Speeding-breeding for wheat

Watson et al 2018[1] and Ghosh et al 2018[2] showed that by using specific growing conditions and premature seed harvesting, one can shorten wheat generation time down to 8-10 weeks. This is particularly useful for population development or to produce double or triple knockout mutants from single mutant lines (see Designing crossing schemes). Briefly, it involves a longer light period of 22 hours followed by a 2 hours recovery in the dark (22 °C / 17 °C, respectively). The spikes are harvested ~ 2-3 weeks post-anthesis and dried at 28-30 °C for 3-5 days prior to threshing. These conditions can be applied in either a controlled-environment chamber or a glasshouse setting (**Figure 1**).

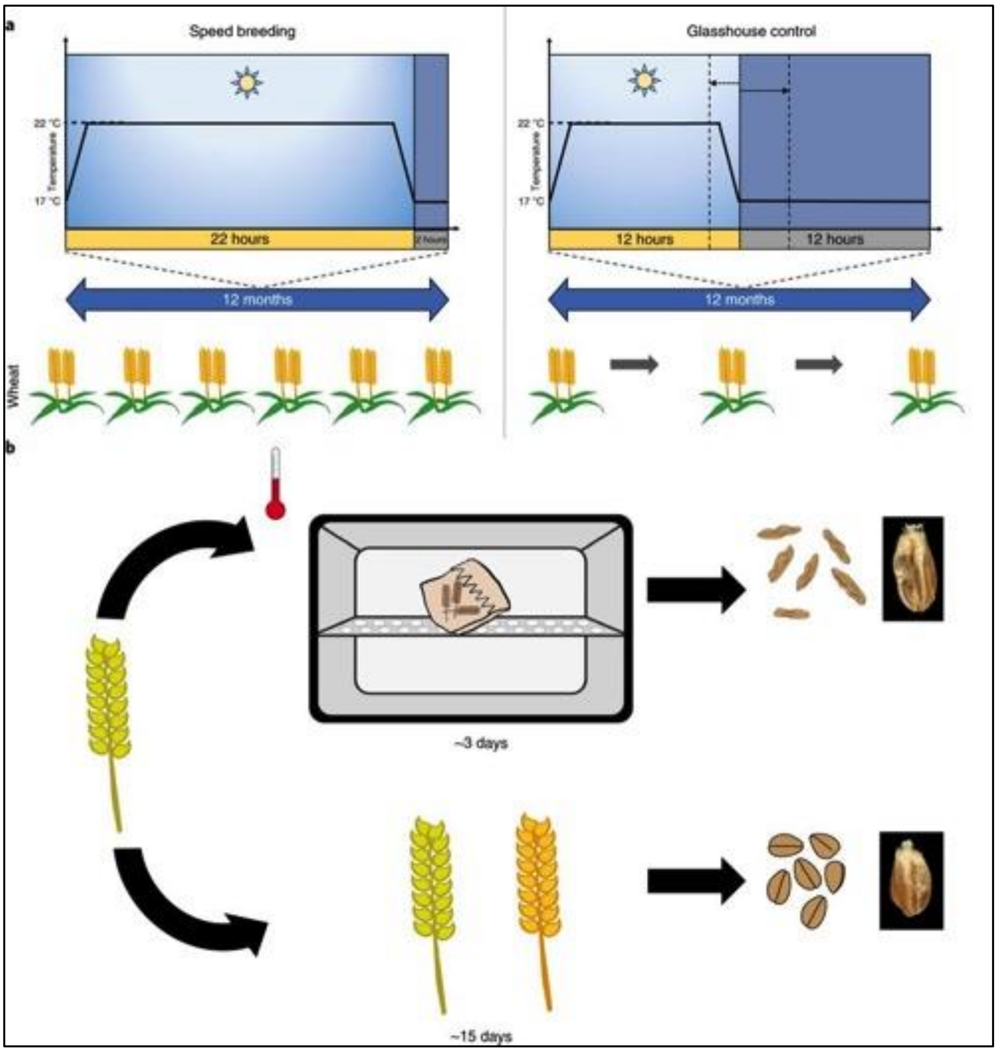

Figure 1. Overview of the speed breeding procedure and comparison to standard glasshouse conditions
(a) Six generations can be achieved for wheat in speed breeding conditions.
(b) Harvesting immature spikes and drying them at 30 °C for 3 days allows for faster seed to seed cycling, although it comes with a loss in grain weight. Adapted from Watson et al., (2018).

Here we extracted and summarised the procedure for wheat. Please do consult the original papers for detailed protocols and conditions as well as experiments showing the effects of speed breeding conditions on plants.

a) Controlled-environment chamber

This section summarises how to set up speed breeding in an existing growth chamber. Details are available in Ghosh et al. 2018.

Lights: As for any plant growth chamber, it is crucial that the lighting system covers the photosynthetically active radiation (400-700nm) with special focus on blue, red and far-red regions of the spectrum. It was shown that LED and ceramic halogen lights (or a combination of the two) are suitable for this, although LEDs are more energy efficient and thus more cost-effective in the long term. A photosynthetic photon flux density of 450-500 µmol m^{-2} s^{-1} at plant canopy height is recommended. As mentioned above, a photoperiod of 22 hours with 2 hours of darkness was used. Gradually increasing/decreasing light intensity to mimic dawn/dusk might help.

Temperature/Humidity: The temperature cycling regime to apply is: 22 °C / 17 °C for 22 hours light and 2 hours dark, respectively. Humidity ranging from 60-70 % is ideal.

b) Glasshouse

This section summarises setting-up speed breeding in an existing glasshouse. Details are available in Ghosh et al. 2018.

Lights: LED lamps are energy efficient with little maintenance required and thus the most cost-effective option in the long term. The speed breeding regime uses 22 hours of light and 2 hours of darkness. Ideally, programmable lights allowing intensity modification according to sensor feedback should be used to minimise the effect of environmental variables such as ambient light. Controllable blinds may be used to precisely control photoperiod.

Temperature: Ideally the glasshouse is fitted with a temperature-controlled system to avoid any over-heating or significant drop of temperature. The maximum allowed temperature during the photoperiod ranges from 20-22 °C and the minimum temperature set for the dark period ranges from 15-17 °C.

c) Preparing seed for sowing

See corresponding section in **How to grow wheat**. However, it is important to know that if seeds are too well established in Petri dishes (i.e. already developing green leaves), speed breeding treatment can induce a strong stress that possibly leads to the death of the plants. Thus, ensure sowing seed early if pre-germination is used.

d) Seed harvesting

As mentioned above, immature spikes can be harvested as soon as the grain moves past milk stage into dough stage (see section **Wheat development**). Seed can then be dried at 30 °C for 3-5 days or using a desiccator. The harvested seeds can look quite shrivelled, but normally do not exhibit much grain dormancy. It may be helpful to stratify them at 4 °C for 3 days or so in the dark to reduce the chances of failed germination.

e) Soil and fertilisation

Accelerated growth may produce nutrient deficiency. It is thus very important to supply the plants with enough fertiliser and a proper soil mixture. JIC Cereal Compost Mix and UQ Compost Mix were both shown to be suitable for wheat speed breeding conditions (see **Tables 1** and **2**). Appropriate slow-release fertiliser within the soil is essential in preventing nutrient deficiency.

Calcium deficiency is a common symptom resulting from speed breeding conditions and mostly occurs at early growth stages – it produces bumps and bubbles on the leaves, and some hypersensitive responses as well. Foliar fertiliser application with calcium-containing fertiliser will help to counteract this issue.

Table 1. Compost mix components and fertilisers designed by Mr K. Hayes, Central Glasshouse Services, UQ, Australia.

From Ghosh et al., (2018)

pH is balanced with either FeSO if high or Dolomite if low

Component Measure
Composted pine bark (0-5 mm) **70 %**
Coco peat **30 %**
Fertiliser
Yates Flowtrace (Yates, Padstow, NSW, Australia) **1.0 kg m^{-3}**
Iron sulphate heptahydrate **1.0 kg m^{-3}**
Superphosphate **0.4 kg m^{-3}**
Copper sulphate **0.03 kg m^{-3}**
Gypsum **1 kg m^{-3}**

Table 2. JIC Cereal Compost Mix supplied by Petersfield Growing (Leicester, UK)

From Ghosh et al., (2018)

Component
Medium Grade Peat **40 %**
Sterilised Soil **40 %**
Horticultural Grit **20 %**
Fertiliser
PG Mix™ 14-16-18 + Trace Elements Base Fertiliser **1.3 kg m^{-3}**
Osmocote Exact Mini 16-8-11 + 2MgO + Trace Elements 0.02 % Boron **1.0 kg m^{-3}**
H2Gro (Wetting agent) from ICL Specialty Fertilisers (Ipswitch, UK)
Maglime **3.0 kg m^{-3}**
Insecticide
Exemptor from ICL Specialty Fertilisers (Ipswitch, UK) **300 g m^{3}**

References

1. Watson A., S. Ghosh, M. Williams, W.S. Cuddy, J. Simmonds, M. Rey, M.A.Md. Hatta, A. Hinchliffe, A. Steed, D. Reynolds, N.M. Adamski, A. Breakspear, A. Korolev, T. Rayner, L.E. Dixon, A. Riaz, W. Martin, M. Ryan, D. Edwards, J. Batley, H. Raman, C. Rogers, C. Domoney, G. Moore, W. Harwood, P. Nicholson, M.J. Dieters, I.H. DeLacy, J. Zhou, C. Uauy, S.A. Boden, R.F. Park, B.B.H. Wulff, L.T. Hickey (2018). "Speed breeding: a powerful tool to accelerate crop research and breeding." Nature Plants 4:23-29, DOI: 10.1038/s41477-017-0083-8
2. Ghosh S., A. Watson, O.E. Gonzalez-Navarro, R.H. Ramirez-Gonzalez, L. Yanes, M. Mendoza-Suárez, J. Simmonds, R. Wells, T. Rayner, P. Green, A. Hafeez, S. Hayta, R.E. Melton, A. Steed, A. Sarkar, J. Carter, L. Perkins, J. Lord, M. Tester, A. Osbourn, M.J. Moscou, P. Nicholson, W. Harwood, C. Martin, C. Domoney, C. Uauy, B. Hazard, B.B.H. Wulff, L.T. Hickey (2018). "Speed breeding in growth chambers and glasshouses for crop breeding and model plant research." Nature Protocols 13, 2944-2936, DOI: 10.1038/s41596-018-0072-z