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Review Article

Speed Breeding: An Innovative Method for Crop Improvement

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Received: January 29, 2021; **Published:** February 24, 2021

Abstract

Crop improvement can help us to meet the food requirement and feed for the growing population, which is expected to reach about 10 billion by 2050. Plant breeding plays a key role to mitigate various challenges and increased agriculture production to feed for the growing population. Conventional plant breeding and advanced genomic tools including genotyping, high throughput phenotyping, genome editing, genomic selection, DNA marker and marker- assisted selection enable plant breeder for rapid trait discovery and their introgression in the recurrent background, but all these required longer generation time hence to cop up these problems research developed a new concept of plant breeding "Speed breeding" for advanced generation cycling, the greater genetic gain which accelerate rapid varietal development. In this context, we have summarized a short view of the application and achievement of speed breeding in vegetable crops and their future prospectus.

Keywords: *Speed Breeding; Genomic Selection; Accelerate Breeding; Vegetable Crops; Photoperiod*

Introduction

The exponential growth of the human's population and increasing climatic change a major challenge to global agriculture for sustainable food production to feed the growing population. Plant breeding plays a significant role in resolving agricultural problems and enhanced both production and productivity that need to feed the growing population. But the current pace to increase the yield of major vegetable crops such as tomato (*Solanum Lycopersicon.* L), Pea (*Pisum sativum*. L), Potato (*Solanum tuberosum*. L) and other legumes and leafy vegetable crops such as lettuce (*Lactuca sativa*. L), is insufficient to meet future demand [1]. Breeder and geneticists are under pressure to improve the production of these existing crops and other vegetables that are superior both in terms of quality and quantity and resistance against various abiotic stress such as heat, drought, salinity, low temperature and climate-smart. Evolution of various breeding technologies and development of faster high-throughput phenotyping system, the second and third generation of sequencing platform, DNA markers system can enable to plant breeder for trait discovery, trait dissection and improve both accuracy and selection intensity for desired traits in several vegetable crops [2]. One major drawback plant breeding required long generation time and is facilitated to grow single generation per year that can be alleviated by "Speed breeding" with the use of extended photoperiod and controlled environment

conditions for shortening generation times and genetic gain in vegetable crops. Speed breeding protocol was earlier proposed by Watson., *et al*. [3] and demonstrated that speed breeding in full enclosed glass house chamber with artificial supplement of light or extended photoperiod to all full-day and harvested immature seed for shortening generation times and accelerate research programme on "Speed breeding". Jahne., *et al*. [4] developed a speed breeding protocol for soyabean. Soyabean is a short-day crop hence extending photoperiod more than 12 hr leads to delayed flowering, but another hand may enhance carbon accumulation therefore speed up seed production [5]. In addition, reported that phytochrome deficient genotypes of soyabean, maize, rice and sorghum produced earlier flowering long-day photoperiod. Focusing on soyabean, the first reported enhanced light regime with extended photoperiod > 12 hr combined with low-red to a far-red ratio (< 700 nm; > 700 nm) accelerate earlier flowering with increased photosynthetic efficiency. Craig and Runkle [6] reported far-red lighting to accelerate earlier flowering but in the case of soyabean neither far-red nor blue light promotes earlier flowering but lead to unwanted morphology, elongated petiole and lodging; hence multi-story speed breeding protocol for soyabean must be avoided far-red light (700 nm) and maintaining low red/blue light ratio with optimal light intensity ~500 μmol/m^2 accelerated rapid maturity and earlier flowering in soyabean [4]. Speed breeding enables 5 - 6 generations per year as compared to one generation in field and 2 - 3 generations in winter nursery in soyabean. In general speed breeding, speed breeding accelerates the rapid introgression of monogenic traits that are easily characterized in the growth chamber; obtaining five generations per year through the speed breeding system will lead to approximately double the genetic gain as compared to another conventional breeding programme.

History and evolution of speed breeding

Development of cultivars required long generation times and time-consuming process and it takes around 8 - 10 years for developing an improved cultivar depending on the breeding procedure, traits of interest. A Long year ago, some scientists and geneticists have reported that plants can grow under artificial light with extended photoperiod to cope with rapid generation cycling, generation advancement. Recently speed breeding has been introduced and a majority of more than 100 different plant species, including vegetables, legume crops, cereals and other herb vegetables reported that faster and rapid generation cycling can be obtained under continuous light with extended photoperiod condition. In -1980s NASA makes a joint research programme with Utah State University for the rapid generation advancement of wheat on the space station, which opens a new era of crop breeding and also explores the possibility of growing food in space to meet the requirement of the astronaut's in the space station. USU-Apogee was the first dwarf wheat variety was developed by 'speed breeding" [2,7]. The discovery of Light emitter iodide (LEDs) in the 1990s and their effect on plant growth and development was evaluated at the University of Wisconsin, the USA accelerate more advanced research and application of speed breeding for crop improvement. Inspired from NASA's work, the research of the University of Queensland proposed the term "Speed breeding' in 2003 to accelerate and rapid generation advancement of wheat breeding. Speed breeding coupled with other technology such as double haploid technology, embryo rescue to shortening generation times [8]. Speed breeding utilized continuous supplement lighting in glasshouse condition, with optimum light quantity, light quality, and intensity, and daylength accelerate earlier flowering, photosynthetic rate and rapid generation cycling of crop plants. Generally, different crop responds different growing environment, hence it is necessary to design and development of crop-specific standard speed breeding protocol is necessary [9].

Recently, speed breeding protocol is developed in long-days crops, but lack in short day vegetables, and cereal crops. Meanwhile, speed breeding protocol recently has been demonstrating in some short days crop likely, Soyabean (*Glycine max*), rice (*Oryza sativa*) and Amaranthus (*Amaranthus spp*.) [1]. Speed breeding protocol based on light-emitting diodide (LEDs), hence adjusting photoperiod about 10 hr and blue-light enriched facilitated the growth of short days crop species such as soyabean plants that are flowered 23 days earlier than normal plants and give rise advanced crop maturity within 77 days thus facilitated to grow up to 5 - 6 generation of soyabean per year. Similarly, rice and Amaranthus advanced flowering 10 and 20 days can be achieved by using speed breeding protocol respectively. First spring wheat variety "DS Farady' developed in Australia by using the "speed breeding' protocol.

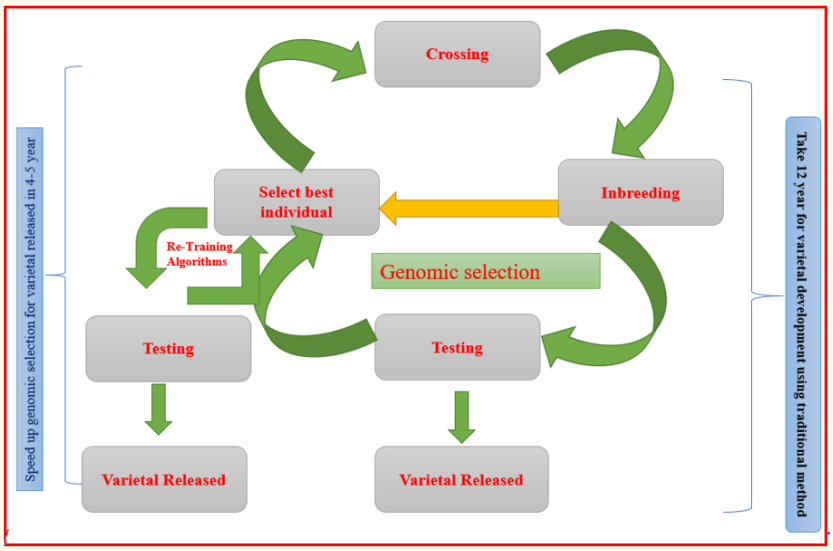

Figure 1: *Speed up genomic selection for earlier varietal development [10].*

Speed breeding with other genomic tools

Speed breeding accelerates research in plant breeding, faster varietal development, traits dissection, crossing, development of mapping population, backcrossing, a pyramiding of multiple traits in to single genotyping for development of multiple disease resistance and explore a new possibility of phenotyping of particular desired traits as well transgenic pipelines. Integration of speed breeding with other Morden breeding tools such as single seed descent (SSD), double haploid, embryo rescue, high-throughput genotyping and phenotyping, genome editing, genomic selection and DNA markers accelerate research in crop improvement, reducing generation time, testing of hybridity, purity of cultivars and rapid introgression of genes in recurrent parent background. Genome editing combined and induced mutagenesis coupled with speed breeding accelerate crop bio-fortification example, increasing the level of Vitamin B9 in rice, removal of saponin from quinoa (*Chenopodium quinoa)*, removal of glucosinolate from *Brassica* spp and neutralize toxin from *Lathyrus sativus* for safe consumption. Speed breeding in conjunction with other technologies facilitated the development of disease resistance and scoring of wheat leaf rust and wheat leaf rust and reported that wheat-rye hybrid is cytologically stable under speed breeding conditions. Speed breeding integration with other biotechnological tools such as genetic transformation accelerates the development of viable seed > 6 weeks in barley than standard control condition and facilitated accurate phenotyping to resistance against pod shattering in canola, which is a major hinder for cultivation in the dry and semi-dry region [3].

Advanced in genomic tools and decreasing the cost of sequencing have enabled plant geneticists to focus from model plants to crop plants. In the breeding context, advance in homozygosity following crossing and rapid cycling allows more rapid production of improved cultivars which can be achieved by using 'speed breeding" technology [2]. Genomic selection (GS) accelerate the selection accuracy of superior genotypes, germplasm enhancement (i.e. pre-breeding) and help in selection for targeted gene for particular traits from gene bank accession to elite lines other approaches like hyperspectral image technology integrated with genomic selection (GS) and pedigree assisted breeding to explore a faster way for rapid introgression of genes from elite or wild accession to cultivated crop [11]. Accelerate genetic gain by using integrative approaches of speed breeding and genomic selection is represented (Figure 2). Genomic selection (GS)

is used to predict the genotypic value, combined with high throughput genotyping systems such as SNPs, sequencing platforms for the improvement of quantitative traits i.e. Yields. Molecular marker such as RAPD, RFPL, SSR, SNP, CAPS etc used to detect phenotypic variation at the genomic level which accelerates marker-assisted selection (MAS) for crop improvement. Marker assisted-selection comprises selection of the QTL-associated markers that have major an effect and significantly contribute to economic traits. The genomic selection offers an opportunity for rapid genetic gain, and reduced breeding cycle to at least half the conventional breeding discovery of QTLs and QTLs associated marker, significantly increased grain yield and performance of cultivars [11] (Figure 2).

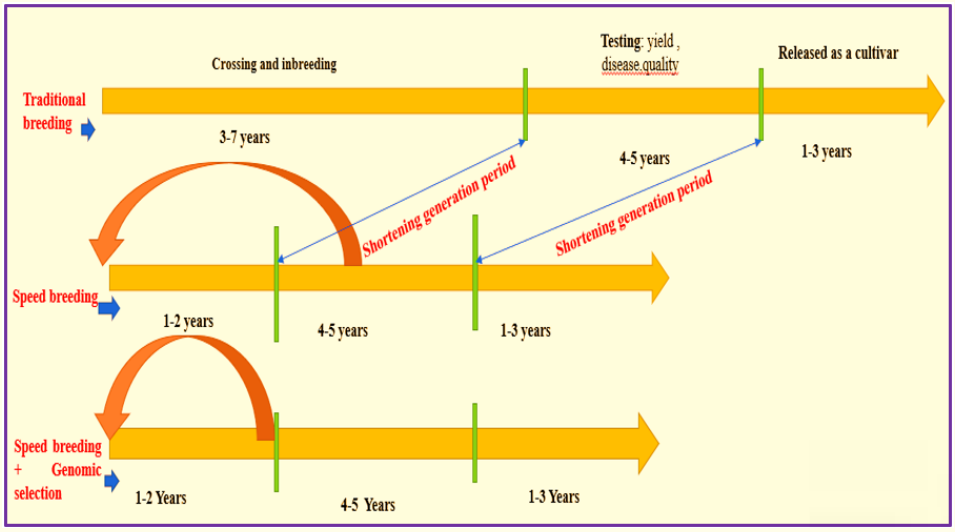

Figure 2: *Boosting genetic gain by using integrative approaches of speed breeding and genomic selection*

Speed breeding in vegetable crops: A future prospectus

Generally, tremendous research work has been made, the application and prospectus of speed breeding in cereals but lack in vegetable crops except for a few ones. Although speed breeding protocol has been standardized for some vegetable crops such as pepper (*Capsicum annuum*), tomato (*Solanum Lycopersicon*), radish (Raphanus sativus), pea (*Pisum sativum*), Amaranthus (*Amaranthus spp*), soyabean (*Glycine max*), Onion (*Allium sativum*) and till yet research work has been progressed for standardized and development of speed breeding protocol in legumes, leafy and herb vegetable crops. Speed breeding methods have already have been standardized to accelerate rapid generation advancement and genetic gain in amaranthus, peanut, wheat, rice, sunflower, tomato etc. Development and standardization of "speed breeding" protocol in several vegetable crops such as radish, pea, tomato (Introgression of continuous light tolerance gene *CAB-13* to increase productivity under continuous light) [9], Amaranthus, Cassava, Potato (Speed breeding with extended photoperiod in development James Hutton Institute), Brassica, Sugar beet, pea [1] and some other leafy vegetables are in progress. Speed breeding is likely to reduce generation time for other crops like tomato, potato, Amaranthus (can produced eight-generations per year instead of two in the field) Pepper (Early flowering and fruiting under continuous light [12]. Onion is biennial (bulb formation in one year and flowering and seed setting takes place in the next year), cross-pollinated (due to the presence of protandry) and suffer serve inbreeding depression [13]; which took 10 - 12 years for varietal development [13,14]. In onion, speed breeding can be hastened by breaking bulb dormancy and subjected with 22-hour photoperiod using Far-red light accelerate bulb initiation within 45 days and caused rapid bulb maturity within

80 days instead of required 5 - 6 months for bulb development [13,14]. Tomato is sensitive against continuous light supplement; hence identification and mapping of continuous light tolerant locus *CAB-13* at the short arm of chromosome 7 and *CAB-13* (Solyc07g063600.2) gene linked with marker 7-20-1B responsible for light tolerant, and its role in continuous light tolerant was further tested in studies [9]. Identification of metabolic pathways and biochemical reactions involved in the mechanism responsible for tolerance against continuous light lead to the identification of differentially expressed proteins by gene expression analysis and gene ontology facilitated the growth of tomato in speed breeding chamber with continuous light supplement. Legumes are the main source of dietary protein, which are essential for growth and development. Speed breeding programme for rapid generation cycling and fast stacking desired trait has been received much attention in several cool-season legumes include chickpea (*Cicer arietinum*), Faba bean (*Vicia faba*), lentil (*Lens culinaris*), and pea (*Pisum sativum*), and some warm legumes include common bean (*Phaseolus vulgaris*), cowpea (*Vigna unguiculata*), pigeon pea (*Cajanus cajan*) [15] soybean (*Glycine max*), and groundnut (*Arachis hypogaea*) [1]. Speed breeding protocol has been standardized for growing of potato with continuous supplementation of long photoperiod in controlled greenhouse conditions accelerated faster plant growth and produced earlier flowering, fruiting and faster seed maturity [16]. Speed breeding integrated with aeroponics technology-facilitated year-round production of disease-free mini tubers suitable for planting purpose.

A Single locus *CAB-13*: Facilitated speed breeding in tomato

Tomato (*Solanum lycopersicum* L.) belongs to the family Solanaceae and acts as a model crop for biotechnological and molecular research. Based on the growth habit, tomato can be classified into two types, (i) Determinate habit: suitable for open field condition and mechanized harvesting, (ii) Indeterminate habit: suited for greenhouse conditions.

Recently, with the discovery and application of speed breeding technology in several kinds of cereals, pulses, millets, generated an idea for their applicability, and suitability has been tested in several vegetable crops. Earlier research regarding the effect of photoperiod and continuous light on growth, yield and quality revealed that tomato is susceptible against continuous prolonged photoperiod and shows pronounced chlorosis and necrosis on leaves and detrimental to fruit yield and quality. However, other vegetable crops such as pepper (*Capsicum annuum* L), lettuce (*Lactuca sativa* L.), vegetable soya bean (*Glycine max*), and a model plants like Arabidopsis did not show such types pronounced effect and give rise higher yield under prolong continuous light conditions. Johne., *et al*. [4] reported that continuous light supplement with far-red light-induced chlorosis and necrosis in tomato leaves. This could facilitate a fundamental research program for understanding the mechanism lying under continuous light tolerance in tomato. The tomato simulation model "*TOMSIM*" was used to predict the performance of tomato genotypes under continuous light proposed that increased yield about 22 - 24% was reported under greenhouse with extending photoperiod about 18 hours/days in continuous light tolerance tomato after introgressing the *CAB-13* gene enable for vegetative growth, reproductive and ultimately enhance total crop yield and double the genetic gain with rapid generation advancement for faster varietal development (Heuvelink., *et al.* 1997). One wild species of tomato such as *Solanum pimpenellifolium* 'LA-1589' showed resistance against the continuous light condition, hence the discovery of the *CAB-13* gene and their introgression in tomato F_1 hybrid, and another interspecific hybrid of eggplant (*Solanum Melongena*), potato (*Solanum tuberosum*) accelerate the application of speed breeding program. Initially, single nucleotide polymorphism (SNPs) marker used for mapping the continuous light tolerance gene in tomato and reported continuous light tolerant gene in tomato was governed by a single dominant gene [9].

The comparison of RNA-Seq derived transcriptome of light tolerant wild species and light-sensitive leads identification of chlorophyll a/b binding protein 13 gene (*LHCB type III CAB-13* or *CAB-13, Solyc07g063600.2*), a putative candidate gene confers tolerant against continuous photoperiod condition [9]. The identification and confirmation regularity role of *CAB-13* against tolerant to prolong photoperiod and identified several putative regularity elements which are responsive against continuous light such as *SORLIP2AT, 10PEHVPSBD*, GATA box, and the *EVENINGAT3* at *CAB-13* promotor region (Chang., *et al*. 2008, Hudson., *et al*. 2003, Thum., *et al*. 2001). Further, more continuous light tolerant locus *CAB-13* has been mapped at the short arm of chromosome 7, and *CAB-13* (Solyc07g063600.2) gene linked with marker 7-20-1B responsible for light tolerant, and its role in continuous light tolerant was further tested in studies [9]. Identification of metabolic pathways and biochemical reactions involved in mechanism responsible for tolerance against continuous light lead to identification of differentially expressed proteins by gene expression analysis and gene ontology. Generally, susceptible prolong photoperiod plants are enriched with carbohydrate metabolic process, chlorophyll biosynthetic process, photosystem I reaction centre, and chlorophyll-binding; while tolerant plants having carbohydrate metabolic process, chlorophyll-binding and heme-binding were enriched in CLT

enrichment. Another experiment was conducted regarding the performance of *CAB-13* gene plants for their usefulness was reported that no difference on flowering truss appearance, fruit set, plant growth except longer stem in *CAB*-13 introgressed plants than normal plants, and fruit yield was reported to compare to normal and introgressed (*CAB-13*) plants [9].

Arnon., *et al*. [9] reported continuous supplementing photoperiod 16 hours in *CAB-13* introgressed plant tend to yield up to 20% in tomato. Several wild species such as *Solanum neorickii, Solanum pennellii, Solanum habrochaites* and *Solanum chilense* are used for resistance breeding programmes for introgression of the continuous light tolerant gene in cultivated tomato [17].

Conclusion

Speed breeding reduces generation cycle and rapidly generates homozygous line through single seed descent, which in some species may be cheaper than generating double haploids, for subsequent field evaluation and selection, thus facilitate genetic gain for key traits and allow more rapid production of improved cultivars. Combining genomic selection, genome editing, and resources with speed breeding will provide a strong incentive for plant scientists to perform research on crop plants directly, thus further accelerating crop improvement research.

Acknowledgement

Authors are grateful to their colleagues, seniors and scientists of Division of Vegetable Science, ICAR-IARI, New Delhi for providing valuable suggestions, guideline, research article, published research peppers from various national and international institutes during writing this manuscript.

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Volume 7 Issue 3 March 2021