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Speed Breeding in Vegetables: a Potential approach for Future Food Security

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According to the United Nation, the global population will top around9.7 billion by 2050. In order to feed the escalating population, there is an urge to develop new technologies to shorten the crop production cycle or development of high yielding varieties to satisfy the consumer needs. However, for improvement of any crop using conventional methods is a time-consuming process hence crop breeding needs to be fastened in order to meet the challenges. Therefore, molecular markers have been introduced in 1990s for artificial selection of specific traits directly in crop season which shortens the time required for evaluation of genotypes for a particular trait. But usage of molecular markers demands for expertise in designing and handling of markers and also, they are highly expensive. Apart from those various other techniques like use of winter nurseries, utilization of the doubled haploids, genetic engineering and genome editing were introduced to reduce crop breeding cycle. However, these approaches have severe disadvantages as winter nurseries are often expensive, difficult to manage and do not assure quality seed production, double haploids are non-available for many crops and often require highly qualified personnel and financial resources whereas transgenic or genome-edited crops are often not a viable option because of political legislation or societal scepticism.

To overcome all these drawbacks, Lee Hickey and co-workers developed a novel technology entitled speed breeding which shortens the breeding cycle and accelerate crop improvement through rapid generation cycling. The first spring wheat variety 'DS Faraday' was developed using speed breeding which was released during 2017 in Australia. Speed breeding involves control of photoperiod (light quality and duration), temperature (crop specific)and Co_2 surrounding the crop to accelerate photosynthesis and early flowering thus shortening seed maturation and harvesting time that ultimately reduces the crop generation time. It increases the number of plant generations per year, using less growth space and more accurate phenomics data compared to conventional methods. This technique has been successfully employed in various economically important crop families like Poaceae, Fabaceae and Brassicaceae.

Strategies of speed breeding:

1. **Manipulation of photoperiod (Light duration and intensity):**

It involves exposure of plants to scheduled light and dark regimes to enhance rapid growth, flowering and seed set which varies from crop to crop. Short photoperiods accelerate flowering whereas long photoperiods delay flower initiation process in plants. Light quality is a prime factor that directly affects plant growth by modifying net photosynthetic rate, stomatal conductance, intercellular CO_2 and transpiration rate. The changes in photoperiodic signals are perceived by photoreceptors located in the leaves and mediate responses by causing changes in the morphology and developmental processes of plants. Particularly the ratio of red to far-red (R:FR) is reported to play an important role in control of flowering in plants. Presence of shade conditions and low red-light causes decrease in red: far-red ratio which mediates shade avoidance responses characterized by elongated internodes and premature flowering. A photoperiod of 22h light and 2h darkness in diurnal cycle of 24 hoursis an ideal photoperiodic regime for speed breeding. This spectrum can be achieved by using light emitting diodes (LED's), sodium vapour lamps andhalogen lamps which can be adjusted at different levels according to requirement of cropin speed breeding.

2. **Temperature regulation:**

Temperature has major impact on the rate of plant development including transition from vegetative to reproductive phase. Altering soil and air temperatures affect germination and growth responses leading to rapid growth, flowering, seed set and maturity. Most of the crops require optimum temperature regime between 12-30℃ for seed germination and 25-30℃ for growth, flowering and seed set which is specific to crop. During light periods, higher temperatures should be maintained while fall in temperatures during dark period can help in stress recovery. Ideal temperature for each crop should be maintained otherwise higher temperature may induce stress conditions and affect growth of the plant. Temperatures can be regulated through fan and pad cooling system, foggers and solar air power battery systems.

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3. **Carbon dioxide(> 400 ppm):**

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 475-600ppm 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.

4. **Humidity:**

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

5. **Plant nutrition and hormones:**

Plant nutrient content and hormones plays a key rolein accelerating growth and induction of flowering, seed set and germination of immature seed in vitro. Varied responses to plant growth regulators (PGRs) can be achieved in controlled environments where photoperiod and temperatures can be timely monitored and regulated. Auxins, gibberellins and cytokinin's plays a pivotal role in pioneering various plant responses. The plant remains in vegetative state, when cytokinin levels are lower than auxin levels. As cytokinin levels increases and auxin levels decreases, the plant transforms into reproductive stage. Gibberellins help to control the transition from vegetative to the reproductive state.

6. **Density of plant population:**

High-density planting entails growing at higher plant densities than the density recommended to produce maximum yield. High plant densities result in tall plants due to light competition, leading to a rapid transition from vegetative to reproductive growth stage.

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This approach is useful to induce early flowering and maturity leading to increase in the number of generations per year.

Advances in speed breeding:

1. **Speed breeding coupled with single seed descent:**Speed breeding combined with single seed descent is more frequently utilised to create excellent inbred lines that can be used to generate superior crop varieties faster and cheaper than Di-haploid production. This technique can provide a platform for integration of other emerging technologies in crop improvement, such as high-throughput phenotyping, marker assisted selection and genomic selection that could accelerate the rate of crop improvement for increasing various nutritional components like protein production and maximizing environmental benefits.
2. **Speed breeding capsules:**The implementation of speed breeding in many crops may be hampered by the high cost of controlled environment chambers and glasshouse facilities. Some indoor farming firms (in the USA and Australia, for instance, Modular Farms, Cropbox, and Podponics) create containers appropriate for large-scale agricultural production. The major goal of these initiatives is to grow crops (mostly high-value herbs and leafy vegetables) closer to the point of demand while utilising less land (approximately 99% less) and other resources (such as water and agrochemicals) than crops that are produced in fields. Custom-built capsules will cost a little more, but shipping containers may be modified with multi-tier greenhouse benches, hydroponic systems, lighting, and air conditioning which can be shifted anywhere in the world.
   - **Controlled environmental chamber:** This type of structure programmed toprovide 22h photoperiod with an optimum temperature of 22^{o}C during the photoperiodand17^{o}C during the 2hr dark period. In this chambers, artificial photoperiod can be maintained by a mixture of white LED bars (Valoya; 6 units per 3.67 m^2), far-red LED lamps (Valoya; 12 units per 3.67 m^2) and metal HQI lamps (Valoya; 32 units per 3.67 m^2) and optimal light intensity should be adjusted to 360-380µmol $m^{-2}s^{-1}$ at bench height, where pots are planted on the bench. Generally, wheat, barley, Amaranthus and pea are suited to grow in this model for speed breeding under controlled environmental conditions.

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- **Glasshouse speed breeding conditions:** A temperature-controlled greenhouse is fitted and maintained at 17/22℃ optimum temperature regime, with sodium vapour lamps and 12hrs of turnover and 22hrs of photoperiod. Light intensity is maintained as 440-650µmol $m^{-2}s^{-1}$at the height of the plant canopy (approximately 45cm above the bench height).
- **Homemade growth room design for low-cost speed breeding:** A homemade structure of about 3m x 3m x 3m with insulated sandwich panelling and fitted with lighting equipment about LED lightboxes (1 lightbox per 0.65 m^2). Light quantity and quality at bench height ranged from 210-260µmol $m^{-2}s^{-1}$and at 50cm above the pot from 340-590 µmol $m^{-2}s^{-1}$. The lights were situated at a height of 140cm above the bench. In this type of structure, the room can accommodate 90 pots of 8-inch diameter and 5litre volumes are maintained.

3. **Speed breeding coupled with other breeding methodologies:**

Recent advancements in genetic tools, breeding methods and development of sequencing technologies provides a flexible platform for sequencing, tagging, mapping and introgression of genes for the desired trait at low cost, but they are time consuming. In such cases, 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 combining other genetic tools like genome editing, marker assisted backcrossing andtransgenic approaches coupled with cost-effective genotyping and rapid phenotyping helps to develop homozygous inbred lines followed by crossing will facilitate rapid cycling, genetic gain and rapid development of improved cultivars. Therefore, speed breeding integrated with other multiple branches can be used to improve desirable traits in various horticultural crops.

Speed breedingprotocols in vegetable crops:

1. **Fava bean (*Viciafaba*):**

The application of 6-benzylaminopurine (BAP) @10^{-5}M at four days after flowering increases seed set at the lower nodes of fava bean. Cold treatment @ 8°C during day and 4°C during night for 2 days after the onset of flowering inducesearly pod set along with increase in pod number. The time to first seed formationreduces and pollen viability also increases in plants exposed to cold treatment. The combinations of 10^{-5}M 6-benzylaminopurine (BAP) and cold treatment together pose similar and independent effects. These findings can accelerate plant breeding in fava bean by providing additional tools for reducing generation time.

2. Pea (*Pisumsativum*):

In Pisum four generations per year can be achieved successfully by adopting in vitro-in vivo system, which shortened the generation cycles with a greater efficiency (51–95%) which can be operated at lower cost. This system consistsof a hydroponic system with 22-h photoperiod supplied by fluorescent T_5 tubes at a temperature of 20±2℃ withflurprimidol and anti-gibberellin results in early grain harvest. This method applies to segregating populations presents higher efficiencies than the traditional single seed descent in the field achieving up to four generations per year.

3. Chilli (*Capsicumannuum*):

Hot pepper plants (variety 'Xiangyan 55' and 'Xiangla 712') blooms at 39days after sowing under photosynthetic photon flux density (PPFD) 420 μmol·m^{-2} s^{-1} and a 12h photoperiod helps to produce seed with acceptable germination rates at 82days after sowing. And blooming also preponed 2-3days earlier than normal when photoperiod was extended to 20h. Supplementation of far-red light (R:FR=2.1) significantly accelerates the ripening of pepper fruit and improves seed germination rate. Modification of the light environment accelerates hot pepper growth and development, reduces breeding cycles and produce up to four generations per year.

4. Amaranthus (*Amaranthus tricolor*):

Speed breeding protocol based on light-emitting diodes (LEDs) allow to modify light quality, and exemplarily demonstrate its effectiveness for the short-day crop likeAmaranthus. Adjusting the photoperiod to 10hrs and using a blue-light enriched with far-red-deprived light spectrum facilitates the growth of Amaranth.These conditions help inflowering within 35days after sowing i.e., advancement in flowering by 10 days prior to normal flowering time inAmaranthus genotypes. In short-day crops speed breeding protocol enables several generations per year using cropspecific LED-based lighting regimes without the need of tissue culture tools such as embryo rescue. Moreover, this approach can be readily applied to a multi-storey 96celled tray-based system to integrate speed breeding with genomics toward a higher improvement rate in Amaranthus breeding.

5. Chick pea (*Cicer arietinum*):

Temperatures at 25±1℃ under 12/12hr light or dark conditionscan be maintained for germination of direct sown immature seeds in chickpea. Temperature regulation allows the germination of immature seeds harvested at 16-24 days after flowering. These seeds can be directly sown in pots which allows for the production of seven generations per year.

Milestones in speed breeding of vegetable crops:

S. No.	Crop	Speed breeding strategy	Days to flowering	Number of generations or cycles per year	References
1.	Chilli	Photoperiod, Light intensity	38-40	4	Liu *etal* (2022)
2.	Amaranthus	Photoperiod and temperature	28	6	Jahne*etal* (2020)
3.	Fava Bean	Plant hormones, photoperiod, light intensity and immature seed	29-32	7	Mobini*etal* (2015 & 2020)
4.	*Brassicaoleracea*	22hours photoperiod	108	-	Ghosh *etal* (2018)
5.	*Brassicanapus*	22 hours photoperiod	87	-	Ghosh *etal* (2018)
6.	*Brassicarapa*	22 hours photoperiod	87	-	Ghosh *etal* (2018)
7.	Chick Pea	Plant hormones, photoperiod and immature seed germination	33	5	Mobini and Warkentin (2016)

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Advantages of speed breeding:

1. Multiple generations in one year
2. Fast way to obtain fixed homozygous lines through single seed descent method
3. Phenotypic selection possible in early segregating generations
4. Rapid introgressionof genes into elite lines by employingmarker assisted selection
5. Allows study of plant-pathogen interaction and flowering time etc.
6. Multi- environmental trail across years can be made easy
7. It can be integrated with genomics selection and genome editing tools, etc.
8. High throughput phenotypic screens for multiple traits
9. Itcan be useful to exploit gene bank accessions and mutant collections for rapid discovery of particular gene

Challenges in Speed Breeding:

1. Lack of trained plant breeders
2. Inadequate infrastructure and unreliable water and electricity supplies for sustainable operations
3. Lack of standard protocols
4. High initial investment
5. Early seedharvest interferes with phenotyping of different seed traits
6. Differential responses of plant species to extended photoperiodic conditions
7. Disease outbreak in controlled environment conditions
8. Incorporation of relatively inherited traits

Conclusion:

Speed breeding can accelerate the development of already existing, high performing cultivars with desirable traits by reducing the time, space and resources required for selection and genetic advancement of superior germplasm. This technique allows plant breeders to deliver improved crop varieties more rapidly. Furthermore, integration of speed breeding with advanced breeding approaches like marker assisted selection and genetic engineeringenables effective selection of elite genotypes with novel traits, such as higher yield and better-quality qualities, together with biotic and abiotic stress tolerance. Incountry like India, where resources are very limited, speed breeding can be one of the most viable options to shorten the breeding cycle and accelerate the research programmes by cutting down the input requirement per unit area.