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Speed Breeding in Vegetable Crops: A New Approach to Feed the Future Generations

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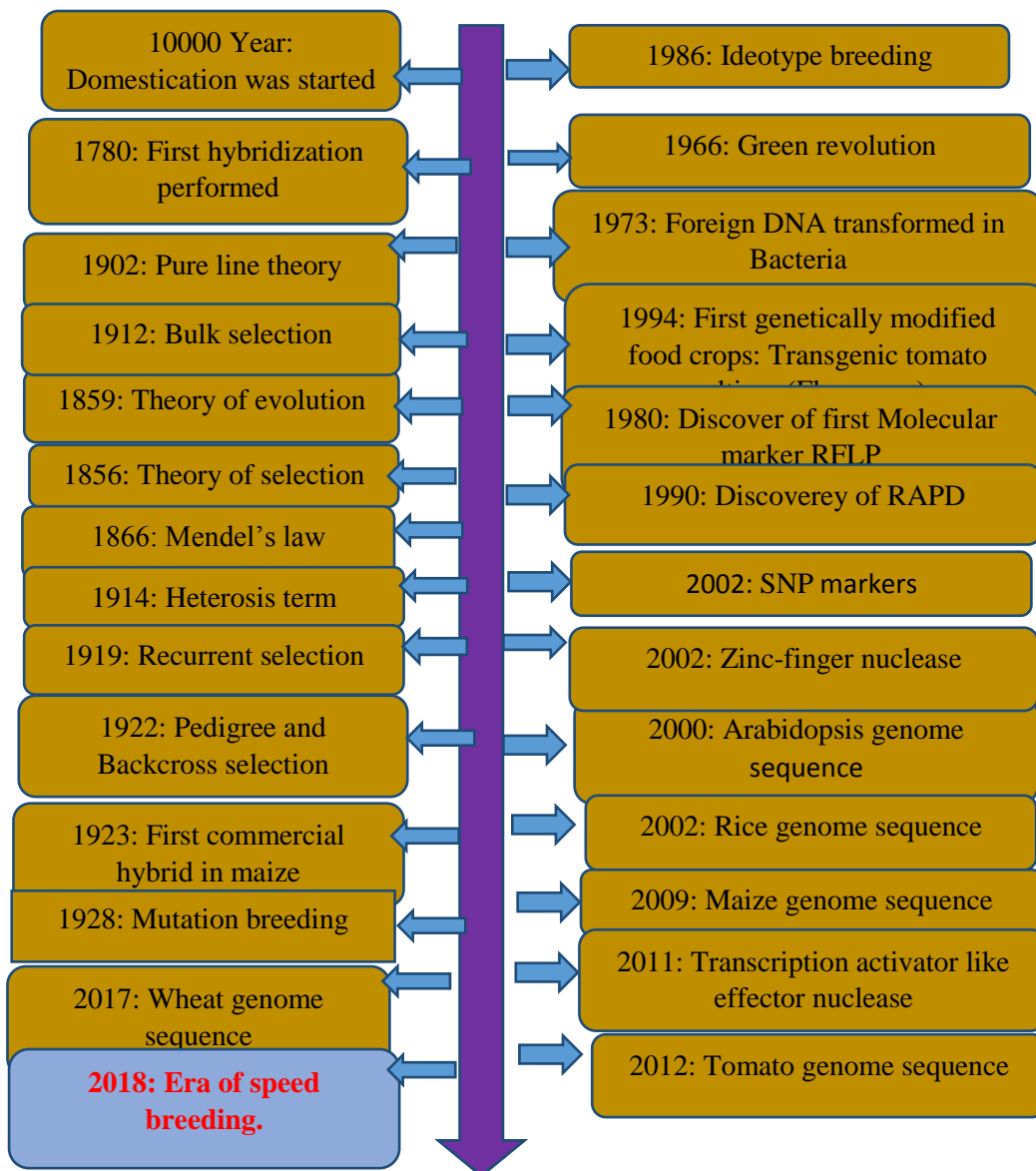
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The growing human population and changing environmental conditions significant concern for global food security, with the current improvement rate of several important crops which are inadequate to meet future demand. This slow improvement rate is attributed partly to the long generation time of crop plants. An alternate technique to counter this hurdle is the use of speed breeding technology that shortens the breeding cycle and accelerates the crop research through rapid generation advancement. It can be carried out in numerous ways, one of which involves extending the duration of plant's daily exposure to light, combined with early seed harvest, to cycle quickly from seed to seed, thereby reducing the generation time for some long-day or day-neutral crops. It can also achieve up to 6 generations per year in growth chambers and normal glasshouse conditions. The use of supplemental lighting using LEDs in a glasshouse environment allows rapid generation cycling through single seed descent (SSD) method and plant density can be scaled-up for large crop improvement programs (Watson *et al.*, 2018).

Speed breeding can accelerate backcrossing and pyramiding of traits as well as transgenic pipelines (Chiurugwi *et al.*, 2018). The idea for growing of wheat in space inspired by senior research fellow Dr Lee Hickey of the University of Queensland, Australia. The first spring wheat variety developed using speed breeding is 'DS Faraday', which was released in 2017 in Australia. Speed breeding carries in an enclosed chamber with artificially provided LED light (e.g. halogen lamps) which is provided PAR of 400-700 nm and photoperiod of 22 hours with 2 hours of darkness in 24 hours of the diurnal cycle and temperature should be maintained accordingly and relative humidity 60-70% also maintained during the entire life cycle (Hickey *et al.*, 2018). Vegetable crops like radish, pea tomato (Introgression of continuous light tolerance gene *CAB-13* to increase productivity under continuous light), Amaranthus, Cassava, Potato brassica, Sugar beet and some other leafy vegetables crops (Chiurugwi *et al.*, 2018). 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) (Kai *et al.*, 2019).



History and evolution of speed breeding technology

Principle: The principle behind speed breeding is to use optimum light intensity (photoperiod) light quality, optimum temperature (varied accordingly crop to crop), and daylight length control (22 h light, 22°C at day/17°C at night with high light intensity) (Ghosh *et al.*, 2018) which leads to accelerate the rate of photosynthesis, stimulates early flowering, seed maturity, harvesting and ultimately shorten the generation time required for crop growth and development. Using speed breeding coupled with single seed descent is more commonly used for developing efficient breeding line and elite inbred line which can be exploited for the development of improved crop varieties in a shorter time, which is cheaper compared to the production of Di-haploids. Speed breeding is also used for gene insertion (common haplotypes) of distinct phenotypes followed by MAS of elite hybrid lines (Alahmad *et al.*, 2018)

Standard protocol, greenhouse design and set up-requirements for speed breeding: To make successful approaches of speed breeding program a proper channel and set-up requirements are



essential. Controlled green-house structure, the light requirements (Photoperiod), temperature, humidity and germplasm are necessary (Ahmar *et al.*, 2020). Photoperiod (Light) requirement differs in different plant species because different plants respond differently with varying wave-length emitted by different lighting sources. Generally, light requirements covering PAR (400-700 nm) is more suitable for speed breeding with 22 hours of photoperiod. The optimum temperature should be maintained according to different plant species with specific growth stage (seedling germination, vegetative growth stage, flowering, fruiting, and fruit setting) with 60-70 % relative humidity (Chiurugwi *et al.*, 2018). The following methods are employed for speed breeding program:

(A) Controlled environmental chamber speed breeding condition: This type of structure programmed is run up to 22-hour of photoperiod, with an optimum temperature of 22°C during the photoperiod, and about 17°C during the 2-hour dark period. An artificial photoperiod can be applied by a mixture of white LED bars (Valoya; 6 units per 3.67 m²), far- red LED lamps (Valoya; 12 units per 3.67 m²) and metal HQI lamps (Valoya; 32 units per 3.67 m²) and optimal light intensity should be adjusted to 360- 380 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (highest value after ramping) at bench height, where pots is planted on the bench. Generally, wheat, barley, amaranthus and pea are suited to model plant for speed breeding under controlled environmental conditions.

(B) Glasshouse speed breeding conditions: A temperature-controlled greenhouse is fitted and maintained at 17/22°C optimum temperature regime, with sodium vapour lamps and 12 hours of turnover and 22-hour of photoperiod (Ghosh *et al.*, 2018). Light intensity is maintained as 440-650 $\mu\text{mol m}^{-2} \text{s}^{-1}$ at adult plant height (approximately 45 cm above the bench height).

(C) Homemade growth room design for low-cost speed breeding: A homemade structure about 3 m x 3 m x 3 m with insulated sandwich panelling and fitted lighting equipment about 7 LB-8 LED lightboxes (1 lightbox per 0.65 m²) from Grow Candy (www.growcandy.com). Light quantity and quality about PAR at bench height ranged from 210-260 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and at 50 cm above the pot from 340-590 $\mu\text{mol m}^{-2} \text{s}^{-1}$. The lights were situated at a height of 140 cm above the bench. In this type of structure the room can accommodate 90 pots of 8" diameter and 5 L volume are maintained at this kind of structure.

Application and constraint of speed breeding in vegetable improvement: Speed breeding can accelerate the rapid generation and develop the breeding population. Mobini *et al.*, (2016) recorded and reported rapid development of mapping population in pea, mutation studied, fast-forwarding genetic trait, faster and better phenotyping. Watson *et al.*, (2018) recorded that the phenotypes associated with the EMS- induced mutation of the awn suppressor B1 locus 9 and the green revolution reduced height gene (Rht) in wheat could be accurately recapitulated, encapsulated in the controlled green-house condition. Marker- assisted selection coupled with speed breeding provides a flexible platform to rapid introgression of the desired gene for targeted trait having resistance to biotic and abiotic stress and pyramiding could help the development of multiple disease resistance cultivars in different cereals and vegetable crops.

Speed breeding has some following limitations eg., applicable speed breeding protocol in various vegetable crops are not yet developed. (A) Most vegetables are long day and day-neutral plants hence, continuous light can accelerate the genetic gain and breeding cycle but, short day plant required limited photoperiod for their growth and development which limits the application of speed breeding for improvement short day vegetable crops. Speed breeding is more straight forward and easier implementation in long day and day-neutral types than short-day plant, but Lee Hickey *et al.*, (2018) have been standardized speed breeding protocol for some short-day plants like millet, sorghum etc, but not in any vegetable crop yet been developed. (B) Different phenotyping: speed breeding accelerates rapid cycling and shorten generation time further early harvest of immature seed



can interfere with phenotyping of some trait (Hickey *et al.*, 2009). (C) High initial investment: speed breeding program are carried in enclosed glass house chambers which requires highly initial investment, purchasing lighting equipment and controlled capabilities and supplementary LED lighting provides more efficient power usage and reduced heat than other lighting types, such as SVLs. (D) Plants which are grown in an enclosed glass chamber with extended photoperiod show various physiological and toxicity symptoms like, chlorosis, necrosis and yellowing due to any micronutrient or heavy metal deficiency or excess.

Summary

Recent advancements in genetic tools, breeding methods and development of NGS sequencing technology provides a flexible platform for sequencing, tagging, mapping and introgression of a gene for the desired trait at low cost, but it is time-consuming. Speed breeding coupled with other approaches tends to shorten the breeding cycle, generation advancement and accelerates the development of cultivars in a very short time. speed breeding and integration with other breeding technologies may lead rapid genetic gain, For example, tomato is sensitive to constant light, but researchers have identified a tomato gene (*CAB-13*) that make the plant to tolerate constant long photoperiod when transferred into a tomato cultivar grown under speed breeding conditions (20% increase in fruit yield). Speed breeding as an alternative solution which can enable breeding in other recalcitrant crops, such as short-day species like maize and biennial species like sugar beet. It can be concluded that speed breeding combining other genetic tools like genome editing, Marker-assisted backcrossing, Transgenic approaches coupled with cost-effective genotyping and rapid phenotyping helps to develop homozygous inbred line followed by crossing will facilitate rapid cycling, genetic gain and rapid development of improved cultivars to researchers or Plant breeder. Therefore speed breeding integrated with other multiple branches can be used to improve Orphans, legumes, vegetables, leafy vegetables and other forage crops.

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