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Opportunities and Challenges of Speed Breeding

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Breeding a new crop variety via conventional approach requires selection of complementary parental genotypes with desired traits, followed by crosses and a series of selection and advancement of superior progenies to release candidate cultivars that meet market demands. Notable breeding goals in crop cultivar development programmes include higher yield potential and nutritional quality, and enhanced tolerance to biotic and abiotic stresses. In any crop improvement programme, the following breeding procedures can be distinguished in the order presented: (a) selection of desirable parents with complementary traits to be combined; (b) crosses involving the selected parents and the development of progenies; (c) selection and genetic advancement of the best progenies based on target traits; (d) selection of the best progenies for screening in multiple target production environments to identify the best performing and stable candidate cultivars; and (e) cultivar registration, and seed multiplication and distribution to growers. These conventional breeding procedures are used in most crop cultivar improvement programmes. However, conventional breeding procedures can take more than 10 years to develop and release an improved variety in the absence of an integrated pre-breeding programme. In most variety design programmes, resources, space and time are invested mainly in Stages 3 and 4 of the breeding procedures. The time taken in these stages significantly delays the pace of cultivar development and commercialization.

Field selection processes take an entire season, and the slow rate of advancement of each generation during the conventional breeding is attributable to the inherent nature of the crop cycles. In the common cereal and legume crops a crop cycle is typically 3 to 6 months per crop, per generation and per year. In other crops such as cassava it takes 15 to 18 months to complete one breeding generation. In some agro- ecologies, variable weather conditions such as extreme temperatures, erratic and poor rainfall distribution, day length, etc. allow for only one crop cycle per year. The duration of each crop breeding cycle can be reduced using modern technologies such as doubled haploid breeding and speed breeding.

Speed breeding is a suite of techniques that involves the manipulation of environmental conditions under which crop genotypes are grown, aiming to accelerate flowering and seed set, to advance to the next breeding generation as quickly as possible. The method saves breeding time and resources through rapid generation advancement (Table 1). Various selection methods can be integrated into speed breeding, such as the single seed descent (SSD), single pod descent (SPD), single plant selection (SPS), clonal selection and marker-assisted selection (MAS) to shorten the breeding cycle and for efficient resource use.

Speed breeding results in ~3 to 9 generations per year compared to 1 to 2 generations per year achieved with conventional selection approaches.

The objective of this review is to present the key opportunities and challenges of speed breeding to guide pre-breeding and breeding programmes. In the first section, the paper discusses plant growth manipulation practices that induce early flowering and early seed maturity, which reduces the intervals between selection cycles. This is followed by highlights on the selection methods available for early generation advancement. The current review highlights the potential advantages of speed breeding for successful development and release of crop cultivars in ~5 years compared with conventional breeding that can take up to 8 to 10 years.

Opportunities of Speed Breeding Techniques

Rapid development of homozygous lines for accelerated breeding

Speed breeding techniques have been used on various crops to rap- idly develop homozygous lines after initial crosses of selected parents with complimentary traits. The technique depends on the manipulation of photoperiod, light intensity, temperature, soil moisture, soil nutrition and high-density planting. These methods have been used to induce early flowering and seed set, reducing the time taken to generate each breeding generation (Table 1). The method allows for the production of 3 to 9 breeding generations per year. This is ideal for accelerated breeding and population evaluation across the target production environments involving various selection methods such as SSD, SPD and SPS Speed breeding relies on deliberate manipulation of various growing conditions that are described below.

Manipulation of photoperiod regime

Photoperiod refers to the length of daily exposure of plants to scheduled light and dark regimes to enhance rapid growth, development, flowering and seed set. Different crop species and genotypes within species have variable photoperiod requirements for flower induction and seed set. Thus, it is important to determine the optimum light quality, intensity and photoperiod that trigger flower initiation in various crops and genotypes.

Light quality, which includes the instantaneous and cumulative amount, delivered per day has a direct effect on plant growth, net photosynthetic rate, stomatal conductance, and intercellular CO_2 and transpiration rate. Also, the daily light to dark hours has an effect on flowering rate and maturity. Light sources emitting photosynthetic active radiation (PAR) within the range of 400–700 nm with an intensity of 360–650 µmol/m^2/s have been successfully used in many crops, including wheat, barley, chickpea, pea and canola to facilitate speed breeding. Light-based speed breeding protocols provide a benefit for sustained photosynthesis for the entire light period, all year round. For example, found that a photoperiod of 22 hr light and 2 hr dark under PAR of 150–190 µE $m^{-2}s^{-1}$ reduced the total number of days to flowering by half when compared with the corresponding wheat genotypes grown with 12/12 hr light/dark. This procedure induced flowering in 35 and 39 days in several wheat genotypes, Paragon, Watkins landrace W352 and a late flowering Paragon x W352 F6 recombinant inbred line. The plants grown with 12/12 hr light/dark were still at the stem elongation growth stage when the corresponding plants grown with 22/2 hr light/dark had started flowering. In a second study, when plants of a sensitive winter wheat genotype, G3116 (AY485969) were grown under short day (SD) conditions (8/16 hr light/dark) for six weeks before being transferred to long day (LD) conditions (16/8 hr light/dark) at a light intensity of 200–270 µmol/ m^2/s, flowering was induced without the need for vernalization. The days to flowering of non-vernalized genotypes grown in SD-LD condition were comparable with genotypes vernalized for six weeks.

Table 1: Techniques for rapid generation advancement with corresponding days to flowering, number of generations achieved per year and selection methods used in different crops

Crop	Techniques	Days to flowering	Number of generation per year	Selection method	Reference(s)
Amaranth	Photoperiod and temperature	28	6	SSD	Stetter *et al.* (2016)
Arabidopsis thaliana	Plant hormones, immature seed germination and photoperiod	20–26	10	—	Ochatt and Sangwan (2008)
Barley	Photoperiod, temperature, soil fertility, immature seed germination and embryo rescue	24 – 36	9	SSD	Zheng *et al.* (2013)
Canola	Photoperiod, light intensity, temperature, immature seed germination and soil moisture	73	4	SSD	Watson *et al.* (2018)
Chickpea	Photoperiod and immature seed germination	33	7	SPD	Samineni *et al.* (2019)
Faba bean	Plant hormones, photoperiod, light intensity and immature seed	29–32	7	SPD	Mobini *et al.* (2015)
Groundnut	Photoperiod and temperature	25–27	3	SPD	O'connor *et al.* (2013)
Lentil	Plant hormones, photoperiod, light intensity and immature seed	31–33	8	SPD	Mobini *et al.* (2015)
Pea	Plant hormones, photoperiod and immature seed germination	33	5	—	Mobini and Warkentin (2016)
Pigeon pea	Photoperiod, temperature, immature seed germination	50–56	4	SPD	Saxena *et al.* (2019)
Rice	Photoperiod, temperature and high-density planting	75–85	4	SSD	Collard *et al.* (2017)
Sorghum	Photoperiod, temperature and immature seed germination	40–50	6	SSD	Forster *et al.* (2014)
Soybean	Photoperiod, temperature and immature seed germination	23	5	SSD	Jähne *et al.* (2020)
Wheat	Photoperiod, temperature, soil fertility, immature seed germination and embryo rescue	28–41	7.6	SSD	Zheng *et al.* (2013)

Abbreviations: SPD, single pod descent; SSD, single seed descent; SPS, single plant selection

Regime reportedly induced early flowering ranging from 37–38, 50– 51 and 60–65 days in early-maturing (i.e., JG 11 and JG 14), medium- maturing (IGGV 10 and JG 16) and late-maturity (C 235 and CDC - Frontier) chickpea genotypes. Depending on the test genotypes, the numbers of days to flowering in early-, medium- and late-maturity genotypes were reduced by 8–19, 7–16 and 11–27 days, respectively. In grain amaranth, flowering was induced in about four weeks by growing plants for two weeks in LD conditions (16/8 hr light/dark) followed by SD conditions (8/16 hr light/dark) using a light intensity of 150 mmol. Continuous exposure to light (24 hr light) with 450 W PAR lamp induced relatively early flowering at 25 to 27 days after germination in groundnut. Adjusting the photoperiod is cost-effective when using low energy, light-emitting diodes (LED), which can use battery-based inverters system, charged from solar panels. The use of solar power systems is an effective and sustainable approach for indoor speed breeding in countries with an unreliable electricity supply.

Regulation of the temperature regime

Adjustments to soil and air temperatures affect germination and growth responses, leading to rapid growth, flowering, seed set and maturity. Low and high temperature extremes activate a wide range of effects on the rate of plant development, including a transition from the vegetative to the reproductive stages. The temperatures required for germination for most crops are between 12 and 30°C, whereas the optimum temperature for growth, flowering and seed set varies from 25 to 30°C for most crops. Temperatures maintained at 25 ± 1°C under 12/12 hr light/dark condition were used for germination of direct sown immature seed in chickpea. Temperature regulation allowed the germination of immature seeds (harvested 16–24 days after flowering), freshly planted into pots, which allowed for the production of 7 generations per year. In winter wheat, vernalization or cold temperature stress is required at the vegetative stage to accelerate the transition to the reproductive stage. Temperatures above 33°C can lead to decreased pollen viability and increase male- sterility in rice, sorghum and soybean. Therefore, temperatures inside the critical limit can facilitate flowering, seed set and maturity for speed breeding. For example, temperatures of 20–22°C were used for the germination of immature seed derived from embryo culture in wheat and barley. After germination, seedlings were transferred to a temperature regime of 25/22°C synchronized with a photoperiod of 16/8 hr light/dark for rapid plant growth and early flowering. In groundnut, use of a temperature regime of 17/32°C, facilitated rapid plant growth, flower induction and seed set under conditions of constant light (24/0 hr light /dark). Photoperiod sensitive genotypes of various crops have shown variable responses to temperature regimes that affect their transition from the vegetative to the reproductive stage. In sorghum, early flowering was induced by exposing sensitive genotypes (BTx642/Tx7000 RIL population and parental lines) to LD conditions (14/10 hr light/dark) or to SD conditions (10/14 hr light/ dark) at 30/23°C day/night temperature. Solar/battery powered air-conditioning systems could provide a cost effective, stable technology for indoor speed breeding programmes in developing countries.

Regulation of soil moisture

Soil moisture stresses can cause significant changes in plant growth and development processes affecting plant height, days to flowering, and seed set and maturity. Drought or flooding stress can trigger early flowering and maturation, which can be used in speed breeding. Drought stress is the most commonly applied technique for crops such as wheat, barley and pearl millet. Drought causes early flowering in pearl millet, which may have evolved as an 'escape mechanism' to produce the next generation. However, in no-tillering, late-flowering genotypes of pearl millet, drought stress can result in sterility, and extend the

flowering period up to 18 days in high tillering genotypes. In cowpea, plants grown under drought stress flowered about 12 days earlier than those grown under well-watered conditions. In wheat and barley, irrigation when plants show wilt symptoms promotes plant growth and development. Combined watering regimes with embryo rescue, adjusted photoperiod and adjusted temperatures to produce 8 and 9 generations per year in wheat and barley, respectively.

Density of plant populations

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. In rice, up to four generations per year were achieved using a high-density planting of 400 plants m^{-2} (with intra-row spacing of 5 cm and inter-row spacing of 5 cm [5 × 5 cm]), compared with the conventional 25 plants m^{-2} (20 × 20 cm). The length of a crop cycle in rice can be reduced by 15 to 40 days, (90 from 105 days, 105 from 145 days using high density planting. However, others have reported that high density planting did not accelerate flowering in rice. In sorghum, found that a plant density of between four and eight plants m^{-2} had non-significant effects on plant growth and grain yield. In a contrasting sorghum study increasing plant density from 16 to 38 plant m^{-2} reduced the days to flowering from 59 to 50 days. In cotton, high-density planting (11 plant m^{-2}) slightly reduced flowering to 25–26 days from 26–31 days c with lower density planting (9 plant m^{-2}). The above studies suggest that genotype differences affect plant responses to high-density planting under field conditions. Therefore, there is need to establish high-density planting requirements of a given genotype through preliminary trials to optimize induction of early flowering for speed breeding. High planting density is one of the low cost speed breeding strategies suitable for rapid.

Modifying carbon dioxide levels

Increased level of carbon dioxide (CO_2) may enhance rapid plant growth and the speed of the transition from the vegetative to the reproductive stage in some plants. However, different crop species and genotypes within a species have varying responses to increased CO_2. For instance, increased levels of CO_2 of 400/700,350/700 and 350/650/100 ppm reduced days to flowering in soybean, rice and cowpea by 2, 7 and 12 days, respectively. In contrast, CO_2 maintained at 20 µmol/ mol^2 delayed flowering in soybean by 11 days. In pigeon pea, when CO_2 level was increased to 550 µmol/mol^2, it delayed flowering by nine days in a short duration cultivar ICPL 15,011.

Use of plant nutrition, hormones and organ tissue culture

Plant nutrition and hormones have been used to accelerate growth and to induce flowering and seed set, and germination of immature seed in vitro. Varied responses to plant growth regulators (PGRs) are achieved when used in controlled environments such as greenhouses and growth chambers in which the photoperiod and temperatures can be monitored and controlled. For instance, combining auxin and cytokinin hormones, in the form of flurprimidol (0.3 µM), indole-3-acetic acid (5.7 µM) and zeatin (2.3 µM), promoted in vitro flowering at 100% and seed set (90%) in faba bean. Additionally, increased seed set was achieved by exogenous application of 6-benzylaminopurine (10^{-5} M BAP) four days after flowering in faba bean. Subsequently, used a combination of flurprimidol (0.9 µM) and 4-chloroindole-3-acetic acid (0.05 µM), resulting in 90 and 80% flowering and seed set in lentils. They also used an integration of adjustments to the photoperiod (18/6 hr light/dark),

temperatures (22/18℃ light/dark), the application of plant growth regulators (i.e., flurprimidol, cytokinin and auxins) and embryo res- cue, to reduce the generation cycle in faba bean and lentil from 102 and 107 days to 54 and 45 days, respectively. This approach allowed up to 8 generations per year. They also used an in vivo protocol involving embryonic seed culture grown under 20 hr light (21°C)/4 hr (16°C) dark in a hydroponic system with a vermiculite substrate, scheduled fertilizer applications and 500 μM/m^2 s^{-1} light intensity (fluorescent light bulbs) When treated with a plant growth hormone, flurprimidol (0.6 μM), treated and untreated pea plants had 100 and 98% flowering, and seed set in 33 and 68 days, respectively. The use of immature embryos grown on Murashige and Skoog (1962) (MS) culture medium supplemented with 0.175 mg/L indole-3-acetic acid (IAA) and 0 mg/L 6-benzylaminopurine (BAP) was found to be suitable for embryo culture of lentil. Embryo culture of wheat on a half strength MS supplemented with ten times the normal levels of potassium dihydrogen phosphate (KH2PO4) and 4% sucrose induced 100 and 92% flowering rate and seed set in two wheat cv. 'Emu Rock' and 'Zippy'.

Amenability with selection methods

Speed breeding is routinely used for generation advancement with- out phenotypic selection. However, modern technologies (e.g. high- throughput genotyping methods, marker-assisted selection, etc.) can be successfully integrated for target traits selection. The combination of speed breeding and effective selection methods should allow for the maintenance of a good breeding population and genetic diversity in the environments that restrict plant growth, and for maxi- mum yield production (Johnston et al., 2019). Conventional selection methods such as bulk, mass, recurrent, pedigree and pure line se- lection require a genetically stable plant population for selection of optimally yielding genotypes. These methods are not ideal for speed breeding due to the long inbreeding and selection cycles that they require. The most appropriate selection methods amenable with speed breeding
are single seed descent (SSD), single pod descent (SPD) and single plant selection (SPS) methods. These methods are briefly described below.

Single seed descent method

Single seed descent (SSD) is geared towards achieving homozygous populations through continuous inbreeding of segregating population by retaining one seed from each F_2 plant and advancing these individuals to the next generation. Each inbred line developed is traced back to an F_2 plant. The time taken to achieve inbred lines with SSD is comparable to that of the doubled haploid (DH) method. The advantages of the SSD selection method include less growing area and labour being required for the handling of early generations. It allows for the advancement of progeny under high-density plantings in small nurseries, growth chambers or greenhouses. The negative aspect is that SSD carries forward more inferior progenies than pure line, pedigree and recur- rent selection methods.
In maize, found non-significant differences in the grain yield of inbred lines developed from the same parental genotypes using the doubled-haploid (DH) and SSD methods. Overall, SSD is the best selection method for speed breeding and can be carried out under both field and indoor conditions.

Single pod descent method

Single pod descent (SPD) method involves selection of one pod per plant from each F_2 – F_4 plant instead of a single seed. Due to there being more than one seed per pod in most legume crops, SPD has a higher chance of maintaining each F_2 plants in the advanced generations than SSD selection.

Another advantage of SPD is that it allows for the early selection of pods, so a smaller population can be advanced. In other re- search, SPD resulted in soybean progeny with mean yields of 7.96 g plant^{-1} compared with the mean yield of 6.42 g plant^{-1} of test lines developed through SSD). Conversely, better yield increases in soybean lines selected using the bulk selection method of 72.6%, followed by pedigree (33%) and SPD (10.4%). Therefore, preliminary trials are required to determine the efficiency of SPD for the crop and trait being selected for under speed breeding.

Single plant selection method

The single plant selection (SPS) method advances each F_2 plant by harvesting all the seeds of each selected plant. Hence the next generation will be advanced as plant-to-row. The SPS method has been used in a modified backcross strategy to develop introgression lines (ILs) within two years in barley. European barley, cv. 'Scarlett', was crossed with other donor parents to develop lines that were resistant to leaf rust, net blotch and spot blotch. A speed breeding procedure of continuous light and temperature maintained at 22°C, with 87 of the BC_1F_3: 4 generation being selected from 5,000 BC_1F_2 plants. The authors reported that the yield of 12 Scarlett ILs selected for superior multiple disease resistance and agronomic traits were significantly higher than cv. 'Scarlett'. In bread wheat, Alahmad *et al*. (2018) used the SPS selection method to enhance foliar disease resistance, grain dormancy, seminal root angle, seminal root number, tolerance to crown rot, resistance to leaf rust and plant height in an approach that is compatible with speed breeding. The study found a better selection response when using SPS in comparison with unselected F_3 plants for crown rot and leaf rust resistance, and for better root angle and number. The SPS involves early selection of plants based on a smaller population than SSD and SPD.

Challenges of speed breeding

The use of speed breeding techniques is a valuable approach to accelerate conventional breeding programmes. However, the technology requires expertise, effective and complementary plant phenomics facilities, appropriate infrastructure and continuous financial support for research and development (Shimelis *et al.,* 2019). For these resource to be in place requires that speed breeding approaches are recognized as essential for conventional plant breeding, marker assisted-selection and genetic engineering. Furthermore, the integrated suite of tools requires skills and expertise in plant breeding and biotechnology, long-term funding and government policy support. For example, in Sub-Saharan Africa (SSA) most public plant breeding programmes use traditional plant breeding approaches. Use of modern breeding tools in the public sector is limited by technical, economic and institutional challenges. Speed breeding methods could accelerate the release of both conventional and genetically modified crop cultivars in SSA. However, the most common challenges hampering the use of speed breeding include: (a) access to suit- able facilities, (b) staff trained in the protocol, (c) adopting major changes to breeding programme design and operations, and (d) the need for long-term funding. Briefly, these challenges are discussed below.

Lack of trained plant breeders and breeding technicians

A major challenge that can hamper the adoption of speed breeding in the public sector is a lack of trained and active plant breeders, and plant breeding technicians in developing countries. The public sector breeding programmes are negatively affected by a high turn-over of plant breeding personnel to private seed companies and training institutes that offer better remuneration than government service. Moreover there are relatively few scientists specializing in plant breeding because postgraduate qualifications in plant breeding are only

offered at a few universities in developing countries. In some countries, the legislative and administrative framework to manage plant breeders' rights and seed regulation have not been developed to encourage plant breeding to benefit the value chain from farmers to consumers. Therefore, developing countries need to adjust their policies and practices related to investments in plant breeding education, research and personnel retention to ensure the viability of long-term crop improvement programmes, and the adoption of scientific innovations such as speed breeding.

Inadequate infrastructure

Speed breeding platforms require sophisticated infrastructure to regulate environmental factors, particularly soil moisture, temperature and photoperiod. Additionally, an overreliance on donor agencies ('donor mind-set') and a lack of harmonization of regional breeding programmes leads to duplications of activities and investments in resources. Therefore, there is a need for active collaboration between national and regional organizations in the development of infrastructure, and for resource and knowledge sharing once the infrastructure is in place.

Unreliable water and electricity supplies for sustainable operations

The manipulation of environmental factors, specifically moisture, temperature and photoperiod, in indoor growing facilities requires reliable water and electricity supplies. Indoor speed breeding facilities require affordable, sustainable and reliable energy for cooling, heating and lighting. Unreliable supplies of electricity are a major problem for the management of temperature and photoperiod for speed breeding in public plant breeding programmes. Growing crops in the field require land preparation, fertilization, irrigation and other standard agronomic practices, which have substantial costs and require substantial infrastructure investments. In developing countries, speed breeding will require innovative solutions to the supply of water and electricity, such as the use of sustainable solar power. A small indoor speed breeding kit consisting of fitted LED lights and temperature controls powered by a solar system with battery backup could be developed using existing technologies.

Conclusion and Outlook

The use of speed breeding can accelerate the development of high performing cultivars with market-preferred traits by reducing the amount of time, space and resources invested in the selection and genetic advancement of superior crop varieties. The technique allows plant breeders to deliver improved crop varieties more rapidly. Streamlined operations that reduce labour and lost-cost facilities are key for effective integration of speed breeding into a crop improvement programme. Furthermore, integration of speed breeding with conventional, MAS and GE breeding approaches can enhance effective selection of elite genotypes and lines with novel traits, such as higher yield and better nutritional qualities, together with biotic and abiotic stress tolerance. The most appropriate selection methods amenable with speed breeding include SSD, SPD and SPS methods. However, the adoption of speed breeding in many developing countries, especially in public plant breeding programmes, is limited by the lack of trained plant breeders and plant breeding technicians, and a lack of the requisite infrastructure and reliable supplies of water and electricity. Currently, there is also a lack of enabling government support at a policy and financial level to initiate and sustain speed breeding in public plant breeding programmes.