 h	ND
<i>Brachypodium</i> <i>distachyon</i>	Supplementary Tables 39, 40	22 h	31.5 ± 5.2
		16 h	44.0 ± 5.2
Quinoa <i>C. quinoa</i>	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

<i>A. sativa</i>		16 h	66 ± 0.0
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628 ¹Days to flowering/anthesis (GS65) from sowing⁴⁰.

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

630 ³Days to first flower opening from sowing.

631 ⁴Days to the first flower bud from sowing.

632 ⁵Days to anthesis (growth stage 6 according to BBCH scale⁴²).

633

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/m², 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.

651

652

653 **Table 7 | Mean days to reproductive stages³⁻⁵ of single seed descent (SSD) sowing densities under**
654 **speed breeding using the JIC-GH-LED¹ or UQ-GH-LED² 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 <i>T. aestivum</i>	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 <i>T. durum</i>	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 <i>H. vulgare</i>	UQ-GH-LED	30-cell (300 plants/m ²)	22 h	27.3 ± 1.2 ⁵

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

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).

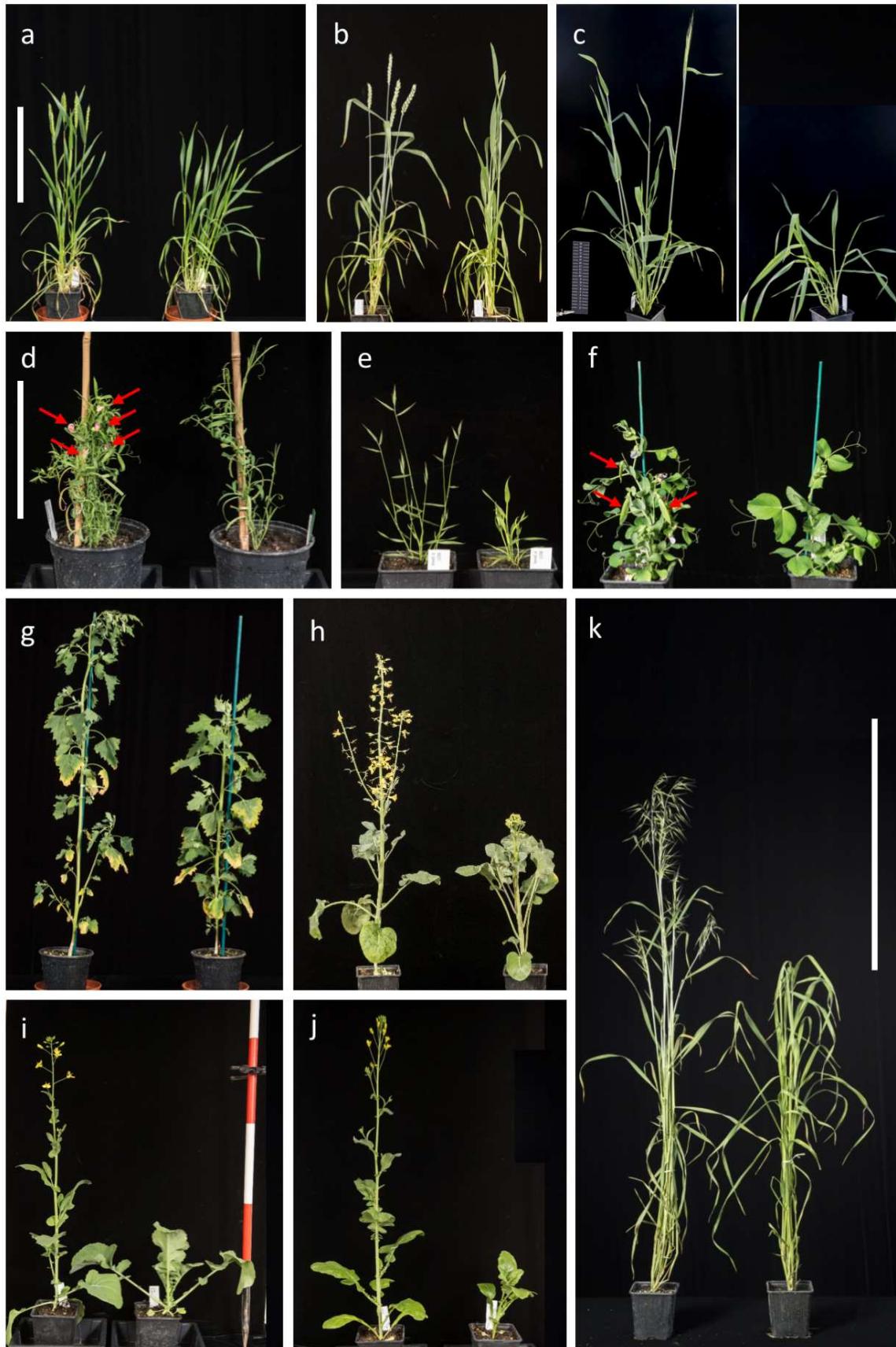
662 ³ Days to 50% ear emergence from sowing (GS55)⁴⁰.

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

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

665

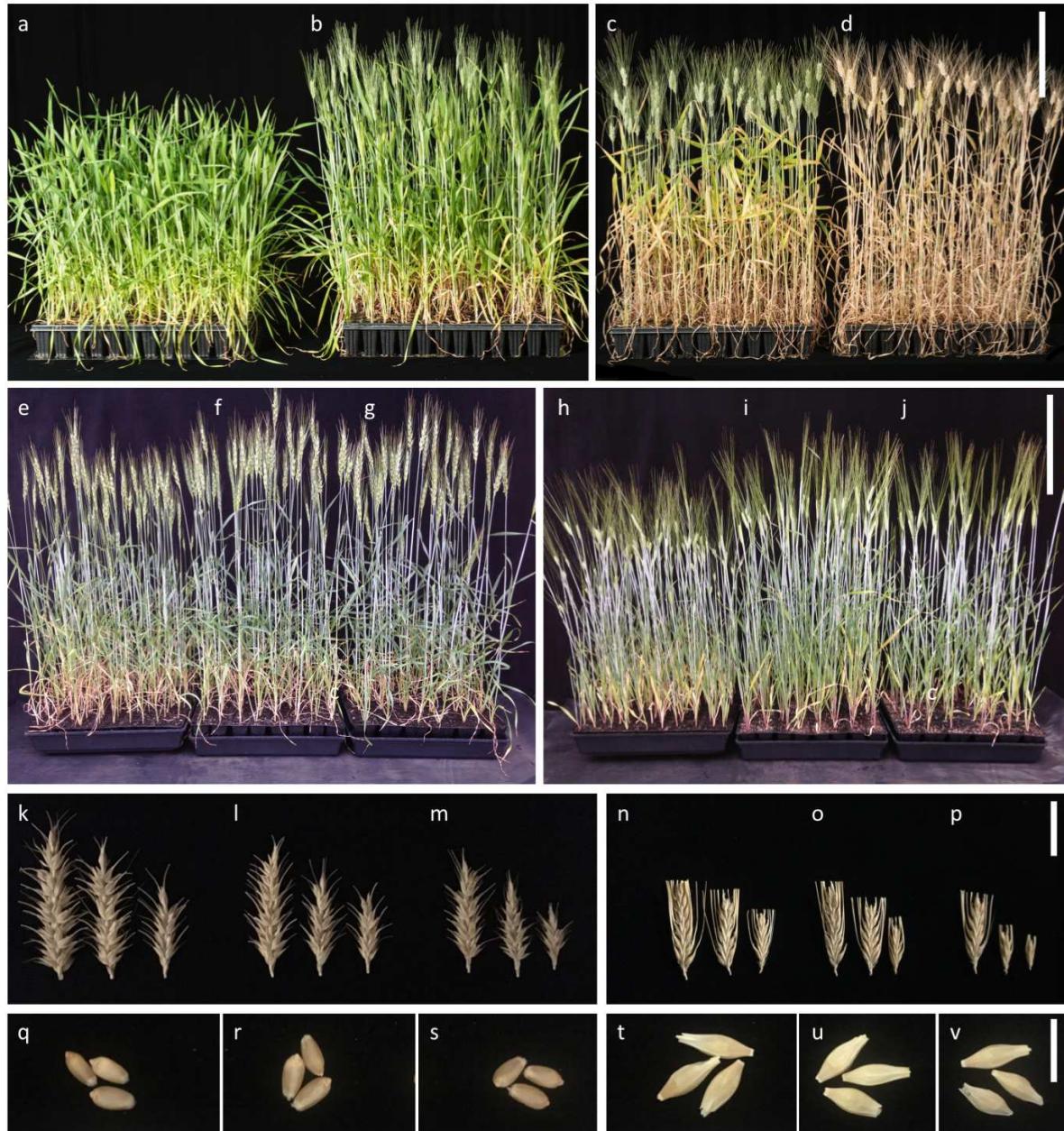
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668 **Figure 1. Accelerated plant growth and development under speed breeding (22 hour photoperiod**
669 **conditions) (left) compared to standard long-day conditions (16 hour photoperiod) (right) in LED-**

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.
680
681



682
683 **Figure 2 | Single seed descent sowing densities of wheat (spring and durum) and barley under LED-**
684 **Supplemented Glasshouse setup at JIC, UK and UQ, Australia.** Durum wheat (*T. durum* cv. Kronos)
685 grown under the LED-Supplemented Glasshouse setup, JIC, UK, in 96-cell trays: **a**, Forty-three days
686 after sowing under 16-hour photoperiod; **b**, Forty-three days after sowing under 22-hour
687 photoperiod; **c**, Seventy-nine days under 16-hour photoperiod; **d**, Seventy-nine days under 22-hour
688 photoperiod. Scale bar is 20 cm. Spring wheat (*T. aestivum* cv. Suntop) grown under LED-
689 Supplemented Glasshouse setup, UQ, Australia, at 37 days after sowing: **e**, plants in a 30-cell tray; **f**,
690 plants in a 64-cell tray; **g**, plants in a 100-cell. Barley (*H. vulgare* cv. Commander) grown under LED-
691 Supplemented glasshouse setup, UQ, Australia, at 34 days after sowing: **h**, plants in a 30-cell tray; **i**,
692 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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