



Speed Breeding for Crop Improvement

(*Rahul Kumar and Veerala Priyanka)

Department of Genetics and Plant Breeding, Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut, UP-250110

*rahul.gujjar.3009@gmail.com

The human population is projected to reach nine billion by the year 2050 and will strain global resources. The onset of climate change and new diseases and pests threaten the productivity of agriculture worldwide. Meeting the future demand for agricultural products will depend in part on developing cereal cultivars that are adapted to ever-changing environments. Plant breeders are now targeting durable forms of genetic resistance to diseases as well as tolerance to abiotic stresses such as drought, heat and frost. However, annual breeding cycles and other biological and genetic constraints restricts the ability of traditional plant breeding to meet these demands. 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 in turn particularly time consuming for field-grown crops that are often limited to only 1–2 generations per year.

Cereal breeding programs typically requires 10–15 years to transfer novel genes into adapted germplasm. Application of biotechnology is seen as key to reduce delivery time. Breeding strategies involving the application of molecular markers that are being applied to enhance progress include: marker assisted recurrent selection (MARS; Johnson 2004) and Doubled-haploid (DH) technology that can reduce the time to develop genetically stable lines from a new cross (Forster and Thomas 2005). Shuttle breeding uses diverse ecological environments to develop improved varieties with higher adaptability. Alternate generations of early breeding materials are grown under different environments. The basis of shuttle breeding is cooperative research among nations and institutions. The benefit is increased breeding efficiency. A new methodology for rapid trait introgression in spring bread wheat was developed at the University of Queensland (Australia), which combines the use of speed breeding and high-throughput phenotypic screens for multiple traits. The approach was initially trialed by selecting for resistance to tan spot (*Pyrenophora tritici-repentis*) in wheat between 2005 and 2006 (I. DeLacy, personal communication). However, over the last 10 years it has been extended to a number of important traits in spring bread wheat, including: grain dormancy (Hickey *et al.* 2009, 2010) and for resistance to stripe rust (*Puccinia striiformis*; Hickey *et al.* 2012).

Speed breeding in 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 using LEDs in a glasshouse environment allows rapid generation cycling through single seed descent (SSD) and plant density can be scaled-up for large scale crop improvement programs. ‘Speed breeding technology’ shortens the breeding cycle and accelerates crop research through rapid

generation advancement. Speed breeding can be carried out in numerous ways, one of which involves extending the duration of plants by daily exposure to light, combined with early seed harvest, to cycle quickly from seed to seed, thereby reducing the generation times for some long-day or day-neutral crops. 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. The technique has also been successfully adapted to oat, various Brassica species, grass pea, quinoa, *Medicago truncatula* and *Brachypodium distachyon*.

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). A light/dark period was chosen over a continuous photoperiod to support functional expression of circadian clock genes. Growth was also 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*. 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 and both wheat and barley plants produced a healthy number of spikes per plant, despite the rapid growth. The viability of harvested seeds appeared to be unaffected by speed breeding, with similar seed germination rates observed for all species. Moreover, crosses made between wheat cultivars under speed breeding conditions produces viable seeds, including crosses between tetraploid and hexaploid wheat. 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 2822 (Domoney, C. et al).

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, speed breeding enables 4–6 generations of these crops to be grown in a year. These values are representative of relatively rapid cycling cultivars of each crop. 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. The combination of speed breeding techniques and a single seed descent breeding strategy has the potential to significantly reduce the time in developing new cultivars compared to conventional systems where field based pedigree breeding strategies are commonly employed. 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 programmes. The speed breeding system is also less susceptible to adverse biotic and abiotic stresses such as reduced rainfall, low diurnal temperatures and foliar diseases and allows the breeder more flexibility in the generation of new breeding material.

Fast-Forwarding Genomic Selection

For more complex traits, including drought tolerance or yield, phenotyping must be undertaken in the field in the target environment. The potential for genomic selection to save

time and resources is greatest for traits that are typically measured late in the variety development pipeline and are costly to phenotype, such as yield.

Express Editing for Crop Improvement

In CRISPR gene editing, the sg RNA directs the Cas9 enzyme to the target DNA site, and Cas9 cuts the DNA at this site. 'CRISPR ready' genotypes that contain a heterologous Cas9 gene can be created. For example, a transformed plant harboring a Cas9 transgene can be used as a donor to create a range of elite inbred lines using speed marker-assisted backcrossing. Editing and mutagenesis combined with speed breeding could also be applied to create healthier foods by biofortification—for example, increasing levels of vitamin B9 in rice or removal of deleterious proteins such as saponins from quinoa (*Chenopodium quinoa*), anti nutritional glucosinolates from Brassica seeds, and neuro toxins from grass pea (*Lathyrus sativus*).

Conclusion

State-of-the-art innovations in agriculture will light the way for the accelerating selection and establishment of future crops in the upcoming years here on planet Earth and will create a novel revolution in agriculture. Commercial breeding companies now using speed breeding need to invest in infrastructure. We can take speed breeding off the grid. Speed breed Genomic Selection involves bigger grants possible through redesign of program, some traits can be screened in SB system. Also exciting opportunities to improve different crops by combining speed breed GS with CRISPR can also be developed in future.

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