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Rapid Generation Advance Methods to Fast-track Crop Breeding: A Review

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ABSTRACT

Rapid generation advance is a collection of breeding tools for quicker development of homozygous lines from the segregating generations raised from a cross of two divergent parents. An array of techniques including single seed descent, speed breeding, shuttle breeding, doubled haploidy, marker assisted breeding and genomic selection were designed to achieve the maximum genetic gain through reducing the time required for development of such homozygous lines. In addition, utilization of genome editing in agronomically important crops further enhances the genetic advance to alleviate the food supply shortage. Therefore, utilization of these techniques could be more advantageous than the conventional breeding approaches in terms of speed and logistic support. Combination of these methods allows breeders to develop agronomically superior varieties in a very short period of time.

Key words: Genetic gain, Homozygous lines, Rapid generation advance, Speed breeding.

With the increase in global population at an alarming rate, there is an urgent need to increase food production at the same rate or even faster to fulfill the food and nutritional requirements. As there is a little scope for increase in the area of cultivation, plant breeders need to focus on development of new varieties with increased productivity as well as ability to tolerate various biotic and abiotic stress. Besides, recent climate change and global warming demand climate-smart agriculture with new crop varieties that can minimize crop loss due to adverse conditions and emergence of new pests and diseases. In this context, plant breeders should focus on stability and sustainability of crop yields. However, development of new varieties is a tedious and time-consuming process. The time required in a breeding program majorly depends on number of years required to develop homozygous lines from segregating generations from crossing of two parents. Conventional pedigree breeding method takes around six to seven years or even more to develop homozygous lines if only a single crop is grown in a season. In addition, subsequent few more years are required for evaluation of the advanced generation breeding materials for release as a new variety. Therefore, special tools and techniques are required for modern-day crop improvement programmes to meet the increasing demands. Rapid generation advance (RGA) is a novel way of reducing the time for the breeding cycle to enhance the genetic gain (Li *et al*. 2018).

Rapid generation advance

Rapid generation advance (RGA) is a bunch of breeding tools for faster development of fixed lines from the segregating generations (Fig 1). RGA allows several generations per year by manipulating growth conditions of plants such as temperature, relative humidity, photoperiod and daylength for rapid induction of flowering and seed set. Often, this is achieved by shortening the crop growth cycles and producing immature seeds (Carandang *et al.* 2006). The main objective of RGA is to reduce time required to develop lines for breeding program. RGA is superior to other approaches in terms of speed, technical simplicity, cost and logistic requirement. It reduces the time for variety development through a stringent selection cycle and thereby enhances the genetic gain. An array of methods can be employed for RGA to fast-track crop breeding. Therefore, the current context aimed for a comprehensive discussion of various breeding schemes which allow us to shorten the overall time required for varietal developmental process.

Single seed descent method

Single seed descent (SSD) method is a modification of bulk breeding method for handling segregating populations. SSD method mainly targets at maintaining maximum possible genotypes from the original segregating populations by avoiding early generation selections and thereby creating a large genetic variation among the individual homozygous lines after F_6 or later generations (Brim, 1966). In SSD method, following crossing two suitable parents, the F_1

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generation is grown to generate F_2 generation from where a single seed from each plant is selected and bulked seeds to grow the next generation. The same process is repeated up to F_6 or F_7 generation to obtain nearly homozygous lines. Furthermore, it allows off-season nurseries to advance segregating material rapidly by taking two to three or even more generations in a single year to attain desired level of homozygosity. The F_1 generation is grown in the field for obtaining F_2 seeds followed by subsequent generation's advancement in off-season nurseries. SSD method allows the maintenance of maximum amount of genetic diversity and thereby permits breeder for development of recombinant inbred lines (RILs). As a routine practice, development of RILs can be hastened by growing three generations in a single year (Gaur *et al*. 2007). For instance, the application of SSD method in rapid generation advance has been undertaken in rice breeding for advancing the breeding materials (Ikehashi and Hille Rislambers 1977). Currently, SSD method is being used for RGA for breeding rice varieties conducted by IRRI, Philippines for photoperiod sensitivity, cold and submergence tolerance (Suh *et al*. 2003; Sarkar *et al*. 2021).

Speed breeding

Speed breeding is another novel approach that can be followed for long day to day-neutral crops. This technique involves extending photoperiod and controlled conditions such as temperature, soil media and appropriate spacing in glasshouses to shorten breeding cycles, thereby leading to RGA. With speed breeding, almost three to nine generations can be grown in a year, as compared to one to two generations per year in case of conventional method (Ghosh *et al*. 2018). Thus, it promotes rapid development of stable and homozygous genotypes to facilitate development and release of new crop varieties (Watson *et al*. 2018). The original concept of speed breeding came from NASA having an objective to grow crops quickly in space. This method may include manipulation of temperature and photoperiod regime, soil moisture, high-density planting, modification of composition of media (nutrients and minerals), use of plant growth regulators and often increasing the level of carbon dioxide in the growth chambers for promoting quick growth from vegetative to reproductive stage (Mobini *et al*. 2015; Jagadish *et al*. 2018). Generally, light of 400-700 nm wavelength with LED is used with temperature 22°C/ 17°C for 22 hours supplemented with an alternating 2 hours darkness under 60-70% relative humidity condition. Speed breeding is highly amenable to phenotypic selection for different traits. Several quantitative traits including leaf rust and collar rot in durum wheat were screened using this method (Alahmad *et al*. 2018) and also amenable for rapid introgression of multiple disease resistance in barley (Hickey *et al*. 2017). However, conventional selection methods *viz*, pedigree, bulk and recurrent selection can hardly be used in speed breeding as it does not allow breeders to exert their skill in the selection process. Hence, single plant selection (SPS), single seed descent (SSD) and single pod descent (SPD) may be merged with speed breeding for RGA (Wanga *et al*. 2021).

Shuttle breeding

During the phenological stages, crop plants are exposed to several types of stress conditions, like salinity, drought, nutrient deficiency or toxicity, diseases and pest attacks. Therefore, for evaluation of breeding lines for tolerance to these stresses, early generation breeding materials should be exposed to these actual stress environments, if not available in the breeding station. In this context, the concept of shuttle breeding originated from this criterion to evaluate the lines rapidly by shuttling in other locations to facilitate

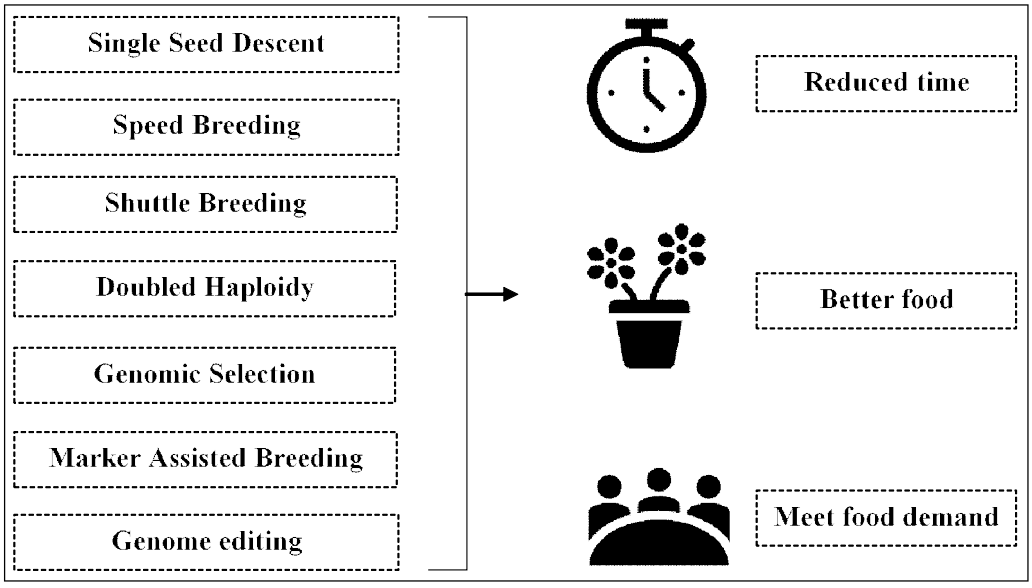

Fig 1: Rapid generation advancement to meet the global food demand.

faster development of cultivars with the ability to adapt to those environments (Khush, 1989). Many international institutes including IRRI used this method for crop breeding in collaboration with other partner organizations. In shuttle breeding, selected improved variety or good parental lines are crossed with a locally adapted parent to generate F_1 progenies which are grown at experimental station followed by raising F_2 generation in the targeted environment where the materials were exposed to the desired stress conditions. Subsequently, better performing adapted lines are selected to obtain F_3 generation. In subsequent generations, grain yield and quality are evaluated to select desired segregants having tolerance to stress environments (IRRI 1985). Using this approach, varietal improvement was done in rice under lowland rice growing ecosystem in eastern India (Singh *et al*. 1998; Mallik *et al*. 2002). In shuttle breeding, crop cycle can also be maintained in off-season by planting the crop in multiple field locations. Alongside yield and grain quality, this method improves selection efficacy to a particular environmental condition, including diseases, pests and climate. It was primarily designed by CIMMYT and later also used by Dr. Norman Borlaug for development of superior lines in wheat.

Doubled haploidy

Doubled haploidy is one of the most fascinating tools in plant breeding where haploid set chromosomes are doubled to get diploids, hence called doubled haploids (DH). This can help to obtain completely homozygous lines in just 1-2 years by colchicine treatment to double the chromosome sets. Haploid plants may be obtained through tissue culture techniques like anther, pollen culture (Heberle-Bors 1989) or ovule culture (Keller and Korzun 1996) or by chromosome elimination method using Bulbosum technique in barley (Ho and Kasha 1975) and haploid inducer lines like in maize (Chaikam *et al*. 2019). Although apparently looks easy, many crops are not amenable to tissue culture and regeneration protocol is not yet available. Artificial chromosome duplication is accomplished by treating with chemicals that restrict mitotic activity of the haploid seedlings. For this purpose, colchicine is most widely used for artificial chromosome doubling in DH development pipelines (Chaikam *et al*. 2019). Colchicine treated seedlings generally show lack of microtubules in the meristematic cells of the shoot apex during mitosis as colchicine binds to β-tubulin forming tubulin dimers resulting in prevention of chromosome separation towards the polar region.

Genomic selection

Genomic Selection (GS) is a form of marker assisted selection (MAS) that simultaneously estimate the effects associated with all the marker loci covering the entire genome, irrespective of whether the effects are significant or not and selects favorable individuals based on genomic estimated breeding values (GEBVs) (Crossa *et al*. 2017). GS uses a 'training population' of individuals that have been genotyped and phenotyping is done to build a model to estimate marker effects which in turn is used to predict GEBVs. It has been predicted for over two decades that molecular marker technology would reshape breeding programs and facilitate rapid gains from selection. Despite important strides in marker technologies, the use of MAS has stagnated for the improvement of quantitative traits. Current MAS methods are effective for the manipulation of large effect alleles with known association to a marker, but ineffective for small effect alleles of quantitative traits. However, in GS, GEBVs say nothing about the function of the underlying genes but they are used as selection criterion. A key to the success of GS is that it incorporates all marker information in the prediction model, thereby avoiding biased marker effect estimates and capturing even small-effect QTLs (Cerrudo *et al*. 2018). Several statistical approaches *viz*., Step-wise regression, Ridge regression-best linear unbiased prediction (RR-BLUP) and a Bayesian regression, kernel regression and non-parametric regression models have been proposed for the prediction of GEBVs. The accuracy of GS prediction methods is largely affected by various factors. GS is expected to accelerate the breeding cycle (selection gain per unit time) and change the role of phenotyping. GS may be regarded as a potent, attractive and valuable approach for plant breeding that leads to the next phase of MAS. Machine learning (ML) is a field of computer science that uses algorithms and existing samples to capture characteristics of target patterns. Several ML methods like Artificial Neural Network (ANN), Convolutional Neural Networks (CNN), Multilayer Perceptron (MLP), random forest (RF), Support Vector Machines (SVM), Radial Basis Function Neural Network (RBFNN) could be applied in GS for estimating the GEBV along with higher order interaction (Gonzalez-Camacho *et al*. 2018). Haploid breeding pipeline conjugated with GS in CIMMYT has revolutionized the Maize Breeding.

Marker assisted breeding

Conventional breeding mostly utilizes phenotypic evaluation for a trait of interest. This is often inefficient, time consuming, biased, destructive, dependent on threshold conditions and specific to developmental stages. Marker assisted selection is one of the means of indirect selection where a desirable allele or quantitative trait loci (QTLs) are selected based on some markers tightly linked to it (Soller and Beckmann 1983; Das *et al*. 2022). The use of molecular markers helps to select the desired ones more efficiently, thereby reducing time as compared to conventional methods which usually take several years for phenotype-based selection (Frisch and Melchinger, 2005; Dutta *et al*. 2020a, 2021a and b). Marker assisted backcrossing is a process of incorporating traits into a well-established variety with the help of molecular markers for selection for the target trait and simultaneously recovery of the recurrent parental genome (Jiang, 2015). Marker assisted backcross breeding efficiently detects targeted trait or QTL with maximum recovery of recurrent

parent genome. Plant breeders regularly use marker assisted backcross breeding to integrate various traits like grain yield and submergence tolerance (Pandit *et al*. 2021), fertility restorer gene (Ponnuswamy *et al*. 2020), disease resistance like for bacterial blight in rice (Sundaram *et al*. 2011). By employing this method, selection of desired plants can be done more rapidly, thereby reducing time required for varietal improvement. Furthermore, marker assisted backcrossing can be used for gene pyramiding and breeding for tolerance or resistance to abiotic and biotic stresses.

Genome editing in plant breeding

The ability to precisely edit DNA sequences within the genome of living cells has been a major challenge for past few decades (Adli 2018). Precision genome editing, including altering single bases, is become a versatile tool to accelerate crop improvement worldwide (Zhang *et al*. 2018). RNA guided CRISPR-associated (Cas) nucleases have been used to achieve this goal in various major crops ranging from cereals (Hillary and Ceasar 2019; Ansari *et al*. 2020), pulses (Wang *et al*. 2017), oilseeds (Jiang *et al*. 2017) and horticultural crops (Karkute *et al.* 2017). Genome editing primarily occurs due to double strand breakage followed by cellular repair responses like non-homologous end joining and microhomology-mediated end joining (Jeggo *et al*. 1998; Rouet *et al*. 1994). In addition, precise DNA editing can also be achieved through cellular homology-directed repair by supplementing exogenously supplied DNA template with desired DNA change flanked by sequence homologous to the regions upstream and downstream of the double strand break (Rudin *et al*. 1989; Rouet *et al*. 1994). Additionally, the edited line can further be utilized in studying the function of a particular gene family spatially and temporally across the species (Dutta 2018; Dutta *et al*. 2019 a and b).

CONCLUSION AND FUTURE PROSPECTS

The use of molecular techniques and genomics in modern plant breeding has made selection easier, indirect and more accurate than traditional crop breeding. Rapid generation advances can accelerate the development of high-yielding cultivars by reducing the number of selection cycles and thereby enhancing genetic gain. Integration of speed breeding and other methods with marker assisted breeding and/or genomics assisted breeding can further reduce the time and make selection for novel traits like nutritional qualities, resistance of diseases and pests and tolerance to abiotic stresses more efficient. Speed breeding with employing embryo culture and crop management practices, like irrigation, control of temperature and light can reduce generation cycles in wheat and barley (Zheng *et al.* 2013). They showed that maximum of 8 generations per year in wheat and 9 generations per year in barley can be achieved by employing speed breeding techniques. RGA methods enabling speed breeding may lead to growing up to Seven generations per year in case of chickpea (Samineni *et al.* 2020). These methods advance generations with sufficient genetic diversity that may lead to rapid development of RILs and NILs as compared to conventional methods. It was reported that increased flower development in some crops like peanut was achieved by SSD under a controlled environment (O'Connor *et al*. 2013). However, lack of infrastructure, funding and trained human resources are the major constraints towards adoption of RGA methods to fast-track crop breeding.

Authors' contribution

Swarnadip Ghosh, Abhik Roy and Suman Dutta drafted the article; Swarnadip Ghosh and Suman Dutta reviewed the manuscript; Swarnadip Ghosh and Suman Dutta conceptualized the article.

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Conflict of interest

The authors declare no conflict of interest.

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