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Speed Breeding as a Bridge Between Conventional and Modern Methods for Wheat Improvement

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Abstract: ***In traditional wheat breeding, developing a new variety takes many years because plants are grown under natural field conditions which allow only one generation per year. This slow cycle delays selection, crossing and the stabilization of desirable traits, making the entire breeding process long and labor-intensive. To overcome these limitations, modern crop science has introduced faster and more efficient approaches. One of the most promising among these is Speed Breeding (SB). SB is a technique that uses controlled environments to accelerate plant growth from the vegetative stage to flowering and seed production, even under high-density planting. The concept was pioneered by Australian scientist Dr. Lee Hickey, whose research led to the development of rapid-growing wheat varieties such as 'USU-Apogee' and 'USU-Perigee'. Notably, USU-Apogee was even grown successfully on the International Space Station in 2003. This method works by extending the photoperiod and carefully regulating temperature, soil media, spacing, CO_2 concentration and other growth conditions in greenhouses or growth chambers. These adjustments significantly shorten the breeding cycle, enabling multiple generations within a single year. SB can be implemented through three systems: a fully controlled growth chamber (SB I), a temperature-controlled glasshouse (SB II) and a low-cost homemade growth room (SB III). SB supports rapid development of genetic mapping populations, speeds up QTL discovery and enhances marker-assisted selection. It also accelerates genome-edited and genetically modified crop development by allowing quicker validation of CRISPR-based edits. Overall, SB enables breeders to efficiently combine traits like stress tolerance, yield and disease resistance, making it a powerful tool for future wheat improvement.***

Keywords: Speed breeding, wheat improvement, controlled growth conditions, rapid plant generation, Crop improvemen

1. Introduction

In traditional crop breeding, developing a new variety usually takes many years. Plants are grown in natural field conditions, so breeders can produce only one generation per year in most crops. This slow process increases the time required for selection, crossing and stabilizing desirable traits. To overcome these limitations, modern breeding has introduced faster methods. One of the most effective among them is Speed Breeding. Speed Breeding (SB) is one such technique that involves utilizing controlled environments, which promotes rapid and accelerated growth and development from the vegetative to the reproductive stage in high-density planting (HDP). It is a technique which involves extending photo period and controlled growing conditions such as temperature, soil media, spacing *etc*. in glasshouses or greenhouse, allow rapid generation advancement by shortening the breeding cycle. It greatly shortens generation time and accelerates breeding and research programs (Ghosh *et al*., 2018).

This concept was developed by an Australian scientist Dr. Lee Hickey at University of Queensland (Australia) in collaboration with University of Sydney (Australia) and John Innes Centre (UK). This concept was first applied for durum wheat and peanut. In 1996, the National Aeronautics and Space Administration (NASA), USA, in association with Utah State University explored the possibilities of growing rapid cycling of wheat; this has led to the development of new fast growth variety 'USU-Apogee'. They also develop 'USU-Perigee' variety. USU-Apogee was successfully grown on International Space Station in the year 2003. The seed-heads emerge just 23 days after germination and it is only 40cm tall (suitable for growth in confined space in controlled environment). 1st spring wheat variety - In 2017, DS Faraday developed through Speed Breeding. Suited for northern zone in early and main season it has high protein content, milling quality, tolerant to pre harvest sprouting and excellent triple rust resistance (Ghosh *et al*., 2018). Alahmad *et al*. (2018) evaluated large segregating F_2 population and weighted selection index. Transgressive segregation was found in the Outrob4/Caparoi F_2 group. A significant mean difference between the "selected" and "unselected" F_3 families for CR tolerance, LR resistance, RA and RN indicates that the SI was successful in shifting the population mean for four characteristics. No discernible change in PH was noted. An effective technique for quickly selecting early filial generations and describing fixed lines during off-season is multi-trait phenotyping. By testing several features on a single plant set, it makes effective use of resources. It enhances recombinant inbred lines with desired alleles when done in tandem with speed breeding.

2. Fundamentals behind speed breeding

1) Manipulation of photoperiod regime

Photoperiod refers to the length of daily exposure of plants to scheduled light and dark regimes to enhance rapid growth, development, flowering and seed set (Kouressy *et al*., 2008; Samineni *et al*., 2019) Light quality, which includes the instantaneous and cumulative amount, delivered per day has a direct effect on plant growth, net photosynthetic rate, stomata conductance, intercellular CO_2 and transpiration rate. Also, the daily light to dark hours has an effect on flowering rate and maturity. Light sources emitting photosynthetic active radiation (PAR) within the range of 400–700 nm with an intensity of 360–650 µmol/m^2/s have been successfully used in many crops, including wheat, barley, chickpea, pea and canola to facilitate speed breeding. For example, photoperiod of 22 hr light and 2 hr dark under PAR of 150–190 µE $m^{-2}s^{-1}$ reduced the total number of days to flowering by half when compared with the corresponding wheat genotypes grown with 12/12 hr light/dark. This procedure induced flowering in 35 and 39 days in several wheat genotypes, Paragon, Watkins landrace W352 and a late flowering Paragon × W352 F_6 recombinant inbred line. The plants grown with 12/12 hr light/dark were still at the stem elongation growth stage when the corresponding plants grown with 22/2 hr light/dark had started flowering. In a second study, when plants of a sensitive winter wheat genotype, G3116 were grown under short day (SD) conditions (8/16 hr light/dark) for six weeks before being transferred to long day (LD) conditions (16/8 hr light/dark) at a light intensity of 200-270 µmol/m^2/s, flowering was induced without the need for vernalization. The days to flowering of non-vernalized genotypes grown in SD-LD condition were comparable with genotypes vernalized for six weeks.

The use of solar power systems is an effective and sustainable approach for indoor speed breeding in countries with an unreliable electricity supply. Vikas *et al.* (2021) used ten popular cultivars of wheat. The cultivars were kept in a polyhouse with natural temperatures between 17 and 22°C and relative humidity levels between 75 and 80% for 22 hours of prolonged light using red LED lights and 2 hours of darkness. With the exception of HD 2967, all cultivars saw plants attain physiological maturity in 67-73 days and heading in 36-42 days. On the other hand, in field circumstances, the same cultivars needed 53-72 days to head and 105-132 days to reach physiological maturity. Compared to two generations in the field, we may have five generations annually using SB.

2) Regulation of the temperature regime

Temperature changes in the soil and air have an impact on germination and growth responses, resulting in quick development, flowering, seed set, and maturity. Low and high temperature extremes activate a wide range of effects on the rate of plant development, including a transition from the vegetative to the reproductive stages (McClung *et al*., 2016). Most crops require temperatures between 12 and 30°C for germination, but most crops prefer temperatures between 25 and 30°C for growth, flowering, and seed set. Temperatures maintained at 25 ± 1 °C under 12/12 hr light/dark condition were used for germination of direct sown immature seed in chickpea [14]. Temperature regulation allowed the germination of immature seeds (harvested 16-24 days after flowering), freshly planted into pots, which allowed for the production of 7 generations per year.

In winter wheat, vernalization or cold temperature stress is required at the vegetative stage to accelerate the transition to the reproductive stage (Dubcovsky *et al*., 2006; Yan *et al*., 2004). For speed breeding, temperatures within the critical range can thereby promote blooming, seed set, and maturity. For example, temperatures of 20-22°C were used for the germination of immature seed derived from embryo culture in wheat and barley (Zheng *et al*., 2013). After germination, seedlings were transferred to a temperature regime of 25/22°C synchronized with a photoperiod of 16/8 hr light/dark for rapid plant growth and early flowering.

3) Regulation of soil moisture

Soil moisture stresses can cause significant changes in plant growth and development processes affecting plant height, days to flowering, seed set and maturity. Stress from drought or flooding can cause early maturation and flowering, which is advantageous for speed breeding. The method most frequently used for crops including wheat, barley, and pearl millet is drought stress (Shavrukov *et al.,* 2017). When wheat and barley exhibit signs of wilting, watering encourages the growth and development of the plants. Watering regimes with embryo rescue, adjusted photoperiod and adjusted temperatures to produce 8 and 9 generations per year in wheat and barley, respectively (Zheng *et al.*, 2013).

After flowering, rapid grain filling and maturation can be facilitated by gradually reducing soil moisture contentA number of crops, including wheat, barley, canola, and chickpeas, have been speed-bred by reducing the frequency of watering from daily to twice a week, four to six weeks after flowering, and not watering in the final week before harvesting. Soil moisture management approaches are applicable under both field and indoor growing environments.

4) Density of plant populations

Growing at densities higher than those necessary to achieve maximum yield is known as high-density planting. High plant densities result in tall plants due to light competition, leading to a rapid transition from the vegetative to the reproductive growth stages. This approach is useful to induce early flowering and maturity, increasing the number of generation cycles per year. In rice, up to four generations per year were achieved using a high-density planting of 400 plants m-2 (with intra-row Spacing of 5 cm and inter-row spacing of 5 cm [5 × 5 cm]), compared with the conventional 25 plants m^{-2} (20 × 20 cm). The length of a crop cycle in rice can be reduced by 15 to 40 days, (90 from 105 days, 105 from 145) days using high density planting. However, others have reported that high density planting did not accelerate flowering in rice. Genotype differences affect plant responses to high-density planting under field conditions. Therefore, there is need to establish high-density planting requirements of a given genotype through preliminary trials to optimize induction of early flowering for speed breeding. High planting density is one of the low-cost speed breeding strategies suitable for rapid advancement of generations, while maintaining the large population size required for advanced selections.

Schoen *et al*. (2023) tested the vernalization length, light and temperature requirements, and viability of seeds collected at varying durations post-anthesis in prolonged daylight circumstances in order to enhance the SB technique using a collection of 48 different soft red winter wheat (SRWW) cultivars. they discovered that fast generation progress occurs when high-density 50-cell trays are placed to a 22-hour setup (22 hours day/2 hours night, 25˚C/22˚C). They prove that this methodology may enable the progression of four generations annually under controlled settings for winter wheat varieties, experimental lines or emerging cultivars using a collection of 48 SRWW cultivars and material from Maryland and four additional public breeding projects. For winter wheat, enabling a faster transition from the first cross to experimental genotypes that are genetically stable and suitable for field testing.

5) Modifying CO2 levels

Increased level of carbon dioxide (CO_2) may enhance rapid plant growth and the speed of the transition from the vegetative to the reproductive stage in some plants (Jagadish *et al*., 2016). However, different crop species and genotypes within a species have varying responses to increased CO_2. For instance, increased levels of CO_2 of 400/700, 350/700 and 350/650/100 ppm reduced days to flowering in soybean, rice and cowpea by 2, 7 and 12 days, respectively. In contrast, CO_2 maintained at 20 µmol/ mol_2 delayed flowering in soybean by 11 days. In pigeon pea, when CO_2 level was increased to 550 µmol/mol_2, it delayed flowering by nine days in a short duration cultivar ICPL 15,011 (Sreeharsha *et al*., 2015). Speed breeding involving modification of CO_2 levels requires appropriate facilities such as growth chambers, CO_2 cylinders and regulators and operational costs. Also, there is need to adhere to health protocols and safety guidelines while handling and using CO_2 cylinders and valves Wanga *et al*., 2021).

6) Use of plant nutrition, hormones and organ tissue culture

Plant nutrition and hormones have been used to accelerate growth and to induce flowering and seed set, and germination of immature seed in vitro (Bermejo *et al*., 2016). Varied responses to plant growth regulators (PGRs) are achieved when used in controlled environments such as greenhouses and growth chambers in which the photoperiod and temperatures can be monitored and controlledSeed dormancy in immature seeds of several crops can be broken by drying and chilling (Ghosh *et al*., 2018). For example, drying of immature seed of wheat and barley at 28– 35°C for 3– 5 days in an oven or dehydrator followed by chilling (4– 5°C) for 3 days resulted in germination rates of 80 and 100% for seeds harvested at 2 and 4 weeks after flowering, respectively (Watson *et al*., 2018). This procedure was subsequently used in combination with a long photoperiod (22/2 hr light/dark) and temperature regulation (22/17°C light/dark) to achieve 4–6 generation cycles per year for wheat, barley.

3. Methods of Speed Breeding

1) 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. Humidity was set to 70%. Lighting was supplied by a mixture of white LED bars (6 units per 7.6 m^2), far -red LED lamps (12 units per 7.6 m^2) and ceramic metal hydrargyrum quartz iodide lamps (32 units per 7.6 m^2). Light intensity was adjusted to 360–380 µ mol m^{-2} s^{-1} (highest value after ramping) at bench height, where the pots were kept, and 490– 500 µ mol m^{-2} s^{-1} (highest value after ramping) at adult plant height (with reference to wheat, *T. aestivum* cv. Paragon) (Watson *et al*., 2018).

2) Speed breeding II (Glasshouse conditions)

Temperature controlled glasshouse fitted with high-pressure sodium vapour lamps (Philips SON-T 400 W E 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. The 2-hour period without lamps operating and 17 °C cycle occurred during the night. Light intensity was 440–650 µ mol 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 (Watson *et al*., 2018).

3) Speed Breeding III (Home-made growth room design for low-cost)

A room of 3 m × 3 m × 3 m with insulated sandwich paneling fitted with seven LB-8 LED light boxes (one light box per 0.65 m^2) from Grow Candy 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 photo synthetically active radiation (PAR) at bench height ranged from 210–260 µ mol m^{-2} s^{-1} and at 50 cm above the pot from 340–590 µ mol 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 (Watson *et al*., 2018).

4. Applications of Speed Breeding

1) Genetic Mapping Populations

Traditionally, generating diverse segregating populations for genetic mapping was a time-consuming process. However, SB's ability to rapidly cycle through generations has transformed this area. Large and genetically diverse mapping populations are now easily produced by researchers, giving them the tools they need to identify the genes linked to important characteristics. This breakthrough has significantly accelerated the field of quantitative trait locus (QTL) analysis and MAS (Gaba *et al*., 2021). Studies have demonstrated the use of a mapping population developed through a biotron SB

system to enhance salinity stress tolerance in rice. The University of Queensland's "SB" method accelerates line generation, and genotyping with DArTseq markers helps validate known QTL and discover new ones for these traits (Potts *et al*., 2023).

2) Genetic modification crop development

Researchers can progress the entire GM crop development process, from gene insertion to field trials, thanks to controlled settings and faster generation periods. This not only reduces the time to market for GM crops but also empowers researchers to respond swiftly to emerging agricultural issues. SB, therefore, acts as a catalyst for innovation in the biotechnology sector, fostering advancements in crop biotechnology that can benefit both farmers and consumers. It introduces "preassembled Cas9-sgRNA ribonucleoproteins" into plant shoot apical meristems using techniques like particle bombardment or biolistic DNA delivery. Cas9-lacking plants with the desired trait can be bred using Marker-Assisted Backcrossing (MABC). These "CRISPR-ready" plants can undergo further modifications with targeted sgRNA, advancing crop breeding and agricultural productivity [16]. Furthermore, the use of CRISPR/Cas9 technology has proven highly effective in enhancing yield-related traits by disrupting negative regulators that influence factors determining crop yieldtele

This involves increasing the size, quantity, weight, panicle size, and number of tillers in crops like wheat and rice by targeting genes like OsGS3, OsGn1a, OsGW5, TaGW2, TaGASR7, OsGLW2, TaDEP1, OsDEP1 and OsAAP3 (Potts *et al*., 2023).

3) Trait stacking for Resilient crop Varieties

SB plays a pivotal role in streamlining this process. With the ability to rapidly generate and assess multiple generations of plants, breeders can efficiently create and evaluate multi-trait combinations. These multi-trait crops can withstand diverse challenges, ranging from changing climatic conditions to evolving pest pressures. The accelerated trait stacking made possible by SB contributes to the development of crop varieties capable of meeting the demands of a dynamically changing world. Early-generation selection was used to enhance the population with desirable allelic combinations for multiple traits in wheat breeding. This innovative multi-trait phenotyping method incorporates root system architecture, leaf rust resistance and plant height for swift selection of favorable allelic combinations. This approach aligns seamlessly with speed breeding, enabling up to four consecutive screens each year and significantly boosting breeding efficiency (Potts *et al*., 2023) (Alahmad *et al*., 2018)

4) Enhancing Research across Disciplines

Speed breeding's unique capabilities transcend traditional crop improvement and genetic research. It finds applications in diverse fields, including ecology, physiology, and agronomy. The rapid generation cycles allow for quicker hypothesis testing and the collection of critical data, enabling researchers to make informed decisions and develop innovative solutions to address global agricultural challenges. Developing new and improved breeding cultivars for optimal yield in field environments is a complex task, often hindered by the intricate interplay between genetic traits and environmental conditions. Additionally, systems biology unravels the intricate genetic regulatory networks governing plant development, enabling the identification of key genes like high response to photoperiod (HR) and late flowering (LF) in crops like winter peas, facilitating the precise manipulation of critical developmental stages like flowering. Cloning flowering key genes in peas, such as LF and LATE 1, has provided valuable insights into the molecular mechanisms governing flowering (Potts *et al*., 2023) (Foucher *et al*., 2003) (Hecht *et al*., 2007).

Cha *et al.* (2023) used Speed breeding (SB) and speed vernalization (SV) techniques for spring and winter wheat. They aimed to establish and evaluate the performance of a breeder-friendly and energy-savin generation acceleration system by modifying the SV + SB system. In this work, an energy-efficient extended photoperiod treatment was used to create and assess a four-generation advancement system for wheat (independent of its growth habits). Accelerated generation was achieved using a glasshouse with a 22-hour photoperiod that employed 10 hours of natural sunshine and 12 hours of LED lights, with little temperature management throughout the winter. When compared to conventional field-based techniques, modified speed breeding (mSB) when used with a speed vernalization system (SV + mSB) cut breeding time in half, even with just one or two field tests. Significant relationships were found between the SV + mSB and field conditions in terms of the number of days to heading (DTH) and culm length (CL); breeding materials, genetic resources and recombinant inbred lines that displayed shorter DTH and CL values under SV + mSB conditions showed the same pattern in the field. The SV + mSB system reduced energy use by 80% to maintain a 22-hour photoperiod when compared to the current SV + SB system.

Nannuru *et al*. (2025) compared various breeding schemes, including genomic selection and speed breeding, against conventional phenotypic selection. Results showed significant increases in genetic gain with GS-based programs compared to phenotypic selection, regardless of the selection strategies employed. Speed Breeding + GS outperformed others, generating the highest genetic gains. This study highlights the advantages of genomic selection in accelerating breeding gains for wheat, particularly in combating FHB, by leveraging genomic information and innovative techniques like speed breeding.

5. Limitations of speed breeding

Extended photoperiods may cause injury in some crops. A lack of trained plant breeders and breeding technicians. Inadequate infrastructure to regulate environmental factors, particularly soil moisture, temperature and photoperiod. Unreliable water and electricity supplies for sustainable operations. As there is great diversity on earth therefore the protocols are different for different crops. The need for long term funding.

6. Conclusion

Speed Breeding has emerged as a successful technology that aims to shorten the breeding cycle and accelerate crop improvement through rapid generation advancement. In

wheat, speed breeding relies on creating highly controlled environment that provide conditions for plant growth, flowering and seed formation which is 22 hours light, 22/17˚C (25/22˚C for winter wheat) temperature and immature seed harvest. In speed breeding phenotyping approach allow rapid testing of genotypes that can fast track the development of wheat variety by rapid advancement of generation per year. They also accelerated the breeding procedure for the traits that is difficult to screen under field condition *i.e*. disease resistance. Speed Breeding is advisable but still improvement is necessary for the adoption in plant breeding programs.

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