Speed Breeding In Vegetable Crops And For Colour Development

[1]Rashmi S. R., [2] R. M. Hosamani, [3] G. O. Manjunath
[1]M. Sc. (Agri.) student, [2]Professor of Horticulture, [3]Professor of Horticulture
Department of Horticulture
College of Agriculture, University of Agricultural Sciences, Dharwad, India.

Abstract - Globally, micronutrient malnutrition is a big burden in public health spread across the geopolitical regions and socio-economic strata. The efforts for challenging this "hidden hunger" are dietary diversity and increase in nutraceutical value of vegetable crop. The focus on breeding for yield traits affected quality attributes seriously, hence it become essential to breed varieties having high yield and better quality. Speed breeding is one of the techniques for colour development in vegetable crops. Speed breeding is regarded as the future of plant breeding, offering a rapid generation advancement technology aimed at reducing crop cycle times. This innovative approach significantly shortens the traditionally lengthy life cycles of crop plants and also in colour development in vegetable crops. With speed breeding, it becomes possible to obtain up to six generations per year for photo insensitive crops and 2-3 generations per year for others. Central to speed breeding is the manipulation of photoperiodic conditions, ensuring optimal light exposure for accelerated growth. Additionally, this technique effectively manages the temperature requirements of crops cultivated in controlled environments such as poly houses or glass houses. By providing an ideal environment for crop growth, speed breeding accelerates the breeding process and facilitates the development of new varieties. Moreover, speed breeding complements other modern technologies such as high throughput genotyping and genome editing platforms. By integrating these tools, speed breeding enhances the pace and scale of variety development. Originally inspired by NASA's efforts to rapidly grow food in space, speed breeding has evolved into a versatile technique applicable to terrestrial agriculture. The feasibility of employing speed breeding for a particular crop can be assessed using the breeder's equation, which considers various factors influencing crop development. The primary objective of speed breeding revolves around optimizing photoperiodic regimes, manipulating light, temperature and humidity to create an optimal growing environment. The applications of speed breeding are diverse, ranging from accelerated breeding programs to enhancing transgenic and *CRISPR* pipelines. Additionally, speed breeding facilitates the rapid implementation of genomic selection strategies and enables the study of physiological traits in plant species. In essence, speed breeding represents a transformative approach to plant breeding, offering unprecedented efficiency and agility in variety development.

I. INTRODUCTION

Meeting the growing demands of an expanding global population necessitates a significant increase in crop yields. Projections suggest a 25 % rise in global population within the next three decades, reaching around ten billion (Hickey *et al*., 2018). Urgent action is required to swiftly develop cultivars with improved yields and resistance to prevalent pests and diseases. Despite existing conventional methods, the current rate of genetic progress in enhancing productivity falls short of future needs. Worldwide, crop breeding programs have stagnated, often limited to specific environments, impeding their effectiveness. Plant breeders face mounting pressure to revamp programs, focusing on timely yield enhancements and bolstering resistance to diseases, pests, and climate change. Climate change poses a significant threat to global food security, marked by its unpredictability and associated impacts like rising CO_2 levels, temperature shifts and extreme weather events. These challenges underscore the necessity for plant scientists to adapt breeding programs to thrive in harsh conditions and sustain global food security.

This review article is based on Master's Seminar- I presented during semester-II of year 2022-2023.

Speed breeding has emerged as a method to accelerate the processes of backcrossing, trait pyramiding, and transgenic pipeline development (Chiurugwi *et al*., 2018). Inspired by Dr. Lee Hickey of the University of Queensland, Australia, the concept of growing wheat in space led to the inception of this technique. The initial release of a spring wheat variety, 'DS Faraday,' in Australia in 2017 marked a significant milestone achieved through speed breeding. This approach involves growing plants in enclosed chambers with artificially provided LED light, typically halogen lamps, emitting photosynthetically active radiation (PAR) within the range of 400-700 nm. The photoperiod is maintained at 22 hours of light with a 2-hour dark period within a 24-hour diurnal cycle, while temperature and relative humidity are carefully regulated throughout the plant's life cycle (Hickey *et al*., 2018).

Various vegetable crops, including radish, pea, tomato (incorporating the continuous light tolerance gene CAB-13 to enhance productivity under continuous light conditions), amaranthus, cassava, potato, brassica, sugar beet, and several leafy vegetables, have been successfully grown using speed breeding techniques (Chiurugwi *et al*., 2018). Additionally, speed breeding holds promise for reducing generation time in other crops such as tomato, potato and amaranthus, enabling up to eight generations per year compared to two in traditional field conditions.

II. COLOUR DEVELOPMENT :

The presence of color in vegetables serves various vital functions such as aiding in photosynthesis, attracting pollinators for pollination, enhancing nutritional content and appealing to consumer preferences. Plant pigments, which encompass chlorophylls, carotenoids, flavonoids, and betalains contribute to the vibrant and diverse hues observed in nature. These pigments selectively absorb certain wavelengths of light while emitting others, thereby producing visible colors. Beyond visual allure, pigments play crucial roles in plant physiology and ecosystem dynamics, facilitating processes like photosynthesis, pollination and seed dispersal. Moreover, many pigment-rich fruits and vegetables are integral to human and animal diets, providing essential nutrients and potential health benefits. Chlorophylls, responsible for the green color in plants, primarily capture light for photosynthesis, while carotenoids, flavonoids, and betalains serve as accessory pigments with diverse structures and functions, offering nutritional and medicinal advantages. This discussion focuses on the biological significance and dietary contributions of carotenoids, flavonoids, and betalains.

Table 1: Major plant pigments and the colour exhibited

Pigment	Compound Types	Compound Examples	Typical Colours
Chlorophylls	Chlorophyll	Chlorophyll a and b	Green
Carotenoids	Carotenes	Lycopene, α-carotene, β-carotene, γ-carotene etc.	Yellow, Orange, Red
	Xanthophylls	Lutein,, Zeaxanthin, Neoxanthin, Violaxanthin etc.	Yellow, Orange
Betalains	Betacyanins		Red - Violet
	Betaxanthins		Yellow -Orange
Flavonoids	Anthocyanins	Cyanidin, Delphinidin, Malvidin Pelar gonidin, Peonidin, Petunidin	Red, Blue, violet
	Flavone	Luteolin, Apigenin, Tangeritin	Yellow
	Flavonol	Quercetin, Kaempferol, Myricetin	Yellow
	Flavanone	Hesperetin, Naringenin, Eriodictyol, Homoeriodictyol	Colour less co- pigments

III. DIFFERENCE BETWEEN SPEED BREEDING AND ACCELERATED BREEDING

•	• **Accelerated breeding**	• **Speed breeding**
• **Definition**	• Accelerated breeding refers to techniques that expedite the breeding process by reducing the time required for each generation.	• Speed breeding leverages controlled environmental conditions to accelerate plant development, allowing for the multiplication of many generations per year.
• **Purpose**	• It aims to achieve faster crop improvement by shortening the breeding cycle.	• It facilitate rapis growth homozygosity and switch progression of generation, ultimatelty hastening the advancement and release of novel variety crop
• **Methodology**	• Induces changes in physiological processes • Customizes phenotyping protocols for specific traits. •	• Grows plants under controlled • environments optimized for light, temperature, and other growth factors. • Integrates high throughput Phenotyping technique with high genotype platform to ease multiply traits improvement in short span

•

IV. HOW SPEED BREEDING IS BETTER THAN OTHER ACCELERATED BREEDING?

When the question arises of reducing the breeding cycles many methods come into consideration and few such methods amongs them are discussed here with their major drawback. The acceleration of breeding is done through different breeding methods.

Rapid generation advancement shortens the development to variety by two years through the manipulation of growing conditions like good seed set in less time.

In **shuttle breeding**,one can take breeding by growing it in different suitable locations. In off season also Therefore, two generations per year can be taken which reduces the time of the cycle to just half.

The **double haploid** production through bulbosum technique, anther or ovary culture or chromosome elimination techniques. It reduces the time of cycle from seven-year to just two years but it also reduces the genetic gain as the variability is lost due to homozygosity.

Now speeding breeding is better than all because it reduces the time of crossing and breeding from 3-7 to 1-2 years with the potential to double rate the of genetic gain. The speeding up of the method without loss of potential gain makes it the popular and advanced future method.

V. WAYS TO ACHIEVE SPEED BREEDING

a. Photoperiod and Light
b. Temperature
c. Plant nutrition
d. Density of plant population
e. Humidity
f. Carbon dioxide concentration
g. Physiological stress
h. Embryo rescue technique

a. Manipulation of photoperiod and light

This method involves subjecting plants to controlled light and dark cycles to expedite growth, flowering and seed production, a process that varies depending on the crop. Shorter photoperiods stimulate flowering, while longer ones delay this process. Light quality significantly impacts plant growth by altering photosynthetic rates, stomatal conductance, CO_2 levels and transpiration rates. Photoreceptors in leaves perceive changes in photoperiodic signals, subsequently influencing plant morphology and development. The red to far-red (R:FR) ratio, in particular is crucial in regulating flowering. Reduced red-light levels under shade conditions lead to a decrease in the R:FR ratio, triggering shade avoidance responses like elongated internodes and early flowering. A 22-hour light and 2-hour dark photoperiod within a 24-hour cycle is considered optimal for speed breeding. This lighting regimen can be achieved using various light sources such as LEDs, sodium vapor lamps and halogen lamps adjustable according to crop requirements in speed breeding.

In pepper (*Capsicum annum*) micropep red variety under conditions of a low red to far-red (R:FR) ratio of 0.3 and an extended photoperiod (Epp) lasting 20 hours, the time to first harvest for pepper plants was significantly reduced by 75 days compared to the control treatment. Additionally, a speed breeding system was established in a greenhouse with a 20-hour photoperiod and a 3.8 R:FR ratio, effectively shortening the breeding cycle of *Capsicum annuum* by 110 days from seed to seed. To elucidate the accelerated flowering response to Epp and supplemented far-red light, genome-wide association studies (GWAS) and gene expression analyses were conducted. The GWAS revealed a novel flowering gene locus for pepper, identifying four candidate genes (*APETALA2*, *WUSCHEL*-RELATED *HOMEOBOX4*, *FLOWERING LOCUS T*, and *GIGANTEA*). Expression analysis indicated that Epp and far-red light induced flowering by up-regulating the flowering-promoting gene *GIGANTEA* and down-regulating *FLOWERING LOCUS T* (Choi *et al*., 2023).

When *brassica sp* were exposed to LED light Genus-specific changes in plant weight and on the photosynthetic pigment levels as well as transcript levels have been detected. Interestingly, the transcript levels of the three investigated carotenoid biosynthesis genes phytoene synthase (*PSY*), β-cyclase (*βLCY*) and β-carotene hydroxylase (*βOHASE1*) were increased under the combination of blue & white LEDs in the majority of the Brassica sprouts (Frede *et al*., 2023).

This study was conducted on development of protocol for microrhizome production included influence of growth regulators, medium strength and photoperiod. Best response was obtained in medium containing BAP 1mg/l with NAA 0.2 mg/l (31.33 days) followed by BAP 1 mg/l alone (43.33 days) for early induction of microrhizomes. More number of microrhizomes and bigger microrhizomes were produced in MS medium having lower concentration of BAP. The study also revealed that full strength MS medium recorded higher number of microrhizomes, weight of microrhizomes and nodes per microrhizome which was followed by half strength medium. Further, intraction between photoperiod condition and media types showed that dark condition with liquid and semisolid medium produced highest number of microrhizomes per shoot (7.00), followed by four hour light condition with liquid and semisolid medium (Chougule *et al*., 2011)

b. Manipulation of temperature

Temperature plays a critical role in influencing plant development, particularly in the transition from vegetative to reproductive phases. Variations in soil and air temperatures have profound effects on germination, growth patterns and the timing of flowering, seed production, and maturity. Most crops exhibit optimal growth within temperature ranges specific to their species, typically between 12-30 °C for seed germination and 25-30 °C for overall growth, flowering and seed set. It's essential to maintain higher temperatures during light periods while allowing for a decrease in temperatures during dark periods to facilitate stress recovery (Went 1953). Failure to maintain ideal temperatures can lead to stress conditions, adversely impacting plant growth. Temperature regulation methods include fan and pad cooling systems, foggers, and solar-powered air battery systems.

Seven different pigment strains of tomatoes were analysed by Tomes *et al*. (2010). Each strain had a number of fruits removed and matured at either 23.5 °C or 32.0 °C and the levels of various pigments and polyenes were measured. It was observed that, except for β-carotene and potentially prolycopene, the production of all measured pigments and polyenes decreased at 32 °C. Some pigments were more sensitive to higher temperatures than others, with lycopene synthesis being significantly inhibited at 32 °C in all strains producing this pigment. When these tomatoes were genetically modified to enhance pigment production (high pigment factor, *hp*), the increased β-carotene fraction remained unaffected by temperature.

c. Plant nutrition and hormones:

Nutrient content and hormonal balance in plants are crucial factors in promoting accelerated growth and triggering processes such as flowering induction, seed set and in vitro seed germination. Controlled environments allow for varied responses to plant growth regulators (PGRs), with photoperiod and temperature being closely monitored and adjusted as needed. Key hormones including auxins, gibberellins, and cytokinins play pivotal roles in orchestrating various plant responses. When cytokinin levels are lower than auxin levels, the plant remains in a vegetative state; however, as cytokinin levels increase and auxin levels decrease, the plant transitions into the reproductive stage. Gibberellins are instrumental in regulating this transition from vegetative to reproductive states.

Study was conducted on Shortening the generation cycle in faba bean (*Vicia faba*) by application of cytokinin and cold stress to assist speed breeding by Mobini *et al*. (2020). Application of 6-benzylaminopurine (BAP) (cytokinin) improved pollen germination. The application of 10^{-5} M BAP 4 days after flowering increased seed set at the lower nodes. Cold treatment (8/4°C day/night for 2 days) after the onset of flowering induced the formation of more pods and faster pod set compared to the non-cold treatment. The combinations of 10^{-5} BAP and cold treatment together had similar and independent effects. These results will accelerate plant breeding in faba bean by providing additional tools for reducing generation time.

Experiment conducted during kharif green chilli cultivars Pusa Jwala, Nagavi and Kadrolli were fertilized with N at 150, 200 and 250 kg/ha (half before transplanting and half 30 days after transplanting). Flowering was early in Pusa Jwala (Revanappa *et al*., 1998)

An experiment was conducted to understand the influence of seed pelleting on crop growth, seed yield and quality (Manjunath *et al*., 2009). The experimental results indicated the superiority of seed pelleting with $ZnSO_4$ (300 mg/kg) + Captan (2.5 g/kg) + Imidacloprid (2.5 g/kg) mas compared to all other treatments gave 50 per cent flowering (40.34 days).

The study was conducted on influence of growth regulators and stages of spray on sex expression of ridge gourd. Among all the treatment combinations, application of NAA 50 ppm took minimum number of days to first pistillate flower appearance (37.98 and 31.59) and 50 per cent female flowering (59.76 and 50.25) compared to other growth regulators during both summer and kharif seasons respectively (Hilli *et al*.,2008)

Study showed that, short day condition induces tuberization with more efficiency when sucrose is used as a source of carbon than maltose. Further, 50 g/l of sucrose was found to be very effective, while increased concentration was rather lethal for growth and micro-tuberization in potato. Although, both BAP @ 2 ppm and kinetin @ 1 ppm were found to be effective as a source of cytokinin to induce micro-tuberization, kinetin can be a better source of cytokinin as its requirement is only 1 ppm as against 2 ppm of BAP (Bharath and Raju, 2023).

d. Humidity

Relative humidity is controlled to a limited extent in protected environmental chambers. Optimum RH of 60-70 % is ideal for crop growth which can be modified according to the crop.

e. **Carbon dioxide**

Carbon dioxide concentration is important in regulating the opening of stomatal pores through which plants exchange gases with the external environment. Elevated CO_2 concentrations ranging from 475-600 ppm increases average leaf photosynthetic rates by 40 %. High carbon dioxide (CO_2) levels enhance growth of the plant and speeds up the transition from vegetative to reproductive phase in angiosperms. However, different crop species shows varying responses to increased CO_2 levels. Such as the plants that adopts C_4 pathway for photosynthesis, shows less response than others due to elevated CO_2 levels that decreases stomatal conductance leading to indirect enhancement of photosynthesis by avoiding water stress under drought conditions. For altering CO_2 levels growth chambers, CO_2 cylinders and regulators are required that adds to additional operational costs. Also, there is need to adhere to health protocols and safety guidelines while handling and using CO_2 cylinders and valves**.**

f. Exerting them to physiological stress:

1. Dense planting :

High-density planting entails growing at higher plant densities than the density required to produce maximum yield. 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.

2. **Moisture stress**

Drought is considered as one of the major constraints in sustainable crop production. Moisture stress has been found to limit productivity by reducing stem length, leaf area, plant height and plant biomass in different crop. It restricts the crop vegetative growth. Drought or flooding stress can trigger early flowering and maturation, which can be used in speed breeding. Drought also helps in accumulation of phytochemical in plants

The carotenoid contents in three different-colored carrot cultivars, 'Kurodagosun' (orange), 'Benhongjinshi' (red) and 'Qitouhuang' (yellow) were determined by ultra-high-performance liquid chromatography (UPLC) after 15 % polyethylene glycol (PEG) 6000 treatment. Increases in β-carotene content in 'Qitouhuang' taproots under drought stress were found to be related to the expression levels of *DcPSY2* and *DcLCYB*. Increases in lutein and decreases in α-carotene content in 'Qitouhuang' and 'Kurodagosun' under PEG treatment may be related to the expression levels of *DcCYP97A3*, *DcCHXE* and *DcCHXB1*. The expression levels of *DcNCED1* and *DcNCED2* in the three cultivars significantly increased, thus suggesting that *NCED* genes could respond to drought stress (Zhang *et al*., 2020).

g. **Embryo rescue**

Embryo rescue in speed breeding refers to a technique used to rescue embryos from developing seeds and cultivate them in vitro under controlled conditions to accelerate their growth and development. This method is particularly useful in speed breeding programs where the goal is to shorten the breeding cycle and rapidly generate new plant varieties. By rescuing embryos from seeds, researchers can bypass the longer processes of seed maturation and germination, allowing for quicker generation turnover and selection of desirable traits. This technique facilitates the rapid production of new plant varieties, enabling breeders to efficiently introduce and test genetic modifications or crossbreeding combinations.

Several agronomic treatments are evaluated for reducing the generation time of tomato in the M82 (determinate) and Moneymaker (indeterminate) varieties. In the experiment, using XL containers in the autumn-winter cycle, evaluated cold priming at the cotyledonary stage, water stress, P supplementation, and K supplementation on generation time. It was found that, compared to the control, cold priming significantly reduced the number of leaves and plant height to first inflorescence as well as DSA (2.7 d), while K supplementation reduced DAR (8.8 d). Embryo rescue during the cell expansion cycle (average of 22.0 d and 23.3 d after anthesis for M82 and Moneymaker, respectively) allowed shortening the generation time by 8.7 d in M82 and 11.6 d in Moneymaker compared to the in planta fruit ripening. The combination of agronomic treatments with embryo rescue can make an effective contribution to increase the number of generations per year for speed breeding in tomato from the current three to four (Gimeno *et al*., 2023).

Two hot pepper varieties, 'Xiangyan 55' and 'Xiangla 712' were investigated for their growth and development under different light intensities, photoperiods and red-to-far-red ratios. Hot pepper plants bloomed at 39.88 ± 0.74 days after sowing under photosynthetic photon flux density (PPFD) 420 $\mu mol \cdot m^{-2}s^{-1}$ and a 12-h photoperiod and had seed with acceptable germination rates at 82 days after sowing. Blooming was 2–3 days earlier when the photoperiod was extended to 20 h, but the fruit and seed development were not significantly improved. Supplementation of far-red light (R:FR = 2.1) significantly accelerated the red ripening of pepper fruit and improved seed germination rates. The modification of the light environment accelerated hot pepper growth and development, reduced breeding cycles, and could produce up to four generations per year (Liu *et al*., 2022)

VI. A SINGLE LOCUS CAB-13: FACILITATING SPEED BREEDING IN TOMATO

Tomato (*Solanum lycopersicum* L.), a member of the Solanaceae family, serves as a pivotal model crop for biotechnological and molecular research. Its growth habit is distinguished into two types: determinate, ideal for open field conditions and mechanized harvesting, and indeterminate, suited for greenhouse environments. The advent of speed breeding technology initially applied in cereals, pulses, and millets, sparked interest in its potential applicability to various vegetable crops. However, preliminary investigations unveiled tomato's susceptibility to prolonged photoperiods, leading to leaf chlorosis and necrosis, ultimately impacting fruit yield and quality. Surprisingly, other vegetable crops and model plants such as pepper, lettuce, soybean, and Arabidopsis did not exhibit such pronounced effects under continuous light conditions. To delve into the underlying mechanisms of continuous light tolerance in tomato, fundamental research programs have been initiated. The utilization of the tomato simulation model "TOMSIM" predicted a significant increase in yield (up to 22 – 24 %) under greenhouse conditions with an extended photoperiod of 18 hours/day, particularly in continuous light-tolerant tomato genotypes bearing the *CAB-13* gene. *Solanum pimpenellifolium* 'LA 1589', a wild tomato species, exhibited resistance to continuous light, paving the way for the discovery and subsequent introgression of the *CAB-13* gene into tomato F_1 hybrids and interspecific hybrids of eggplant and potato, thereby accelerating speed breeding programs.

The continuous light tolerance gene in tomato was identified through single nucleotide polymorphism (SNP) markers, revealing its dominance. Further analysis of the transcriptome pinpointed the chlorophyll a/b binding protein 13 gene (*CAB-13*) as a putative candidate responsible for conferring tolerance to prolonged photoperiods. *CAB-13* was mapped to chromosome 7 and linked with marker 7-20-1B, demonstrating its role in continuous light tolerance.

Moreover, the study elucidated various regulatory elements responsive to continuous light conditions within the *CAB-13* promoter region. Gene expression analysis and gene ontology highlighted the metabolic pathways and biochemical reactions associated with continuous light tolerance, providing insights into the mechanisms underlying this trait.

Field experiments evaluating *CAB-13* introgressed plants revealed longer stems and increased yield (up to 20 %) under continuous supplemental photoperiods, further emphasizing the potential of *CAB-13* in enhancing tomato productivity. Wild tomato species have been explored for resistance breeding programs aimed at introgressing the continuous light-tolerant gene into cultivated tomato varieties, promising advancements in crop improvement.

The discovery and characterization of *CAB-13* represent a significant milestone in tomato breeding, offering new avenues for enhancing productivity and resilience in this essential crop (Chiurugwi *et al*., 2018).

Table 2: Milestones in speed breeding of vegetable crops

Sl.no	Crop	Speed breeding strategies	Days to flowering	Normal days for flowering	Number of generation or cycle per year	References
1.	Chilli	Photoperiod, light intensity	38-40	45-47	4	Liu *et al*. (2022)
2.	Amaranthus	Photoperiod and temperature	28	30-32	6	Jahne *et al*. (2022)
3.	Faba Bean	Plant harmones, photoperiod, light intensity and immature seed	29-32	35	7	Mobini *et al*. (2020)

4.	*Brassica oleracea*	22 hours photoperiod	108	112	-	Ghosh *et al*. (2018)
5.	*Brassica canapus*	22 hours photoperiod	87	90	-	Ghosh *et al*. (2018)
6.	*Brassica rapa*	22 hours photoperiod	87	90-95	-	Ghosh *et al*. (2018)

VIII. SET UP OF SPEED BREEDING

General set up for speed breeding

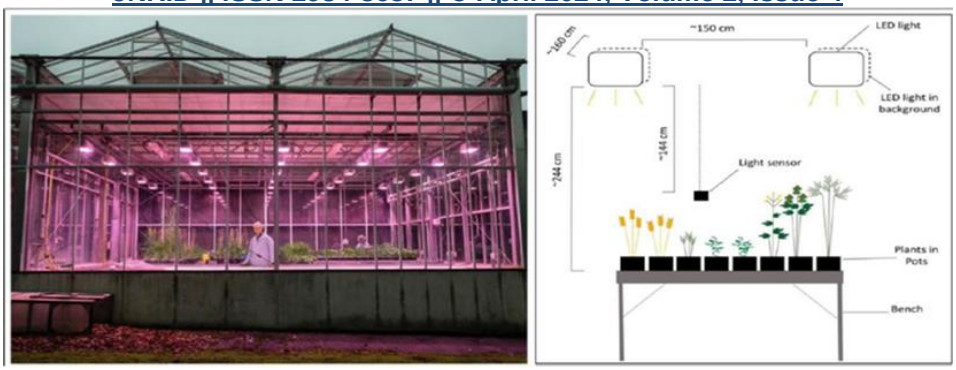
• Light: Photosynthetically Active Radiation (PAR) region (400-700 nm), environmental lighting with LED.
• Photoperiod: 22 hours with 2 hours of darkness
• Temperature: 22 °C for light period and 170 °C for 2 hours dark period
• Humidity: Ideally 60-70 %

i. Speed breeding I- Controlled environment chamber

- It is a convir on BDW chamber.
- Temperature is 22 °C during photoperiod and 17 °C during 2 hours of dark period
- Humidity is 70 %
- Lighting: Mixture of far-red LED lamps, white LED bars and a iodine lamp like ceramic metal hydrargyrum quartz iodine lamps.
- Light intensity was adjusted or controlled to 360-380 µmol m^{-2} s^{-1}

ii. Speed breeding II- Glasshouse speed breeding conditions:

• A temperature controlled or regulated glasshouse fixed with high pressure sodium vapour lamps (22 hours photoperiod).
• Temperature is 17/22 °C regime with 12 hours turnover
• Light intensity was given: 440-650 µmol m-2 s-1
• The two hours' time without lamps operating and 17 °C (glass house temperature) cycle occurred during night.



iii. Speed breeding III- Homemade growth room design for low-cost speed breeding

- A room of 3 m x 3 m x 3 m with insulated sandwich panelling fixed with seven LB-8 (LED light boxes).
- Lightning was set up for run a 12- hour's photoperiod for 4 weeks and then increased to 18 hours.
- 1.5 Horsepower (HP) inverter split system domestic air conditioner (18º C in darkness and 21° C when LED lights on) is adjusted inside the chamber.
- Automatic watering was done with irrigation controllers.

IX. LIMITATION OF SPEED BREEDING

The widespread adoption of speed breeding in developing countries faces several challenges that hinder its potential impact on plant breeding programs. One significant obstacle is the scarcity of trained plant breeders and technicians, essential for implementing and maintaining speed breeding techniques. Moreover, the lack of adequate infrastructure, especially in terms of regulating environmental factors like soil moisture, temperature and photoperiod poses a considerable barrier. Many public plant breeding programs in these regions struggle with insufficient institutional support, limiting their ability to embrace advanced methods such as speed breeding and biotechnological tools.

Another critical issue is the unreliable supply of water and electricity, crucial for sustaining indoor speed breeding operations. These facilities require consistent and sustainable energy sources for maintaining optimal conditions including cooling, heating and lighting. Additionally, the inherent differences in the photoperiodic requirements of various plant species present a challenge. While continuous light can accelerate the genetic improvement of long-day and day-neutral plants, short-day varieties require specific photoperiodic conditions that may not align with speed breeding methods.

Furthermore, the accelerated growth and shortened generation times associated with speed breeding can complicate phenotyping efforts, particularly when immature seeds are harvested prematurely. Each plant species responds differently to extended photoperiodic conditions, making it challenging to establish universal rules for speed breeding. Overall, addressing these challenges is essential for maximizing the potential of speed breeding in enhancing crop improvement efforts in developing countries.

X. CONCLUSION

The utilization of speed breeding has the potential to expedite the creation of high-performing cultivars featuring desired market traits by reducing the time, space and resources required for selecting and advancing superior crop varieties genetically. This technique empowers plant breeders to swiftly introduce improved crop varieties. For seamless integration of speed breeding into crop improvement programs, efficient operations that minimize labor and utilize low-cost facilities are imperative.

However, the widespread adoption of speed breeding, particularly within public plant breeding programs in many developing nations is hindered by the scarcity of trained plant breeders and technicians as well as inadequate infrastructure and unreliable access to water and electricity supplies. Additionally, there is currently a lack of supportive government policies and financial backing to initiate and sustain speed breeding within public plant breeding programs.

XI. Reference

Ahmar S, Gill R A, Jung K H, Faheem A, Qasim M U, Mubeen M and Zhou W, 2020, Conventional and molecular techniques from simple breeding to speed breeding in crop plants: recent advances and future outlook. *International Journal of Molecular Sciences*, 21(7): 2590.

Bharath S R and Raju B M, 2023, Optimization of photoperiod, carbon source and plant hormones for effective micro-tuberization in potato. *Mysore Journal of Agricultural Sciences*, *57*(1).

Chiurugwi T, Kemp S, Powell W, and Hickey L T, 2019, Speed breeding orphan crops. *Theoretical and Applied Genetics*. 132(3): 607-616.

Choi H, Back S, Kim G W, Lee K, Venkatesh J, Lee H B, Kwon J K and Kang B C, 2023, Development of a speed breeding protocol with flowering gene investigation in pepper (*Capsicum annuum*). *Frontiers in Plant Science*, *14*: 1151765.

Chougule P S, Hegde R V, Mokashi A N, Venugopal C K and Ramesh Bhat R B, 2011, Microrhizome production in turmeric (*Curcuma longa* L.). *Karnataka Journal of Agricultural Sciences*, 12: 493-496.

Frede K, Winkelmann S, Busse L and Baldermann S, 2023, The effect of LED light quality on the carotenoid metabolism and related gene expression in the genus Brassica. *BMC Plant Biology*, *23*(1): 1-11.

Gimeno-paez E, Prohens J, Moreno-cervero M, De luis-margarit A, Diez M J and Gramazio, P, 2023, Agronomic treatments combined with embryo rescue for rapid generation advancement in tomato speed breeding. *BioRxiv*, 28: 202-232.

Ghosh S, Watson A, Gonzalez-Navarro O E, Ramirez-Gonzalez R H, Yanes L, Mendoza-Suárez M, Simmonds J, Wells R, Rayner T, Green P and Hafeez A, 2018, Speed breeding in growth chambers and glasshouses for crop breeding and model plant research. *Nature Protocols*, 13(12): 944-2963.

Hickey L T, Hafeez A N, Robinson H, Jackson S A, Leal-Bertioli S C, Tester M, Gao C, Godwin I D, Hayes B J and Wulff B B, 2019, Breeding crops to feed 10 billion. *Nature Biotechnology*, 37(7): 744-754.

Hilli J S, Vyakarnahal B S and Patil S S, 2008, Influence of growth regulators and stages of spray on sex expression of ridge gourd (*Luffa acutangula* L. *Roxb*). *Karnataka Journal of Agricultural Sciences*, 21(2): 198-201.

Jahne, Felix, Volker Hahn, Tobias Würschum, and Willmar L. Leiser, 2020, Speed breeding short-day crops by LED-controlled light schemes. *Theoretical and Applied Genetics*, 133(8) : 2335-2342.

Liu K, He R, He X, Tan J, Chen Y, Li Y, Liu R and Liu H, 2022, Speed breeding scheme of hot pepper through light environment modification. *Sustainability*, 14(19): 122-125.

Manjunath S N, Deshpande V K, Sridevi O, Uppar D S, Babalad, H B and Rao M S L, 2009, Influence of seed pelleting on crop growth, seed yield and quality of paprika chilli (*Capsicum annuum* L.). *Karnataka Journal of Agricultural Science*, 22(4): 762-764

Mobini S, Khazaei H, Warkentin T D and Vandenberg A, 2020, Shortening the generation cycle in faba bean (*Vicia faba*) by application of cytokinin and cold stress to assist speed breeding. *Plant Breeding*, 139(6): 1181-1189.

Revanappa R, Nalawadi U G and Madalageri B B, 1998, Influence of nitrogen on root growth, flowering and yield of green chilli. *Karnataka Journal of Agricultural Sciences*,11(4): 1014-1018

Went F W, 1953, The effect of temperature on plant growth. *Annual Review of Plant Physiology*, 4(1): 347-362.

Tomes M L, 2010, Temperature inhibition of carotene synthesis in tomato. *Botanical Gazette*, 124(3): 180-185.

Zhang R R, Wang Y H, Li T, Tan G F, Tao J P, Su X J, Xu Z S, Tian Y S and Xiong A S, 2021, Effects of simulated drought stress on carotenoid contents and expression of related genes in carrot taproots. *Protoplasma*, 258: 379-390.