

Speed breeding is a powerful tool to accelerate crop research and breeding

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The growing human population and a changing environment have raised significant concern for global food security, with the current improvement rate of several important crops inadequate to meet future demand¹. This slow improvement rate is attributed partly to the long generation times of crop plants. Here, we present a method called ‘speed breeding’, which greatly shortens generation time and accelerates breeding and research programmes. Speed breeding can be used to achieve up to 6 generations per year for spring wheat (*Triticum aestivum*), durum wheat (*T. durum*), barley (*Hordeum vulgare*), chickpea (*Cicer arietinum*) and pea (*Pisum sativum*), and 4 generations for canola (*Brassica napus*), instead of 2–3 under normal glasshouse conditions. We demonstrate that speed breeding in fully enclosed, controlled-environment growth chambers can accelerate plant development for research purposes, including phenotyping of adult plant traits, mutant studies and transformation. The use of supplemental lighting in a glasshouse environment allows rapid generation cycling through single seed descent (SSD) and potential for adaptation to larger-scale crop improvement programs. Cost saving through light-emitting diode (LED) supplemental lighting is also outlined. We envisage great potential for integrating speed breeding with other modern crop breeding technologies, including high-throughput genotyping, genome editing and genomic selection, accelerating the rate of crop improvement.

For most crop plants, the breeding of new, advanced cultivars takes several years. Following crossing of selected parent lines, 4–6 generations of inbreeding are typically required to develop genetically stable lines for evaluation of agronomic traits and yield. This is particularly time consuming for field-grown crops that are often limited to only 1–2 generations per year. Here, we present flexible protocols for ‘speed breeding’ that use prolonged photoperiods to accelerate the developmental rate of plants², and the harvesting and germination of immature seed, thereby reducing generation time. We highlight

the opportunity presented by speed breeding and detail protocols to inspire its widespread adoption as a state-of-the-art breeding and research tool.

To evaluate speed breeding as a method to accelerate applied and basic research on cereal species, standard genotypes of spring bread wheat (*T. aestivum*), durum wheat (*T. durum*), barley (*H. vulgare*) and the model grass *Brachypodium distachyon* were grown in a controlled-environment room with extended photoperiod (22 hours light/2 hours dark) (Fig. 1; Methods: Speed breeding I; Supplementary Table 1). A light/dark period was chosen over a continuous photoperiod to support functional expression of circadian clock genes³. Growth was compared with that of plants in glasshouses with no supplementary light or heating during the spring and early summer of 2016 (Norwich, UK). Plants grown under speed breeding progressed to anthesis (flowering) in approximately half the time of those from glasshouse conditions. Depending on the cultivar or accession, plants reached anthesis in 35–39 days (wheat, with the exception of Chinese Spring) and 37–38 days (barley), while it took 26 days to reach heading in *B. distachyon* (Fig. 2a–d; Supplementary Tables 2–4). Concurrently, the corresponding glasshouse plants had only reached the early stem-elongation or three-leaf growth stage, respectively. Wheat seed counts per spike decreased, although not always significantly, in the speed breeding chamber compared to the glasshouse with no supplementary light (Supplementary Table 5) and both wheat and barley plants produced a healthy number of spikes per plant, despite the rapid growth (Supplementary Table 6). The viability of harvested seeds appeared to be unaffected by speed breeding, with similar seed germination rates observed for all species (Supplementary Table 7). Moreover, crosses made between wheat cultivars under speed breeding conditions produced viable seeds, including crosses between tetraploid and hexaploid wheat (Supplementary Table 8). These conditions were also used to successfully reduce the generation time of the model legume *Medicago truncatula* and the rapid-cycling pea (*P. sativum*) variety JI 2822⁴ (Supplementary Fig. 1a,b; Supplementary Tables 9, 10).

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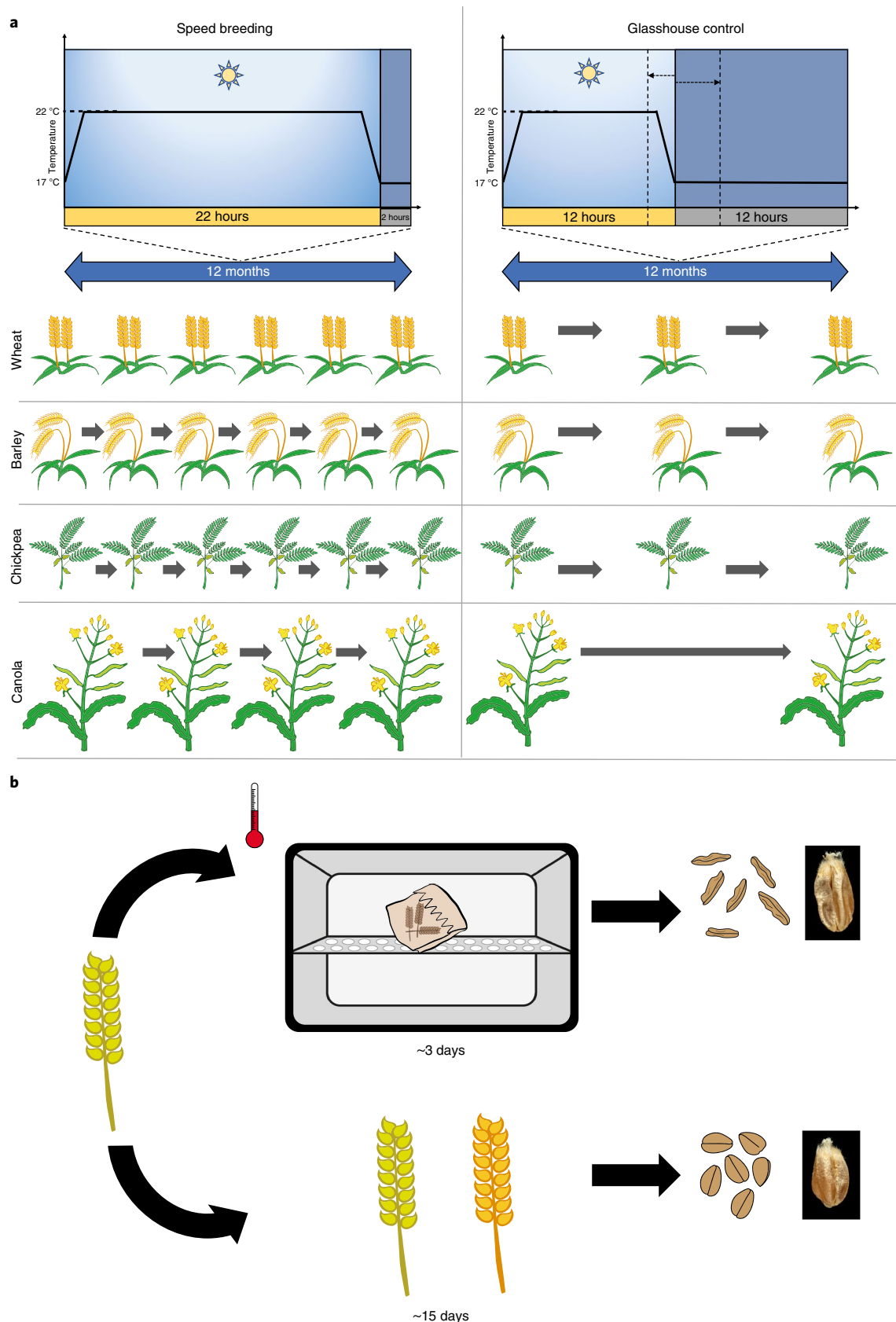


Fig. 1 | Speed breeding accelerates generation time of major crop plants for research and breeding. a, Compared to a glasshouse with a natural variable photoperiod (10–16 hours), where only 2–3 generations of wheat, barley, chickpea and canola can be achieved per year (right), speed breeding enables 4–6 generations of these crops to be grown in a year (left). These values are representative of relatively rapid cycling cultivars of each crop. **b**, Harvesting of immature spikes and drying them in an oven/dehydrator (~3 days) enables faster seed to seed cycling compared to the normal seed ripening process, which takes about 15 days, although it comes with a loss of grain weight.

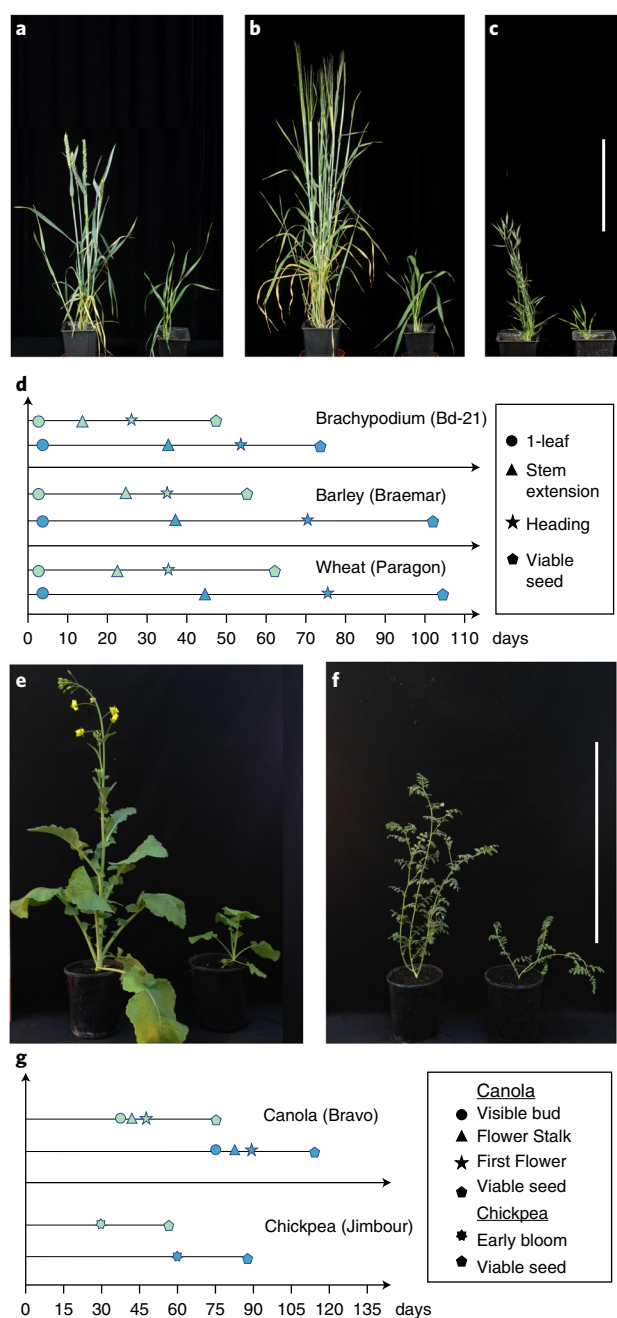


Fig. 2 | Accelerated plant growth and development under speed breeding (left) compared to control conditions (right). **a**, Wheat (*T. aestivum* cv. Cadenza) at 38 days post sowing. **b**, Barley (*H. vulgare* cv. Braemar) at 41 days post sowing. **c**, Brachypodium (*B. distachyon* accession Bd21) at 36 days post sowing. Scale bar = 20 cm. **d**, Representative graph depicting the development stages of wheat, barley and brachypodium (x axis in days) under speed breeding (top line for each crop) conditions in a controlled environment chamber compared to control conditions (bottom line for each crop) in a glasshouse during UK summertime with no supplementary light or heat. **e**, Canola (*B. napus* cv. Bravo), pictured at 50 days post sowing. **f**, Chickpea (*C. arietinum* cv. Jimbour), pictured at 35 days post sowing. Scale bar = 50 cm. **g**, Representative graph depicting the development stages of canola and chickpea (x axis in days) under speed breeding conditions in a supplemented glasshouse (top line for each crop) compared to control conditions (bottom line for each crop) in a glasshouse in Queensland, Australia, with no supplementary light.

In an alternative, yet similar, protocol for rapid generation cycling, we evaluated a collection of spring wheat, barley, canola and chickpea varieties in Queensland, Australia, in a temperature-controlled glasshouse fitted with high-pressure sodium lamps to extend the photoperiod to a day-length of 22 hours. A control treatment in a glasshouse used a natural 12-hour control photoperiod. Both used the same temperature regime (22/17 °C; Fig. 1; Methods: Speed breeding II; Supplementary Table 1). Time to anthesis was significantly reduced for all crop species relative to the 12-hour day-neutral photoperiod conditions, where the average reduction was, depending on genotype, 22 ± 2 days (wheat), 64 ± 8 days (barley), 73 ± 9 days (canola) and 33 ± 2 days (chickpea) (Fig. 2e–g; Supplementary Tables 11–14). Analysis of growth stage progression revealed normal, though accelerated, development for all species (Fig. 2e–g; Supplementary Tables 15–18) compared to the day-neutral conditions. Wheat plants produced significantly more spikes than those in day-neutral conditions and grain number was unaffected by the rapid development in both wheat and barley (Supplementary Tables 19, 20). Notably, time to anthesis was more uniform within each species under speed breeding conditions (Supplementary Tables 11–14)—an important feature, as synchronous flowering across genotypes is desirable for crossing. Additionally, wheat seed was harvested before maturity: 14 days post anthesis in speed breeding conditions, and following a 4-day cold treatment seed viability was high (Supplementary Table 21), indicating that generation time can be further reduced by harvesting immature seed without the need for labour-intensive embryo rescue⁵. Seed viability of all other species under Speed breeding II conditions was either unaffected or improved compared with day-neutral conditions (Supplementary Tables 22–24). Since temperature greatly influences the rate of plant development⁶, generation time may be further accelerated by elevating temperature. This may, however, induce stress and affect plant performance.

Seed production (g per plant) of canola and chickpea was similar between speed breeding and day-neutral conditions (Supplementary Tables 25, 26). Plant growth and seed yield in wheat and barley plants grown for a second generation under speed breeding I and II were unaffected, except for a 2 day delay in early plant development, as well as a final reduction of total number of seeds harvested per plant, when plants were grown from immature seed (Supplementary Fig. 2–3; Supplementary Tables 27–29). Considering the time taken to produce viable seed under speed breeding in the glasshouse, wheat, barley, canola and chickpea, depending on the variety, could produce between 4 and 6 generations per year (Supplementary Table 30). These results highlight the opportunity to apply speed breeding across several important crop species without jeopardising the production of subsequent generations. The application of speed breeding conditions in a glasshouse fitted with supplementary lighting exemplifies the flexibility of the approach and may be preferred over growth chambers if rapid generation advance is to be applied to large populations, such as in breeding programs.

SSD is commonly used in breeding and research programs to facilitate development of homozygous lines following a cross⁷. This process only requires one seed per plant to advance each generation. To investigate the ability of speed breeding to accelerate SSD, where plants are typically grown at high density, wheat and barley genotypes were grown in 100-cell trays under both speed breeding and 12-hour day-neutral photoperiod conditions in the glasshouse. This equated to a density of approximately 900 plants per m². Generation time was shorter than for plants grown at lower density in the previous speed breeding experiments (Supplementary Tables 31, 32). This was probably caused by stress or plant competition as a result of the higher density, which is known to hasten flowering⁸. In most cases, each plant produced a single viable tiller containing on average 19.0 ± 1.3 and 18.6 ± 1.8 seeds for wheat and barley, respectively. Seed viability was 80% for wheat harvested at 2 weeks post anthesis

and 100% for both wheat and barley harvested at 4 weeks post anthesis (Supplementary Tables 31, 32). Therefore, integrating speed breeding and SSD techniques can effectively accelerate the generation of inbred lines for research and plant breeding programs. This method could facilitate sample collection for genetic studies with seed sowed in a 96-cell format to conform to a 96-well DNA plate.

In an additional protocol (Supplementary Table 1; Methods: Speed breeding III), we successfully implemented a low-cost speed breeding growth room design, lit exclusively by LEDs to reduce the operational cost of lighting and cooling (Supplementary Table 33), which permits 4–5 generations a year, depending on genotype and crossing plans (Supplementary Fig. 4). These results highlight the flexibility of tailoring the speed breeding ‘recipe’ to suit the local purpose and resources.

Besides exploring the various ways in which speed breeding can be used to accelerate generation time, we also evaluated the ability to phenotype some key adult plant phenotypes of wheat and barley. We observed that phenotypes associated with the ethylmethane sulfonate-induced mutation of the awn suppressor *B1* locus⁹ and the Green Revolution *Reduced height* (*Rht*) genes in wheat¹⁰ could be accurately recapitulated in the controlled-environment room conditions (Fig. 3a,b). We also evaluated the effects that speed breeding might have on disease by inoculating resistant (Sumai 3) and susceptible (Timstein) wheat spikes with *Fusarium graminearum*, the causal agent of fusarium head blight (FHB). Consistent with expectations, we found clear signs of FHB progression in the susceptible cultivar, and little to no disease progression in the resistant cultivar (Fig. 3c; Supplementary Table 34). Previously, it has been shown that adult plant resistance to wheat leaf rust (caused by *Puccinia triticina* f. sp. *tritici*) and wheat stripe rust (*Puccinia striiformis* f. sp. *tritici*) can also be scored accurately under speed breeding conditions^{11,12}. We also evaluated the effect of loss of function of *FLOWERING LOCUS T-B1* in the F_6 recombinant inbred line of Paragon x W352 under speed breeding conditions. We observed the expected late flowering phenotype in the recombinant inbred line, albeit within fewer days and with fewer leaves produced (Fig. 3d; Supplementary Table 35). To study the effect of speed breeding on cuticular β -diketone wax production in barley, we grew wild type and *Eceriferum* mutants¹³. The mutant leaf sheaths exhibited a clear, decreased glaucous appearance in the flag leaf stage, compared to the control (Fig. 3e). We also studied polyploid wheat chromosome pairing at metaphase I in meiosis in the presence and absence of the *PAIRING HOMOELOGOUS 1* (*Ph1*) locus¹⁴. We grew wheat carrying *Ph1* and wheat-rye hybrids either carrying or lacking *Ph1* in speed breeding and control conditions, and observed no major differences in chromosome pairing and recombination in meiocytes at metaphase I (Supplementary Table 36). The chromosome behaviour suggests that both wheat and wheat-rye hybrids are cytologically stable under speed breeding conditions (Supplementary Fig. 5). In summary, all the above adult plant phenotypes could be recapitulated accurately and much faster than in the corresponding glasshouse conditions.

We further investigated the potential for using speed breeding in conjunction with genetic transformation. Seeds of barley (*H. vulgare* cv. Golden Promise) were sown and immature embryos harvested and transformed (Supplementary Fig. 6). The transformation efficiency of speed-grown and normal plants was comparable (Supplementary Table 37). Moreover, we observed that transformed barley explants could be grown under speed breeding conditions and viable seed obtained >6 weeks earlier compared to standard control conditions (Supplementary Table 38; Supplementary Fig. 7).

We investigated the ability to phenotype canola, grown under speed breeding conditions, for pod shattering resistance, a significant factor causing yield loss in the field. Using an established method which measures the energy absorbed by each pod when shattered by a pendulum¹⁵ (Supplementary Fig. 8), we tested five



Fig. 3 | Adult plant phenotypes in wheat and barley under speed breeding conditions. **a**, Loss of function of the awn suppressor *B1* locus in *T. aestivum* cv. Paragon (mutant on left, wild type on right). **b**, Green Revolution *Reduced height* (*Rht*) dwarfing genes under speed breeding conditions. From left to right, *T. aestivum* cv. Maringá wild type, Maringá *Rht-1*, Maringá *Rht-3*. Scale bar = 100 cm. **c**, FHB progression in spikes of the resistant *T. aestivum* cv. Sumai 3 (right) and the susceptible cv. Timstein (left) at 7 and 10 days post inoculation (dpi). **d**, Two instances depicting the flowering time of the F_6 hybrid (right in both panels) derived from *T. aestivum* cv. Paragon (left in both panels) and *T. aestivum* landrace W352 (centre in both panels). In the left panel, the parents flower while the F_6 is just beginning to boot at 44 days; in the right panel, the F_6 hybrid is shown with developed spikes at 67 days when the parents have moved into senescence. **e**, Reduced glaucousness of *Eceriferum* (*Cer*) mutants in *H. vulgare* cv. Bonus. WT, wild type.

canola cultivars considered susceptible to shattering. All displayed varying degrees of pod strength comparable to measures obtained from field-grown pods (Supplementary Table 39). Due to the lack of shatter resistance in cultivars, new alleles from related Brassica species could be transferred to canola¹⁶. Our results suggest that speed breeding could accelerate this objective.

Speed breeding is likely to reduce generation time for other crop species, for example, sunflower (*Helianthus annuus*), pepper (*Capsicum annuum*), and radish (*Raphanus sativus*), which have been shown to respond well to extended photoperiod². Speed breeding methods have already been successfully applied to accelerate breeding objectives for amaranth (*Amaranthus* spp.)¹⁷, peanut (*Arachis hypogaea*)¹⁸ and pea (*P. sativum*)¹⁹. In the latter study, the gibberellic acid biosynthesis inhibitor flurprimidol was applied to induce a compact growth habit, permitting an increased cultivation density. While the direct application of our speed breeding protocols to short-day species such as maize or rice are unlikely to be successful, there is probably room for optimization to achieve faster generation times in these species. For instance, other growth parameters could be tweaked to accelerate the generation time, such as optimizing photoperiod, temperatures, plant density and watering regime. In a breeding context, it should be noted that phenotypes studied under controlled or glasshouse conditions do not always correlate with field observations; in particular, complex traits such as yield and abiotic stress tolerance. Yet, phenotypic selection in early segregating generations of wheat (F_2 and F_3) has been successfully implemented for some desirable traits such as stripe rust resistance¹² and grain dormancy²⁰. Alternatively, speed breeding could be used to rapidly generate fixed populations through SSD, which in some species may be cheaper than generating double haploids, for subsequent field evaluation and selection, thus facilitating genetic gain and production of improved cultivars. For genetically well-defined

traits, speed breeding could be used to rapidly introgress genes or haplotypes into elite lines using marker-assisted selection²¹.

Recent advances in genomic tools and resources^{22–26} and the decreasing cost of sequencing have enabled plant researchers to shift their focus from model to crop plants. Despite such advances, the slow generation times of many crop plants continue to impose a high entry barrier. We envisage that combining these tools and resources with speed breeding will provide a strong incentive for more plant scientists to perform research on crop plants directly, thus further accelerating crop improvement research. In a breeding context, rapid generation advance to homozygosity following crossing will facilitate genetic gain for key traits and allow more rapid production of improved cultivars by breeding programs.

Methods

Speed breeding I—Controlled-environment chamber speed breeding conditions.

A Conviron BDW chamber (Conviron, Canada) was programmed to run a 22-hour photoperiod, with a temperature of 22°C during the photoperiod, and 17°C during the 2-hour dark period. Light and temperature were set to ramp up and down for 1 hour 30 minutes to mimic natural dawn and dusk conditions (as illustrated in Fig. 1). Humidity was set to 70%. Lighting was supplied by a mixture of white LED bars (Valoya; 6 units per 7.6 m²), far-red LED lamps (Valoya; 12 units per 7.6 m²) and ceramic metal halide quartz iodide lamps (Valoya; 32 units per 7.6 m²). Light intensity was adjusted to 360–380 µmol m⁻² s⁻¹ (highest value after ramping) at bench height, where the pots were kept, and 490–500 µmol m⁻² s⁻¹ (highest value after ramping) at adult plant height (with reference to wheat, *T. aestivum* cv. Paragon). Light quality (spectral composition) is shown in Supplementary Fig. 9.

A timelapse video of *T. aestivum* cv. Paragon under speed breeding condition I ('speed breeding') and control glasshouse conditions ('control conditions') during the UK summertime with no supplementary light in the spring and summer of 2017 is given in Supplementary Video 1 and the accompanying plant height growth curve in Supplementary Fig. 10.

Sowing, harvesting and germination rate evaluation for speed breeding in a controlled-environment room chamber.

Wheat, barley and *Brachypodium* seeds were imbibed on filter paper wetted with sterile water containing 0.5 ppm gibberellic acid, stratified at 4°C for 3 days and then germinated at room temperature for 2 days before sowing in 1.33 l pots containing 900 ml of soil (one seed per pot). The soil used was the John Innes Centre (JIC) cereal mix, the composition of which is outlined in Supplementary Table 40. Seeds were harvested ~2.5–3 weeks post anthesis. The plants were not watered in the last week leading up to harvest in order to accelerate the grain ripening. The harvested spikes were kept in paper bags and dried at 28–30°C for 3–5 days to reduce the moisture content. The grain was threshed and stored at room temperature. To measure germination rates, seeds were placed in Petri dishes lined with filter paper and wetted with sterile water. The seeds were stratified at 4°C for 3 days and then placed at room temperature, with periodic rehydration of the filter paper lining to maintain moisture levels. Seeds were observed for germination at daily intervals for 5 days.

Pea seeds of the John Innes accession JI 2822 were scarified and sown into pots of approximately 260 ml volume with JIC Cereal Compost Mix (Supplementary Table 40). Pots were placed in a glasshouse with no supplementary light and a minimum temperature of 12°C, or the Conviron chamber under speed breeding conditions, and moved around every 2–3 days in a rotating fashion to ensure uniformity of growth conditions. The number of days after sowing until the first flower opened, flowering node number, number of pods, plant height and seed number were recorded (Supplementary Table 10). At 6 weeks and 9 weeks, respectively, the plants in the cabinet and glasshouse were no longer watered. Mature, dry seeds were chipped and placed on moist filter paper for 5 days at room temperature in the dark to estimate percentage germination. The number of seeds where the radicle and shoot had not emerged were counted as not germinated.

Wild-type *M. truncatula* (genotype Jemalong A17) seeds were scarified with glasspaper, surface-sterilized for 3 minutes in 10% sodium hypochlorite and washed five times with distilled water. Seeds were left to imbibe for 2 hours before being transferred to agar plates for 72 hours at 4°C. Seedlings were sown in JIC Cereal Compost Mix in 24-cell trays (each cell containing 80 ml of soil) and transferred to 1 l pots after 14 days. The number of days after sowing for the first flower to appear was recorded. Watering stopped following maturation of ~90% of the pods. Pods were harvested 14 days later and the resulting seeds subjected to germination tests similar to pea.

Speed breeding II—Glasshouse speed breeding conditions. A temperature-controlled glasshouse fitted with high-pressure sodium vapour lamps (Philips SON-T 400 W E40 in Sylvan High Bay housing with glass diffuser) was programmed to a 17/22°C temperature regime with a 12-hour turnover and 22-hour photoperiod (Supplementary Fig. 11). The 2-hour period without lamps operating and 17°C cycle

occurred during the night. Light intensity was 440–650 µmol m⁻² s⁻¹ at adult plant height (approximately 45 cm above bench height). Light and temperature changes did not include a ramping up/down procedure. Light quality (spectral composition) is shown in Supplementary Fig. 12.

Sowing, harvesting and germination rate evaluation for speed breeding in a light-supplemented glasshouse. Wheat, barley, canola and chickpea seeds were imbibed by placing on water-moistened filter paper in agar plates overnight at room temperature. The imbibed seeds were chilled at 4°C for 4 days to break any dormancy and support uniform germination. Seeds were then left at room temperature for 5 days. All seedlings were sown singly into 1.4 l pots. Soil media consisted of the CGS20 compost mix (Supplementary Table 41) with the addition of Scotts Osmocote Plus trace elements (2 g per l soil) and a final pH of 5.5–6.5. Pots were placed in pot racks in complete randomized block designs and hand-watered daily. Plants were supplemented with a weekly foliar spray of calcium nitrate diluted in filtered water (1 g per l) to mitigate any calcium deficiency, which is common with such rapid growth²⁷.

Anthesis date was recorded for wheat, canola and chickpea, while awn peep was recorded for barley. One spike was harvested from wheat and barley at 2 and 4 weeks post anthesis. Three pods per plant of canola and chickpea were harvested 6 weeks post anthesis. All harvested seeds were placed in paper bags and immediately dried in an air-forced oven for 5 days at 35°C, threshed by hand, then weighed. Germination tests were carried out as per the presowing treatments described above. Percentage germination was calculated following 5 days at room temperature. At approximately 4–6 weeks following anthesis, water supply was reduced to every second day for 1 week, then twice per week for 1 week, followed by complete withholding of water. When plants were fully senesced, all spikes or pods were harvested, counted, dried, threshed and weighed to determine yield. Grain number per spike of wheat and barley plants was counted following threshing of each individual spike.

One cultivar each of wheat (*T. aestivum* cv. Westonia) and barley (*H. vulgare* cv. Commander) was sown in a 100-cell tray (length, 350 mm; width, 290 mm; height, 45 mm; each cell approximately 18 ml) to demonstrate the potential of high-density planting. Three spikes were harvested at 2 and 4 weeks post anthesis and percentage germination determined, as above. The watering regime was also as above. Following complete senescence, all grain was harvested, dried, threshed and weighed to determine yield.

As a control, an identical protocol was carried out on the same species/cultivars in a similar glasshouse maintained at the same temperature settings but with no supplemental lighting.

Speed breeding III—Homemade growth room design for low-cost speed breeding.

A room of 3 m × 3 m × 3 m with insulated sandwich panelling fitted with seven LB-8 LED light boxes (one light box per 0.65 m²) from Grow Candy (www.growcandy.com) and a 1.5 horsepower inverter split system domestic air conditioner was set up as a low-cost alternative to the Conviron BDW chamber. The light spectrum details are outlined in Supplementary Fig. 13. The light quantity of photosynthetically active radiation (PAR) at bench height ranged from 210–260 µmol m⁻² s⁻¹ and at 50 cm above the pot from 340–590 µmol m⁻² s⁻¹. The lights were situated at a height of 140 cm above the bench. The room can accommodate 90 pots of 20.3 cm diameter and 5 l volume. Automatic watering was achieved with the Hunter 10 Station Irrigation Controller, with one solenoid per room and a 13 mm main line with spike drippers (one per 20.3 cm pot). The humidity conditions were ambient.

The lighting was set to run a 12-hour photoperiod (12 hours of darkness) for 4 weeks and then increased to an 18-hour photoperiod (6 hours of darkness). The lights did not ramp up and down during the switch between light and dark periods in the 24-hour cycle. The air-conditioner was set to run at 18°C in darkness and 21°C when the LED lights were on, with fluctuation being no more than ±1°C.

This system was used for speed breeding of wheat (*T. aestivum* cvs Morocco and AvocetR), barley (*H. vulgare* cvs Gus and Baudin), oat (*Avena sativa* cv. Swan) and triticale (*Triticosecale* cvs Jackie and Coorong).

The speed breeding experiments I and II used artificial light sources with enriched PAR in the blue, red and far-red part of the spectrum (Supplementary Figs. 9 and 13). Some manufacturers now produce LED lights that can be programmed to optimize PAR and customize the wavelength and intensity to suit different growth stages and plant species (https://www.heliospectra.com/sites/default/files/botanical_garden_gothenburg_-_case_study.pdf). We predict this will significantly contribute to further increase plant growth performance in the academic and commercial research sector for a large range of species.

Adult plant phenotyping protocols. Canola pod shattering resistance phenotyping.

Five canola cultivars were selected for evaluation (cvs Skipton, CB Argyle, ATR Cobler, ATR Beacon and Bravo TT), where ten mature pods representative of normal development were harvested from each plant grown under speed breeding II conditions. This resulted in 30 pods per genotype (three replicate plants per genotype). Pods from each plant were placed together in 50 ml lidded plastic Falcon tubes with silica beads to absorb any moisture. These were then phenotyped using the pendulum pod shatter protocol¹⁵ at the NSW Department of Primary

Industries, Wagga Wagga Agricultural Institute (WWAI), Wagga Wagga, Australia. The rupture energy measures were adjusted for variation in pod length to calculate rupture energy per length squared, as per²⁸. The five canola cultivars were also grown in field row plots at the experimental farm of WWAI in two replicates¹⁶ and evaluated for shattering resistance using the same technique, where ten fully mature pods were sampled per plot.

Wheat crossing and evaluation of crossing efficiency. Newly emerged spikes were emasculated to remove all immature anthers. The emasculated spikes were bagged for up to 3 days to allow for maturation of the stigma and then pollinated by anthers from a separately selected parent. The spikes were checked for seed setting 3–5 days post pollination. Crossing efficiency was calculated as the percentage of emasculated ovaries that successfully set seed.

Wheat flowering time. For measurement of flowering time of the wheat cultivars Paragon, Watkins landrace W352²⁹ and the late flowering Paragon x W352 F₆ recombinant inbred line, the number of days taken to reach heading was recorded for each replicate of each line. At the same time, the leaves on the main tiller were counted (starting from the three-leaf stage) until the time of heading.

Wheat seed count. Seeds were manually counted in each spike. Images were obtained using a Nikon DSC D90 camera (HFX-DX, Melville, NY) and processed using ViewNX 2 software, produced by Nikon (Nikon, Surrey, UK) (Supplementary Fig. 14). Details are given in the Supplementary Information.

Wheat and barley single seed weight. Three replicates of ten seeds were weighed for each replicate plant within each genotype and averaged. This was then divided by ten to obtain an estimate of single grain weight for each plant. This was averaged across the three genotype replicates.

FHB infections. The test undertaken was for type II resistance, which is resistance to spread from floret to floret within the head. A suspension of 1×10^6 conidiospores per ml of *F. graminearum*, which had been cultured in a mungbean liquid media, was used for point inoculations³⁰. The isolate was a mix of two aggressive deoxynivalenol-producing *F. graminearum* UK isolates (K1-4/K1-5), obtained from the JIC's Facultative Pathogen Collection. At least two spikes on each of three plants of the resistant wheat (*T. aestivum* cv. Sumai 3) and three plants of the susceptible wheat (*T. aestivum* cv. Timstein) were inoculated.

Transformation of barley. Immature embryos of the barley (*H. vulgare* cv. Golden Promise) were inoculated with *Agrobacterium* strain AGL1 and transformed plants were recovered following the protocol of Harwood³¹. Constructs from the pBRAC1 series³² containing selection cassettes conferring resistance to the antibiotic hygromycin were introduced.

Wheat meiosis studies. The anthers used for this study came from hexaploid wheat (*T. aestivum* cv. Chinese Spring) carrying the *Ph1* locus and crosses between rye (*Secale cereale* cv. Petkus) and hexaploid wheat (*T. aestivum* cv. Chinese Spring) either carrying or lacking the *Ph1* locus (*ph1b* deletion)³³.

Seeds were stratified on wet filter paper in the dark for 5 days at 4 °C, followed by a period of germination for 24 hours at 25 °C. The seedlings were vernalized for 3 weeks at 5 °C and then transferred to different controlled-environment rooms until meiosis. Three seedlings from each genotype were transferred to the Convicon speed breeding chamber and an additional three were transferred to a glasshouse with no supplementary lighting or heating, as a control. From each plant, one spike was collected for the determination of meiotic stability in pollen-mother cells. For meiosis studies, suitable young spikes were excised and fixed in ethanol/acetic acid (3:1). The samples were kept at room temperature, for at least 1 week, until slide preparation. Each floret has three synchronous anthers, and so one anther per floret was squashed in 45% acetic acid in water and assigned at metaphase I in meiosis by observation under a Leica DM2000 microscope (Leica Microsystems Ltd., Heerbrugg, Germany; <http://www.leica-microsystems.com>). The two remaining anthers were stained following the Feulgen procedure³⁴. The present observations were limited to this stage. Images were collected using the Leica DM2000 microscope equipped with a Leica DFC450 camera (Leica Microsystems) and processed using LAS V4.4 system software (version 4.4, Leica Microsystems). Four parameters of meiotic stability were studied, including univalents, bivalents, multivalents and crossovers. The number of cells observed in each plant in the two different photoperiods was 80. Further details are given in the Supplementary Information.

Statistical analyses were performed using STATISTIX 10.0 software (Analytical Software, Tallahassee, FL, US). The analysis of variance was based on randomised blocks. In wheat-rye hybrids lacking the *Ph1* locus, tangent transformation was applied to univalents to meet the requirement of homogeneity of variances. Means were separated using the least significant difference test with a probability level of 0.05.

Successive generations under speed breeding I and II. In order to demonstrate the health and normal development of successive generations of wheat and barley plants grown under speed breeding, a second generation was grown from seeds obtained through both speed breeding and control (glasshouse grown) plants.

Wheat (*T. aestivum* cv. Paragon) seed procured from plants grown under speed breeding I and harvested immature (2 weeks post anthesis) and seed harvested at maturity under UK summertime glasshouse conditions were grown under speed breeding I conditions (Supplementary Fig. 2, Supplementary Table 27).

To determine the effect on subsequent generations for speed breeding II conditions, a second generation of wheat (*T. aestivum* cv. Suntop) and barley (*H. vulgare* cv. NRB090257) was grown under these conditions using seed harvested at maturity from plants grown under speed breeding II and glasshouse conditions with no supplementary lighting (Supplementary Fig. 3, Supplementary Tables 28 and 29).

Data availability. The data that support the findings of this study are presented in the supplementary materials and methods and/or available from the corresponding authors upon request.

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References

- Ray, D. K., Mueller, N. D., West, P. C. & Foley, J. A. Yield trends are insufficient to double global crop production by 2050. *PLoS ONE* **8**, e66428 (2013).
- Sysoeva, M. I., Markovskaya, E. F. & Shibaeva, T. G. Plants under continuous light: A review. *Plant Stress* **4**, 5–17 (2010).
- Deng, W. et al. Dawn and dusk set states of the circadian oscillator in sprouting barley (*Hordeum vulgare*) seedlings. *PLoS ONE* **10**, e0129781 (2015).
- Domoney, C. et al. Exploiting a fast neutron mutant genetic resource in *Pisum sativum* (pea) for functional genomics. *Funct. Plant Biol.* **40**, 1261–1270 (2013).
- Zheng, Z., Wang, H., Chen, G., Yan, G. & Liu, C. A procedure allowing up to eight generations of wheat and nine generations of barley per annum. *Euphytica* **191**, 311–316 (2013).
- Went, F. The effect of temperature on plant growth. *Annu. Rev. Plant Physiol.* **4**, 347–362 (1953).
- Chahal, G. & Gosal, S. *Principles and Procedures of Plant Breeding: Biotechnological and Conventional Approaches* (Alpha Science International Ltd, Pangbourne, 2002).
- Wada, K. C. & Takeno, K. Stress-induced flowering. *Plant Signal. Behav.* **5**, 944–947 (2010).
- Derkx, A. P., Orford, S., Griffiths, S., Foulkes, M. J. & Hawkesford, M. J. Identification of differentially senescing mutants of wheat and impacts on yield, biomass and nitrogen partitioning. *J. Integr. Plant Biol.* **54**, 555–566 (2012).
- Hoogendorn, J., Pfeiffer, W. H., Rajaram, S. & Gale, M. D. in *Proc. 7th Int. Wheat Genetics Symp.* (eds Koebner, R. M. D. & Miller, T. E.) 1093–1100 (IPSR, Cambridge, 1988).
- Riaz, A., Periannan, S., Aitken, E. & Hickey, L. A rapid phenotyping method for adult plant resistance to leaf rust in wheat. *Plant Methods* **12**, 17 (2016).
- Hickey, L. T. et al. Rapid phenotyping for adult-plant resistance to stripe rust in wheat. *Plant Breeding* **131**, 54–61 (2012).
- Lundqvist, U. & Wettstein, D. V. Induction of *eceriferum* mutants in barley by ionizing radiations and chemical mutagens. *Hereditas* **48**, 342–362 (1962).
- Riley, R. Genetic control of the cytological diploid behaviour of hexaploid wheat. *Nature* **182**, 713–715 (1958).
- Liu, X. Y., Macmillan, R., Burrow, R., Kadkol, G. & Halloran, G. Pendulum test for evaluation of the rupture strength of seed pods. *J. Texture Stud.* **25**, 179–190 (1994).
- Raman, H. et al. Genome-wide delineation of natural variation for pod shatter resistance in *Brassica napus*. *PLoS ONE* **9**, e101673 (2014).
- Stetter, M. G. et al. Crossing methods and cultivation conditions for rapid production of segregating populations in three grain amaranth species. *Front. Plant Sci.* **7**, 816 (2016).
- O'Connor, D. J. et al. Development and application of speed breeding technologies in a commercial peanut breeding program. *Peanut Science* **40**, 107–114 (2013).
- Mobini, S. H. & Warkentin, T. D. A simple and efficient method of in vivo rapid generation technology in pea (*Pisum sativum* L.). *In Vitro Cell. Dev. Plant* **52**, 530–536 (2016).
- Hickey, L. T. et al. Screening for grain dormancy in segregating generations of dormant × non-dormant crosses in white-grained wheat (*Triticum aestivum* L.). *Euphytica* **172**, 183–195 (2010).
- Hickey, L. T. et al. Speed breeding for multiple disease resistance in barley. *Euphytica* **213**, 64–78 (2017).
- Steuernagel, B. et al. Rapid cloning of disease-resistance genes in plants using mutagenesis and sequence capture. *Nat. Biotechnol.* **34**, 652–655 (2016).
- Sánchez-Martín, J. et al. Rapid gene isolation in barley and wheat by mutant chromosome sequencing. *Genome Biol.* **17**, 221 (2016).
- Krasileva, K. V. et al. Uncovering hidden variation in polyploid wheat. *Proc. Natl Acad. Sci. USA* **114**, E913–E921 (2017).

25. Liang, Z. et al. Efficient DNA-free genome editing of bread wheat using CRISPR/Cas9 ribonucleoprotein complexes. *Nat. Commun.* **8**, 14261 (2017).
26. Cavanagh, C. R. et al. Genome-wide comparative diversity uncovers multiple targets of selection for improvement in hexaploid wheat landraces and cultivars. *Proc. Natl Acad. Sci. USA* **110**, 8057–8062 (2013).
27. Hao, X., Zheng, J. & Brown, J. in *6th Int. Symp. Light in Horticulture* 61 (ISHS, Tsukuba, 2011).
28. Kadkol, G., Halloran, G. & MacMillan, R. Evaluation of *Brassica* genotypes for resistance to shatter. II. Variation in silique strength within and between accessions. *Euphytica* **34**, 915–924 (1985).
29. Wingen, L. U. et al. Establishing the AE Watkins landrace cultivar collection as a resource for systematic gene discovery in bread wheat. *Theor. Appl. Genet.* **127**, 1831–1842 (2014).
30. Gosman, N., Steed, A., Chandler, E., Thomsett, M. & Nicholson, P. Evaluation of type I fusarium head blight resistance of wheat using non-deoxynivalenol-producing fungi. *Plant Pathol.* **59**, 147–157 (2010).
31. Harwood, W. A. in *Cereal Genomics: Methods and Protocols* Vol. 1099 (eds Henry, R. & Furtado, A.) 251–260 (Humana Press, Totowa, 2014).
32. Smedley, M. A. & Harwood, W. A. in *Agrobacterium Protocols: Methods in Molecular Biology* Vol. 1223 (ed. Wang, K.) 3–16 (Springer, New York, 2015).
33. Sears, E. R. Genetics society of Canada award of excellence lecture an induced mutant with homoeologous pairing in common wheat. *Can. J. Genet. Cytol.* **19**, 585–593 (1977).
34. Sharma, A. K. & Sharma, A. *Chromosome Techniques: Theory and Practice* (Butterworth-Heinemann, Oxford, 2014).

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Author contributions

A.W. and S.G. performed the experiments for studying two generations of wheat and barley under Speed breeding I and II, managed and oversaw the other experiments and wrote the manuscript. A.W. performed experiments studying growth of canola and chickpea under Speed breeding II. Speed breeding I: N.M.A. grew and evaluated the phenotype of the barley *eceriferum* mutants; S.A.B. provided advice for growth conditions; J.S. made the crosses and some germination tests; M.-D.R. conducted chromosome association studies during meiosis in wheat; M.A.M.H. studied development of transgenic barley lines; A.H. and W.H. contributed to design and execution of barley transformation tests; A.S. performed the inoculation and phenotyping for wheat *Fusarium* headblight infections; L.E.D. and S.A.B. selected and grew varieties involved in the flowering time phenotype; A.K., A.B. and C.R. performed all testing and measuring for Medicago; T.R. and C.D. performed the same for rapid cycling pea; D.R. and J.Z. filmed plant growth, created timelapse videos and performed growth phenotypic analysis; J.C. performed the energy calculations and provided some necessary data for such; C.U., G.M. and P.N. contributed experiment design, variety choice and phenotypes. Speed breeding II: A.R., D.E. and J.B. designed and conducted the initial pilot experiments on canola under speed breeding; H.R. contributed to the design and phenotyping of canola pod shatter resistance; M.R. and W.M. provided the chickpea varieties suitable for evaluation under speed breeding; M.J.D. and I.H.D. first came up with the idea of speed breeding and developed the initial framework for the protocol at UQ, Brisbane. A.R. and L.T.H. designed and conducted initial pilot experiments investigating harvest of immature wheat seed. Speed breeding III: M.J.W., W.S.C. and R.F.P. were involved in building, testing and implementing the cheaper, full LED version of speed breeding in closed environments. B.B.H.W. and L.T.H. contributed to the idea of conducting and summarizing these experiments in the manuscript, and contributed in terms of intellectual input for the experiments. All authors were involved in the writing of the manuscript and providing corrections and suggestions.

Competing interests

The authors declare no competing financial interests.

Additional information

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► Experimental design

1. Sample size

Describe how sample size was determined.

Under SBI, three replicates (or five replicates in the case of pea) were used. Under SBII, three replicates of each line of wheat (10 lines), barley (10 lines), and canola (8 lines) were used. Five replicates of chickpea (4 lines) were used. These experiments were grown under controlled environmental conditions, so low variation within a genotype was expected.

2. Data exclusions

Describe any data exclusions.

There were no data exclusions within experiments from SBI or SBII results.

3. Replication

Describe whether the experimental findings were reliably reproduced.

In SBI, glasshouse controls cannot be exactly replicated under extreme weather conditions. There were no failures to replicate experimental findings under SBII.

4. Randomization

Describe how samples/organisms/participants were allocated into experimental groups.

Under SBI, plants were moved around on a regular basis to minimize local environmental effects on plant phenotype. Under SBII, all experiments were arranged in a randomised complete block design (see page 15 main text).

5. Blinding

Describe whether the investigators were blinded to group allocation during data collection and/or analysis.

NA

Note: all studies involving animals and/or human research participants must disclose whether blinding and randomization were used.

6. Statistical parameters

For all figures and tables that use statistical methods, confirm that the following items are present in relevant figure legends (or in the Methods section if additional space is needed).

n/a Confirmed

- ☐ ☒ The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.)
- ☐ ☒ A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
- ☒ ☐ A statement indicating how many times each experiment was replicated
- ☐ ☒ The statistical test(s) used and whether they are one- or two-sided (note: only common tests should be described solely by name; more complex techniques should be described in the Methods section)
- ☒ ☐ A description of any assumptions or corrections, such as an adjustment for multiple comparisons
- ☐ ☒ The test results (e.g. *P* values) given as exact values whenever possible and with confidence intervals noted
- ☐ ☒ A clear description of statistics including central tendency (e.g. median, mean) and variation (e.g. standard deviation, interquartile range)
- ☒ ☐ Clearly defined error bars

See the web collection on statistics for biologists for further resources and guidance.

► Software

Policy information about availability of computer code

7. Software

Describe the software used to analyze the data in this study.

Excel, STATISTIX 10.0 and/or R was used to calculate means and standard deviations and perform statistical tests.

For manuscripts utilizing custom algorithms or software that are central to the paper but not yet described in the published literature, software must be made available to editors and reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). *Nature Methods* guidance for providing algorithms and software for publication provides further information on this topic.

► Materials and reagents

Policy information about availability of materials

8. Materials availability

Indicate whether there are restrictions on availability of unique materials or if these materials are only available for distribution by a for-profit company.

Some varieties evaluated in speed breeding I and II are subject to Plant Breeders Rights and may require an MTA for transfer to another party. Vectors used in barley transformation may require an MTA. Image collection and analysis in Speed Breeding video was conducted using a proprietary set-up and software (CropQuant).

9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).

NA

10. Eukaryotic cell lines

a. State the source of each eukaryotic cell line used.

Provide information on cell line source(s) OR state that no eukaryotic cell lines were used.

b. Describe the method of cell line authentication used.

Describe the authentication procedures for each cell line used OR declare that none of the cell lines used have been authenticated OR state that no eukaryotic cell lines were used.

c. Report whether the cell lines were tested for mycoplasma contamination.

Confirm that all cell lines tested negative for mycoplasma contamination OR describe the results of the testing for mycoplasma contamination OR declare that the cell lines were not tested for mycoplasma contamination OR state that no eukaryotic cell lines were used.

d. If any of the cell lines used are listed in the database of commonly misidentified cell lines maintained by ICLAC, provide a scientific rationale for their use.

Provide a rationale for the use of commonly misidentified cell lines OR state that no commonly misidentified cell lines were used.

► Animals and human research participants

Policy information about studies involving animals; when reporting animal research, follow the ARRIVE guidelines

11. Description of research animals

Provide details on animals and/or animal-derived materials used in the study.

NA

Policy information about studies involving human research participants

12. Description of human research participants

Describe the covariate-relevant population characteristics of the human research participants.

NA