International Journal of Graduate Research and Review

ISSN: 2467-9283

Indexing and Abstracting

InfoBase Index, Cosmos, Open Academic Journals Index (OAJI), InfoBase Index, Cosmos, ResearchGate, CiteFactor, Scholar Stear, JourInfo, ISRA: Journal-Impact-Factor (JIF), Root Indexing etc

Impact Factors*

IBI factor: 3

Impact factor (OAJI): 0.201

Vol-7, Issue-1

February 2021

SEM-Biotech Publishing

ISSN : 2467-9283
International Journal of Graduate Research and Review
A Multidisciplinary Journal

Mini Review

Speed Breeding: Potential and Challenges

Suprava Acharya[1*], Mukti Ram Poudel[1], Binju Maharjan[1], Rishav Pandit[1], Bigyan KC[1], Rashmi Regmi[1], Bishnu Bhusal[1], Pritika Neupane[1], Kushal Bhattarai[1]

[1]Institute of Agriculture and Animal Science, Tribhuvan University, Kirtipur – 44600, Nepal

Article Information

Received: 04 January 2021
Revised version received: 15 January 2021
Accepted: 21 January 2021
Published: 25 February 2021

Cite this article as:
S. Acharya et al. (2021) Int. J. Grad. Res. Rev. Vol 7(1): 11-16.

***Corresponding author**
Suprava Acharya,
1Institute of Agriculture and Animal Science, Tribhuvan University, Kirtipur – 44600, Nepal
Email: acharya.suprava32@gmail.com

Abstract

Speed breeding has established itself as a flexible, noble plant breeding approach to enhance the plant breeding process. With the use of pro-longed photoperiod of 22hr duration daylight followed by 2 hrs. of night light, speed breeding has become successful in achieving more generations of crops in a year. Speed breeding has successfully achieved a higher genetic gain of the crops through reduced breeding cycle. Higher genetic gain is the need of today's world to compensate for the impacts of increasing food demand and climate change created due to global population expansion. Focusing on these problems and its consequences, speed breeding can play an impactful role in plant breeding history. There are both challenges and opportunities for speed breeding, whether to be flexible with its protocol and expand to a broader horizon or stick to the limited achievements without flexibility. The changing habit and habitat of the plants in response to the changing weather conditions, the dependency of the generation cycle on the variable climatic and environmental factors, the flexibility of the speed breeding protocols with different cultivars, costing of the approach and its adaptation in the farmers' field can be seen as significant challenges for speed breeding. While speed breeding can take these challenges as opportunities to find a way out for these obstacles through integration with different new breeding technologies like genomic selection, programmable nucleases, Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR), and CRISPR- associated (Cas) proteins.

Keywords: photoperiod; genetic gain; speed breeding; protocol; genomic selection; CRISPR/Cas proteins.

Introduction

Background

Since the early 1900s, plant breeding has played an essential role in ensuring food security worldwide (Tester and Langridge, 2010).

However, the plant breeding programs focusing on high yield and varietal development is facing challenges in meeting the rate of increasing food demand results from a rapidly expanding global population (Ahmar *et al.*, 2020).

Various phases of crossing, testing, and selection give rise to a new variety in breeding programs, and multiple approaches have been adopted for varietal development. The time frame has been the significant bottle-necks for the breeding approaches adopted in past days. With improvements over the traditional plant breeding method, several other approaches were introduced sequentially as shuttle breeding, followed by double haploid lines technology (Ortiz *et al.*, 2007).

The breeding programs for varietal development and improvement take around one or two decades (Voss-Fels *et al.*, 2019), while food insecurity arises due to rapid population growth and sometimes on an immediate basis such as natural calamities, biotic and abiotic stresses in plants, etc. Besides this, the changing climatic scenario has created various distinctions in plant habit and habitat due to biotic and abiotic stresses within the plant (Von Braun *et al.*, 2005). All these conditions urge flexibility in the breeding programs by the breeder according to the situation.

Plant breeder is the one who needs to know everything about the plant regarding its present and future market aspects, farmer-friendly inventions. Most importantly, the breeder needs to be far-sighted of the upcoming circumstances as varietal developments is a decade long process (Singh, 2015). Thus, time limitation in varietal development has been a significant obstacle for breeders to cope with the impending natural crisis or develop a breeding program as per the immediate need. The annual improvement rate in plant yield is around 0.8% - 1.2%, which needs to be doubled to meet the growing population's food demand (Li *et al.*, 2018). To overcome this significant limitation, breeders have approached a novel strategy in plant breeding, famous as Speed breeding.

Speed breeding is a powerful tool to accelerate the plant breeding process for varietal development with the subsequent increase in the number of generations in long-duration crops within a year (Watson *et al.*, 2018). Speed breeding that provides a non-GM route was developed by Dr. Lee Hickey and his colleagues (Voss-Fels *et al.*, 2019).

To develop genetically stable lines for the evaluation of agronomic and yield traits, four to six generations of inbreeding is necessary. This process is time-consuming for field crops, with one to two generations per year (Watson *et al.*, 2018). To overcome this challenge, speed breeding has introduced a flexible approach of increasing the photoperiod duration of field crops, thus achieving 4-6 generations in plants like wheat, which used to have 1-2 generations before. The photoperiod protocol set by speed breeding increases the crop generation and accelerates the germination of immature seeds and harvesting time of the plants (Sysoeva *et al.*, 2010).

Techniques

The evaluation of speed breeding was carried in standard genotypes of wheat (*T. aestivum*), durum wheat (*T. durum*), barley (*H. vulgare*), and *Brachypodium distachyon* in a controlled-environment room with the prolonged photoperiod of 22 hrs. daylight and 2 hrs. of darkness. The experiment results were compared with plants' growth under glasshouse conditions with no supplemental lights during the spring and early summer. The time of anthesis seen in speed breeding plants was half the time earlier than that of the glasshouse conditioned plants.

Similar seed germination rate was observed for all species with the viability of harvested seeds unaffected by the speed breeding. A similar protocol was applied to evaluate speed breeding in barley, spring wheat, canola, and chickpea varieties at the University of Queensland. High-pressure sodium lamps were fitted in the temperature-controlled glasshouse to extend the photoperiod up to 22 hrs. day length. A glasshouse with a natural 12-hr control photoperiod was used as a control treatment. The same temperature regime of 22/17 degrees Celsius was used for both setups. In this experiment, six generations of wheat, barley, and chickpea and four generations of canola were achieved per year through speed breeding with the photoperiod of 22 hrs. light and 2 hrs. of darkness. In contrast, the generation number ranged for 2/3 in the same crops grown in glasshouse control treatment with 12hrs light and 12 hours of the night, as seen in Fig. 1.

Different speed breeding approaches that involved the structure of growth chambers fitted with a mixture of the light-emitting diode (LED) and metal halide lighting for creating prolonged photoperiod were also practiced (Ghosh *et al.*, 2018; Watson *et al.*, 2018) (Table 1).

Speed breeding set up

- ***Light:*** Any light that produces a spectrum covering the Photosynthetic Active (PAR) region (400-700 nm) with particular focus on the blue, red, and far-red ranges is suitable to use in speed breeding (Ghosh *et al.*, 2018; Watson *et al.*, 2018).
- ***Photoperiod:*** photoperiod of 22hr daylight followed by 2 hrs. of darkness has been proved to be useful for speed breeding (Ghosh *et al.*, 2018; Watson *et al.*, 2018; Watson *et al.*, 2018).
- ***Temperature:*** 22 degree Celsius of day temperature with 17 degree Celsius of night temperature has been used in the speed breeding (Ghosh *et al.*, 2018; Watson *et al.*, 2018).
- ***Humidity:*** 60-70% humidity has been maintained while conducting the speed breeding program (Ghosh *et al.*, 2018; Watson *et al.*, 2018) (Fig. 2 & Fig.3).

Achievements

1. *Single seed descent method in speed breeding:*

The single-seed descent method is commonly used on large numbers of segregating populations for several generations to generate homozygous lines with fixed traits. It is an essential step in cultivar development that requires high-density plantings. Rapid cycling with healthy and viable seeds can be achieved by increasing sowing density under Speed breeding. According to the experiment done by Watson and Ghosh in wheat and barley, it has been found that, despite the high sowing density, all plants produced a spike that has enough seeds to perform SSD. The results obtained from these experiment highlights the role of high-density planting under SB conditions to grow plants suitable for significant and resource-efficient generation turnover in SSD programs (Ghosh *et al.*, 2018; Watson *et al.*, 2018).

2. *Development of 6 generations per year in plants like wheat, barley, chickpea without embryo rescue*
3. *Premature harvest of barley and wheat seeds produced under SB at two weeks post-anthesis, followed by a short period of drying, gives rise to uniform germination rates and healthy plants at a faster rate*
4. *Design of low-cost bench-top growth cabinet to trial SB.*

Future aspects

- While different genome editing technologies like CRISPER, Genomic selection (GS) have been introduced, the inclusion of speed breeding, a tool to enhance varietal development and plant breeding with these genome editing technologies, can bring about revolutionary changes in the era of plant breeding.
  With the flexibility in this approach, it can explore new allelic diversity and preservation of extinct or ready to extinct cultivars by tapping into the gene bank. Since the genetic gain is inversely proportional to the length of the breeding cycle interval, integration of genomic selection, and speed breeding approach could fast-track gene bank mining (Li *et al.*, 2018).

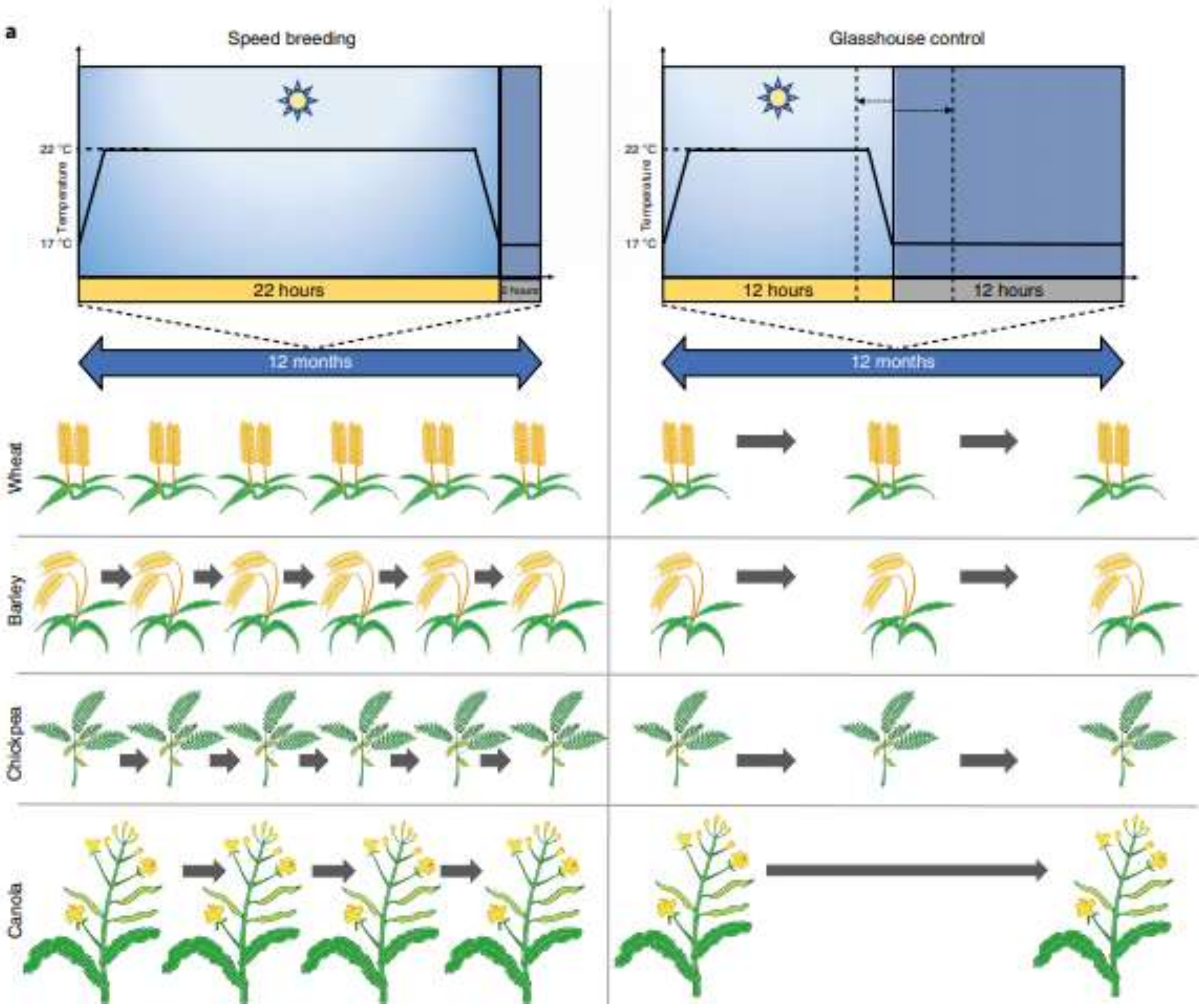

Fig. 1: Comparison of number of generations achieved per year in wheat, barley, chickpea and canola through speed breeding (left) and controlled glasshouse treatment (right) (Adapted from Watson *et al.*, 2018).

Table 1: Mean days to reproductive stages of single-seed densities under speed breeding using the JIC-LED or UQ-GH-LED approach

Species	Approach	Sowing Density	Photoperiod	Mean days to reproductive stage
Spring Wheat (*T. aestivum*)	JIC-GH-LED	96-cell (560 plants/m^2)	22h	45.0 ± 0.0[a]
		96-cell (560 plants/m^2)	16h	58.0 ±0.0[a]
	UQ-GH-LED	30-cell (300 plants/m^2)	22h	31.3 ± 0.7[b]
		64-cell (640 plants/m^2)	22h	30.0 ± 0.0[b]
		100-cell (1000 plants/m^2)	22h	31.0 ± 0.0[b]
Tetraploid wheat (*T. durum*)	JIC-GH-LED	96-cell (560 plants/m^2)	22h	42.0 ± 0.0[a]
		96-cell (560 plants/m^2)	16h	50.0 ± 0.0[a]
Spring barley (*H. vulgare*)	UQ-GH-LED	30-cell (300 plants/m^2)	22h	27.3 ± 1.2[c]
		64-cell (640 plants/m^2)	22h	24.7 ± 0.3[c]
		100-cell (1000 plants/m^2)	22h	24.0 ± 0.6[c]

JIC-GH-LED is the LED-supplemented glasshouse setup at JIC, UK (Equipment setup, “LED-supplemented glasshouse”). It uses a temperature cycle regime of 22h at 22°C and 2h at 17°C to coincide with light and dark times respectively. UQ-GH-LED is the LED is the LED-supplemented glasshouse setup at UQ, Australia (Equipment setup, “LED-supplemented glasshouse”). It uses a temperature cycle regime of 12h at 22°C and 12h at 17°C.

[a] Days to 50% ear emergence from sowing (GS55, Zadoks scale)

[b] Days to mid-anthesis (GS65, Zadoks scale) from sowing.

[c]Days to awn peep (GS49, Zadoks scale) from sowing.

Source: (Ghosh *et al.*, 2018)

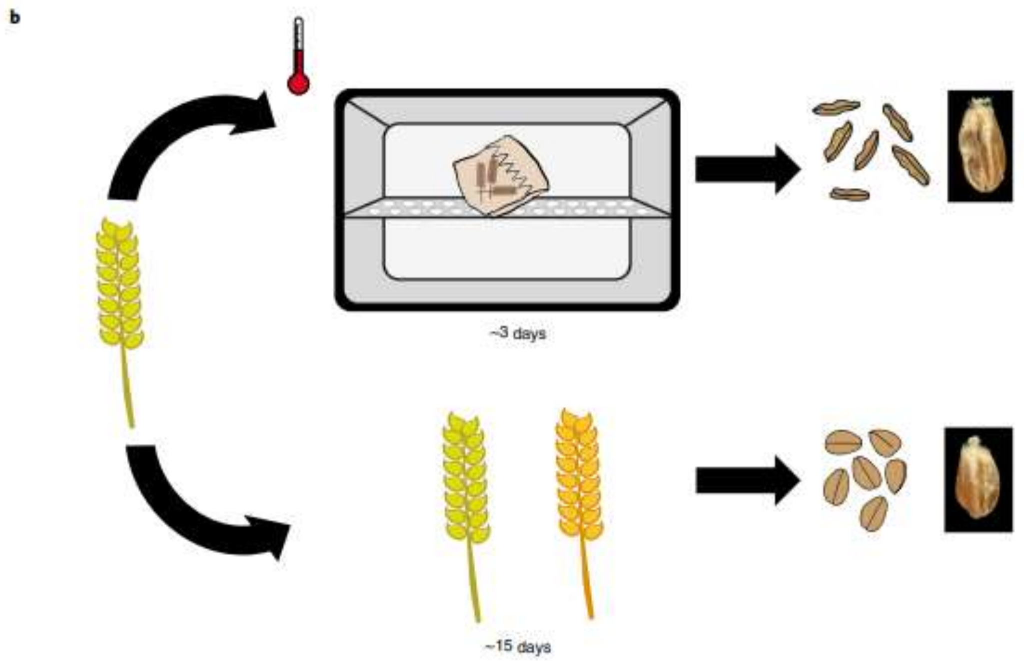

Fig. 2: Speed breeding enables harvesting and drying of immature seeds in dryer promoting rapid seed to seed cycling by shortening the period of drying from 15days to nearly 3 days. (Source: Watson *et al.*, 2018)

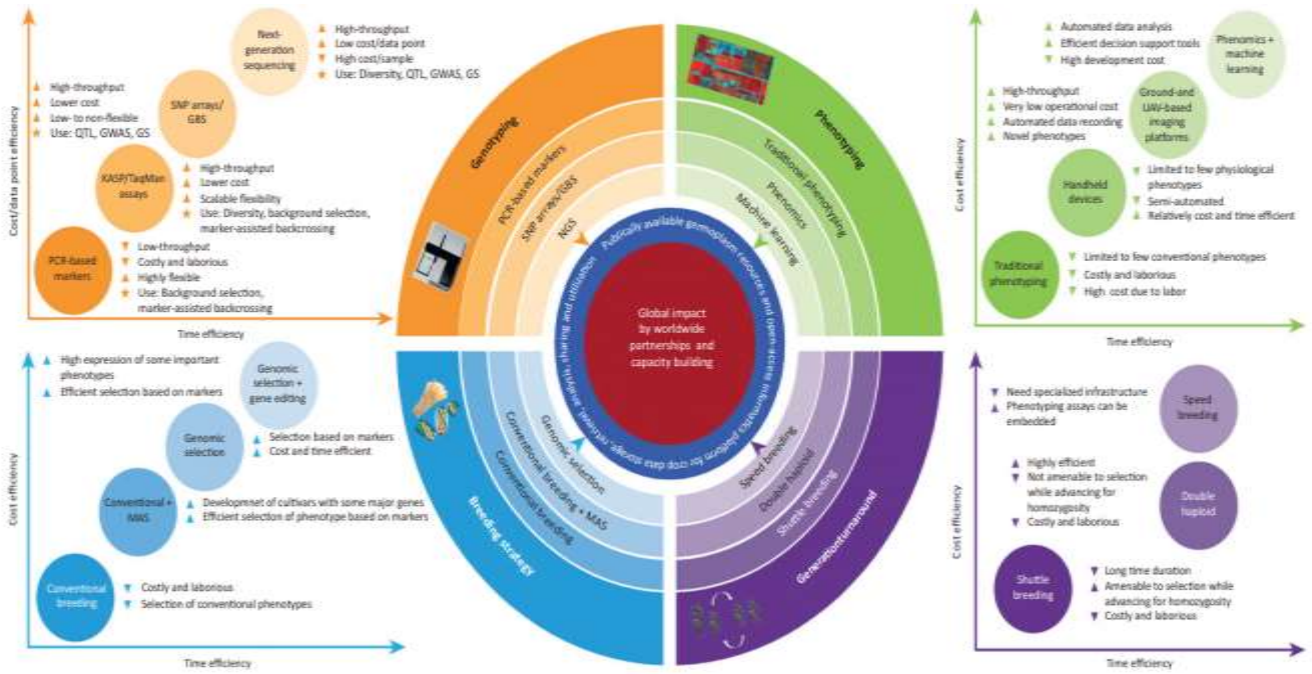

Fig. 3: Integrated strategies to enhance the improvement in plant breeding through genetic gain (Adapted from: Li *et al.*, 2018).

[Note: The features of different aspects have been described in biplots with their key advantages being denoted by ▲ and disadvantages are marked by ▼. The low color intensity indicates the higher possibility of technologies to integrate with other technologies.]

Challenges

- The responses of different species may vary with the extended photoperiods. SB's success in short-duration crops can be a challenge as it needs a photoperiod less than the critical day length to flower (Heide & Sønsteby, 2015).
- A type of rhythmic pattern is followed by plants in gene expression and metabolism corresponding to the day-night cycle (Mcclung, 2001). This rhythmic pattern is governed by the internal circadian clock (Schaffer *et al.*, 1998). The components of the plant circadian clock include the MYB transcription factor genes *LATE ELONGATED HYPOCOTYL (LHY)* and *CIRCADIAN CLOCK ASSOCIATED 1 (CCA1) I* and a series of *PSEUDO RESPONSE REGULATOR (PRR)*genes including *TIMING OF CAB EXPRESSION 1 (TOC1)* (Wang & Tobin, 1998). The *LHY* and *CCA1* are expressed in the early morning while *TOC1* is expressed in the evening time. In a study conducted in Arabidopsis, it was shown that *TOC1* enhanced the positive regulation of *CCA1* and *LHY* expression (Alabadí *et al.*, 2001). The correspondence of diurnal and nocturnal light influencing the expression and transcription levels of clock genes may be a hindrance for some crops (Wang & Tobin, 1998)
- Adaption of speed breeding approach in the farmers' field is the next challenge
- The SB approach cost, which starts at tens of thousands of dollars (Ghosh *et al.*, 2018), maybe a challenge for implementing SB for many projects.
- The generation cycle of a plant may depend upon various factors like variety, duration of light, the strength of sunlight, and soil climate like environmental parameters (Ghosh *et al.*, 2018). So a multi-location trial of speed breeding with the necessary protocol is necessary. Else plant variety that can adjust with speed breeding standards is to be developed and trailed.
- Regardless of the increasing global population, changing climate has been another challenging factor for plant breeders. The high concentration of carbon dioxide in the environment resulting from climate change has directly or indirectly hindered the plant yield (Von Braun *et al.*, 2005). In this scenario, coping with such climatic variations can be challenging and an opportunity for speed breeding. Here the adaptation of speed breeding to cope with this consequence of climate change will be both potential and bottle-neck of plant breeding.

Conclusions

The success of speed breeding on staple cereal crops like wheat, barley, chickpea with the higher genetic gain has no doubt ensure the improvement in yield performance. However, it's still a wonder for the world to know its success in common horticultural crops like fruits and vegetables.

Speed breeding has achieved more vigorous simulations than genomic selection with the high rate of genetic gain in its crops. Coping with the significant challenge of the long duration of varietal development, speed breeding has arisen a hope of more unique breeding strategies and program redesigning in its approach to simplify the challenges and grab the opportunities that may evolve in the path of plant breeding.

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