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Research Article

New Green Revolution - Speed Breeding

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Abstract

Current global population increase coupled with climatic change causes food insecurity. In order to face this challenge, NASA inspired space technology called "speed breeding" was reformed and practiced by scientists in University of Queensland to hasten the generation cycle in plants. In long-day [22 hours of light/2 hours of darkness] and short-day crops [8 to 12 hours of light/16 to 12 hours of darkness] generation cycle has been improved to four to six generations per year in speed breeding. In conventional breeding or glass house without supplementary lighting, it takes minimum of 10 to 15 years to produce hybrid vigor, whereas in speed breeding this could be significantly reduced to half.

Implementing marker assisted breeding as well as genomic approaches along with speed breeding results in quicker development of crops with high genetic gain. In this review, we have briefly discussed different protocols of speed breeding in wheat, barley, rice, canola and few other crops. In addition to this, significance and limitations of speed breeding and its future outlook is also outlined.

Keywords: Speed Breeding; Protocols; Genomic Approaches; Crop Improvement; Crop Protection; Green Revolution

Introduction

The estimated increase in the global population by 25% in 2050 has raised concerns about food security. Severe climatic repercussions on yield and growth of the crops have impacted the global food production rates [9,25] (Ray., *et al*. 2). Climatic change in the early 1940s laid the ground for “The Bengal Famine” in 1943 where two million people perished out of hunger. The unanticipated heavy rainfall in northeastern states of India followed by changes in moisture and humidity has led to the emergence of an epiphytotic disease in rice [22]. Famine across the globe has preached the importance of disease-resistant crops and alleviation of food insecurity. In India, to overcome the food shortage, the embodiment of traditional breeding to produce high-yield varieties using enormous chemical fertilizers, insecticides, and agricultural [1]machinery led to the emergence of the “Green Revolution”. However, even with the high yield varieties, the generation cycle remains the same depending on crops. Like rapid globalization and industrialization, hastening the crop breeding will bring novel solutions to feed growing populations. The research revealed plants are grown under artificial light and CO_2 supply had shown enhanced flowering in almost 100 species comprising grains, vegetables, herbs and garden varieties [1]. Utah State University collaborated with NASA to produce a dwarf wheat,” USU - Apogee” with excessive vegetative growth which can reach the head stage in 23 days under 25°C with continuous light supply [4]. A new technique was inspired by NASA’s effort of growing crops in space inside the controlled environmental chamber with prolonged photoperiods [29]. These modern advancements have brought zealous ideas of shorter breeding cycles using indoor-plantation with increased light duration opening a path for a new green revolution, called speed breeding. Speed breeding, a NASA inspired space technology initially followed by the scientists in the University of Queensland and University of Sydney, Australia with John Innes Centre, UK [11]. The plants were triggered to grow quickly when compared to the crops grown in the glasshouse and normal field conditions. The generation cycle varies about 6 times faster than field and 3 times faster than normal glasshouse condition.

This generation advancement is achieved by growing plants in the regime of high light intensity using low- cost LED's to emit light of specific wavelength as it increases the photosynthesis rate. Speed breeding not only hastens the duration of the breeding cycle it is also useful in improving the crop physiology and morphology like making the plants to produce good quality seeds and to grow in the resilient climatic conditions without a reduction in the yield and quality of the crop. Thus speed breeding will helps us to achieve maximum benefit in minimum space to mitigate food insecurity and usage of arable land associated with population growth in developing countries in Asia.

Speed breeding

Speed Breeding or Accelerated Breeding is a method to accelerate the genetic gain under guided environmental conditions which shortens the breeding cycle by increasing the growth rate of plants in prolonged photoperiod conditions. The breeder's equation [6] which helps us to achieve the genetic gain or the mean change in the value of a trait under selection is,

$\Delta G = (\sigma\_A \ ir)/L$

Where, σA = additive genetic variance, i = intensity of selection,

r = selection advance,

L = length of the breeding cycle.

The accelerated plant development is attained under artificial environment comprising four major components 1) light 2) temperature 3) photoperiod and 4) humidity.

- **Light:** Sources of light such as LED (red, blue), sodium vapour lamp and halogen lamps that induces photosynthetically active radiation (PAR, 400-700 nm) are employed in speed breeding.
- **Temperature:** Suitable temperature depends on the crop variety. Usually, temperature for long day and short day crop is 22°C/17°C and 30°C/25°C for dawn/dusk respectively.
- **Photoperiod:** On a 24 hours' diurnal cycle, 22 hours of light and 2 hours of darkness is provided for long day crop whereas 12,10 or 8 hours of light and 10,12 or 16 hours of darkness respectively for short day crop. This photoperiod variation between short-day, day-neutral and long-day crops promotes early anthesis.
- **Humidity:** 60 to 70% is the ideal humidity provided for crop growth.

Speed breeding technique was elaborated [28] and evaluated in three different conditions, controlled environmental chamber speed breeding, greenhouse speed breeding and homemade growth room design for low-cost speed breeding.

Condition 1

In this method, plants were grown in a Conviron BDW chamber (Conviron, Canada) with programmed lighting (LED, 22 hours of photoperiod and 2 hours of darkness) and temperature (22°C during photoperiod and 17°C during darkness) with ramp up and down settings for 1 hour 30 minutes with 70% humidity to create similar natural environmental conditions. Light intensity during the developmental stage was set to 360-380 mmol m^{-2} s^{-1} and at vegetative stage 490-500 mmol m^{-2} s^{-1}.

Condition 2

Plants were grown in green-house with programmed light [sodium vapour lamp, 22 hours photoperiod, 2 hours of the dark period] and temperature [22°C/17°C, day/night] devoid of ramping up and down. The light intensity received by the adult plant was estimated to be 440-650 mmol m^{-2} s^{-1}.

Condition 3

This method is a cost-effective approach carried out in a 3m x 3m x 3m growth room, insulated with sandwich paneling fitted with seven LB-8LED light boxes 140 cm above the bench. The lights were set initially 12/12 hours of photoperiod/darkness for four weeks and then light duration was increased to 18 hours with 6 hours of dusk conditions. 1.5 horsepower inverter split domestic air conditioner was set to run at 18°C in the darkness and 21°C during the light period with the variation of ±1 °C. Plants received Photosynthetic Active Radiation (PAR) range from 210-260 mmol m^{-2} s^{-1} to 340-590 mmol m^{-2} s^{-1} at 0 to 50cm above the pot respectively. This space can fit 90 pots with 20.3 cm diameter and volume of 5L.

Research carried out in John Innes Centre, UK, on wheat (*Triticum aestivum,* Figure 1), barley (*Hordeum vulgare,* Figure 2) and brachy podium (*Brachypodium distachyon*) under speed breeding condition 1 reveal, plants have reached the stage of anthesis in half time compared to the glasshouse plants with no supplementary light [28]. Immature seeds harvested and dried in an oven or dehydrator for 3 days which enabled faster seed to seed seedling whilst loss of grain weight. Flowering stage in wheat took in 35-39 days (exceptional case: approximately 63 days in Chinese spring wheat), in barley 37-38 days irrespective of the variety and accession number. Nevertheless, the difference in growth time, wheat and barley produced healthy spikes, no impact on seed germination rates in all species but with a decline in wheat seed count compared to the glasshouse variety, although not significantly.

SI. No	Method	Number of generations advanced per year	Crop	Under Day- neutral conditions	Accelerated breeding conditions	References
1	Speed Breeding 1	6	Barley	102-115	55-60	[28]
		6	Pea	84	51	
		6	Bread wheat	105	62	
		6	Durum wheat	102	62	
2	Speed Breeding 2	6	Bread wheat	87	65	
		6	Barley	132	68	
		6	B. distachyon	73	48	
		4	Canola	171	98	
		6	Chickpea	115	82	
3	Biotron breeding system	6	Rice	122	80	[20]
	Modified control biotron speed breeding system	4/5		135	100	[24]
	Rapid Generation Advance (RGA)	4		110-121	95-105	[7]

Table 1: Speed breeding protocols in different crops.

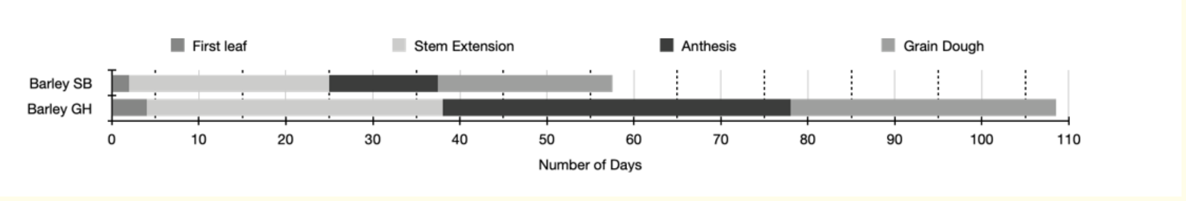

Figure 1: Barley growth rate in Speed Breeding (SB) condition 1 vs Glass House (GH) with day-neutral conditions.

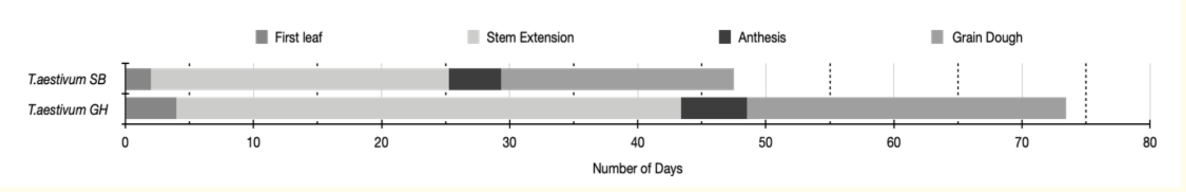

Figure 2: Wheat growth rate in Speed Breeding (SB) vs Glass House (GH) with no supplementary light.

The study conducted by the University of Queensland on wheat, barley, chickpea (*Cicer arietinum*) and canola (*Brassica napus*) is grown in glasshouse fitted with high- pressure sodium vapor lamp revealed occurrence of early anthesis in significant days compared to the crops grown in day-neutral conditions [28]. Increase in the number of wheat spikelet along with unaffected seed count in both wheat and barley were produced in this speed breeding condition. In this method, the flowering stage within the species was uniform which is essential for crossing.

Further, the potential of speed breeding is investigated along with the genetic transformation. In barely the seeds were sown and harvested at the stage of an immature embryo and genetic transformation is carried out to develop the mutant waxy trait. This enhances the growth to 6 weeks earlier when compared to normal plants. Speed breeding also reduced the generation time for crop species like sunflower (*Helianthus annus*), pepper (*Piper nigrum*), radish (*Raphanus sativus*) etcetera as these shows the excellent response to high photoperiod.

Overall, speed breeding is highly suitable for photo insensitive crops (long day, LD) like wheat, barley, chickpea and canola where the generation time of the breeding cycle is greatly reduced to 4 to 6 generations per year (Table 1).

In case of short day (SD) photosensitive crops like soybean (*Glycine max*), rice (*Oryza sativa*), Amaranthus (*Amaranthus viridis*), peanuts (*Arachis hypogaea*) extended photoperiod hampers the early anthesis stage. An alternative approach is used which is known as off-season generation advancement. This aids selection of plants from segregating generation during the offseason that greatly reduces the varietal development duration of crops. For example, soybean offseason generation advancement was successfully carried out in soybean in University of Agricultural Sciences (UAS), Bengaluru collaborative effort with Indian Institute of Soybean Research (IISR), Indore.

Rice is a short day-long duration crop; it requires a large field area for its normal growth. To minimize these consequences, the method of biotron breeding system have been used [20]. This system engages the plant to grow in the artificial environment condition with the daylight for 11 hours maintained at 30°C followed by 13 hours dark maintained at 25°C along with 20% of CO_2 supplied by a gas cylinder. The relative humidity and the light intensity are 70% and 350μmol m^{-2} s^{-1} respectively. As a result, plants started to flower within 50 days after sowing when compared to the normal field environment. The time can be shortened further by using the immature seeds harvested after 7 days of pollination and subjected the process of embryo rescue which reduces the duration required for seed maturity.

Further, this technique is slightly modified to introgress salinity tolerant gene 'hst 1' into high yielding rice variety Yukinko-mai from Kaijin [8,24]. Here the seeds are checked for infection and it is incubated for 2 days in the dark at 20°C.

The seeds are sown in the nursery seedling tray, 10 days old seedlings are transplanted and grown in controlled growth chamber where it is subjected to long day condition of 14 hours light along with 10 hours of dark during the vegetative stage for 30 days and immediately subjected to the short-day condition of 10 hours of light along with 14 hours of dark during the reproductive stages at an ideal temperature of 30°C and 25°C during light and dark respectively. The relative humidity is maintained at 70% and the light intensity is 350μmol m^{-2} s^{-1}. This method is termed as modified controlled biotron sped breeding system.

Speed breeding in rice is faster when they are grown in the red and blue light condition as this improves the photosynthesis [15]. The far-red and red improves the phytochrome activity whereas the blue light improves the cryptochrome and phototropin activity [14]. The far-red helps to inactivate the phytochrome thereby increasing the flowering and the blue light helps to promote flowering in the night increases the plant density and aids the plant to produce more number of seeds that further helps in single seed descent method (SSD) where only one or two seeds were required per plant for its generation advancement. Rapid Generation Advancement (RGA) is a novel method similar to SSD with certain advantages over time, space and money which promote early flowering and improves the seed set under stipulated environmental condition [7].

Speed breeding is also employed in Amaranthus, a short day plant growing in long-day condition helps to improve the vegetative growth and immediate exposure to short-day conditions induces early flowering. This method results in synchronization of flowering across different genotypes, which paves way for better hybridization [27].

In peanut, the speed breeding rapidly increases the duration of inbreeding in F2, F3, F4 generation in less than 12 months so that the cultivar achieves full season maturity earlier [19]. The commercial release could be accelerated in around six to seven years. Speed breeding protocols are not yet developed for root, tubers and banana-like crops where the propagation is through clones. Therefore, it could be achieved by combining speed breeding with Marker Assisted Selection (MAS).

Evolution of markers to speed breeding

The development of molecular markers since the late 1900s brought a revolution in plant breeding. Before the molecular approach, conventional breeding was implemented to develop enhanced crop varieties. In the conventional technique, two diverse parents are hybridized and subsequently bred to develop a hybrid vigor. The morphological feature is the only marker in conventional breeding, which requires a minimum of five years to a decade to produce the desired traits.

Environmental noise makes this approach more unreliable for the traits with complexity and low heritability.

A gene or a DNA sequence associated with the trait- controlling gene in plants located in the same Quantitative Trait Loci (QTL) region is referred to as a genetic marker or molecular marker [18]. MAS in plants is an integrated approach of both marker and its linked phenotypic traits. This method is successful for simple traits which have a small number of QTLs with high impact on the trait. Usually, the trait with economic importance has an association with a plethora of minor QTLs which makes arduous to comprehend [3]. In this scenario, the cost and time effective, high throughput genotyping (HTG) are employed to mitigate the complexity in understanding the complex trait QTLs [13,16,17] suggested an idea of Genomic Selection (GS), which predicts the Genomic Estimated Breeding Value (GEBV) from the training population using the information of whole sequence genome-wide marker association. Genome- Wide Association Studies (GWAS) helps in identifying the QTLs present in the whole genome-based on linkage disequilibrium between the trait and the markers [26]. Among the next-generation technologies, CRISPR-Cas9 is an expressway of editing to develop plant varieties with the desired trait [5,30]. The change initiated by the latest technologies in identifying genes and developing crops with the desired allele, the breeding time remains the same.

Incorporation of speed breeding technologies with Marker- Assisted Breeding (MAB) has two benefits 1) precise mapping of the mutated region to improve the genetic gain 2) reducing the generation cycle of the crops.

Significance of speed breeding

- Speed breeding has helped to develop pre-harvest sprouting resistant wheat variety, “DS Faraday” with high protein and milling [11].
- Speed breeding can be used to develop crops with multiple diseases resistant with quicker generation cycle. For instance, merging novel introgression method and speed breeding, multiple disease resistant H. vulgare cv. Scarlett was developed in 2 years [12].
- The anthesis time is reduced to half in wheat, barley, Brachypodiym distachyon, chickpea and canola serve’s as greater advantage for scientists to develop improved variety in a brief time.
- Combining next-generation technologies to speed breeding, will result in a crop with desired economic traits in a short span.
- Large populations can be grown in the glasshouse with supplementary light overgrowth chambers [28].
- Crops can be speed bred in houses at low cost in a small room and is not limited to the laboratory facilities.
- Artificial growth condition using sodium vapor lamp eliminates environmental noise leading to synchronized flowering within the species which is advantageous for crossing.
- Crops can also be studied for essential adult phenotypes using speed breeding.

Constraints in speed breeding

- Speed breeding is not suitable for short-day crops. Short day or photosensitive crops requires vernalization compared to a long day and day-neutral crops.
- Need to optimize light, temperature, moisture and humidity depending on the crop variety.
- Substantial initial investment to construct speed breeding facility is a major drawback. The maintenance costs vary between $4000-$7000 per annum depending upon the facility features [28].
- Cost efficiency strategies should be considered before selecting energy-efficient lighting and temperature control.

- Use of renewable energy is an essential idea but investing in solar panels will be expensive.
- Extended photoperiod exposure induces plant stress which may lead to chlorosis.

Conclusion and Progression in Speed Breeding

The controlled environment condition for speed breeding relies on high-cost investment growing chambers where soil plays a vital role in successful plant growth. Similarly, hydroponics is an approach which utilizes water for its growth [23]. The unused shipping containers [2], refrigerator can be modified into speed breeding capsules with instalment of solar supported light and temperature control system. This type of capsules strengthens the research areas in developing countries and also in regions of low fertile lands. Speed breeding greatly emphasis on the use of long photoperiod for rapid generation advancement where the different wavelength of light plays a major role in the flowering of a long day and short day crops effectively by LED'S. The monochromatic light, LASER will be the best alternative as it converts 40 to 60% of its energy consumption concerning the light spectrum [21]. Future of speed breeding should focus on advancing research in under-exploited nutritious crops to alleviate malnutrition in developing countries across the globe. Future association with vertical farming will alleviate the need for more space.

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