Advances in Crop Science and Technology

ISSN: 2329-8863

Rehman et al., Adv Crop Sci Tech 2020, 8:3

Review Article

Open Access

Speed Cloning Leading to Speed Breeding through Genome Editing

Nazia Rehman[1*], Safeena Inam[1], Muhammad Munir Shahid[2], Ghulam Muhammad Ali[1,3] and Muhammad Ramzan Khan[1,3*]

[1]*National Institute for Genomics and Advanced Biotechnology, National Agricultural Research Centre, Islamabad, Pakistan*

[2]*Pakistan Agricultural Research Council, Islamabad, Pakistan*

[3]*PARC Institute for Advanced Studies in Agriculture, National Agricultural Research Centre, Islamabad, Pakistan*

[*]**Corresponding authors:** Nazia Rehman, National Institute for Genomics and Advanced Biotechnology, National Agricultural Research Centre, Islamabad, Pakistan, Tel: +92-5190733819; E-mail: naziarehman96@yahoo.com

Muhammad Ramzan Khan, PARC Institute for Advanced Studies in Agriculture, National Agricultural Research Centre, Islamabad, Pakistan, Tel: +92-5190733808; E-mail: drmrkhan_nigab@yahoo.com

Received date: March19, 2020; **Accepted date:** April 06, 2020; **Published date:** April 14, 2020

Abstract

Food security in changing climate is the major challenge to feed the ever growing world population. Advancements in plant breeding in the form of novel techniques is the ultimate solution for the development of high yielding, disease-resistant, nutritious varieties in rapid time. In this scenario speed cloning from wild relatives can lead to manipulation of desirable genes in crops using genome editing which eventually through speed breeding may lead to the development of varieties in half of the time than normal. In this review, we described the novel technical breakthroughs that have great potential and are being employed for enhancing crop improvement.

Keywords: Speed cloning; Gene editing; CRISPR; Speed breeding

Introduction

Modern agriculture must offer adequate food to feed the escalating global population. Currently, we are 7.7 billion and estimated to reach 9.8 billion by 2050. It has been estimated that to fulfill the food demand of a growing population approximately 25%-70% more agricultural production is required by 2050 [1]. Achieving this target becomes more challenging due to crop losses by different environmental factors. The improvement of crops is very essential for increasing crop production to meet global food demands. Plant breeders and scientists are continuously putting efforts to develop improved crops in terms of higher yield, with better nutrient profile, resistant to pest and pathogens and climate-resilient. To improve a crop conventional breeding plays a key role which is prolong and laborious process involved growing and examining large populations of crops over several generations often coupled with co-integration of lethal genes (linkage drag). The limitation of linkage drag demands multiple times of backcrossing and selection to re-establish the elite background [2]. Conventional breeding got momentum with the advent of Marker-Assisted Selection (MAS) and Genomic Selection (GS) as these techniques assisted the breeding program by facilitating the selection efficiency [3,4].

However, with the increase in knowledge about the fundamental genomic features related to quality and yield, the more limitations of traditional breeding techniques become evident. Owing to the random recombination events and undirected mutagenesis, extra improvement of existing elite varieties is an extensive and tedious practice. Furthermore, during domestication there is a lack of functional diversity in elite varieties which is another genetic bottleneck for conventional breeding [5].

Genetic engineering is an alternative to conventional breeding which refers to the direct modification of genetic makeup of any organism *via* biotechnology [6]. The transgenic approaches have also increased the number of elite crop varieties by transferring genes to acquire desirable traits. Despite potential applications of this approach and the promising role of the genetically Modified Crop (GM) in sustainable agriculture and food security, their usage raised bio-safety concerns.

To overcome all the limitations of conventional breeding and genetic engineering scientific community has pushed for new technology which assists to reduce the generation time and speed up crop development. Recently scientists become successful in attaining their goal and found the new grounds which permit them to accelerate the process of new variety development.

Here with the aim of crop improvement, we furnish an overview of innovated techniques of " Association Genetics and Sequence Enrichment" (AgRenSeq) or speed cloning, genome editing and speed breeding.

Speed Cloning or "Association Genetics with R Gene Enrichment Sequencing"

Keeping in view the bottlenecks of conventional plant breeding, an international union of researchers has established a new technique to speedily recruit disease-resistance genes from wild plants for transfer into domestic crops. This technique assures to revolutionize the development of disease-resistant crop varieties which fulfills the global food demands. The newly established technique is known as speed cloning or AgRenSeq "Association Genetics with R gene enrichment Sequencing" was developed by Scientists working at John Innes Centre in UK in collaboration with the scientist in Australia and US. According to Project Director Dr. Brande Wulff, developing disease-resistant crops the new AgRenSeq technique is quite useful to increase yields and reduce pesticide applications. Moreover, the through speed cloning technique the elite crops became more resilient for smart agriculture. The results of AgRenSeq accelerate the struggle against

pathogens which threaten major food crops globally such as wheat, maize, rice, soybean and potato. According to Habans Bariana, this technique strengthens the rapid identification and characterization of new disease-resistant genes. The AgRenSeq technique is a combination of association genetics and R gene enrichment sequencing (AgRenSeq).

To clone R genes scientists selected a diversified panel of wild diploid wheat accessions to test out their resistance against different pathogens. Although positional cloning and mutational genomics can be used to clone R gene but both techniques have limitations which necessitate the R gene to exist as a single gene and also require screening of thousands of recombinant or mutant lines [7-9]. On the other hand, wild relatives of crop plants often hold many R genes and have pre-domestication traits that prevent to clone R genes by existing techniques. Genome-Wide Association Studies (GWAS) facilitate the correlation of traits in a genetically varied population by using already existing recombination events in natural populations. However, the limitation of the dependence of GWAS on a reference genome, obscure the identification of sequences that have considerably deviate from the reference, for example, R genes. Considering this limitation, the trait associations on sub-sequences (k-mers) have been used [10].

So, for speed cloning of R genes from diverse panel of plants k-mer-based association genetics was used in combination with R gene enrichment sequencing (AgRenSeq) would enable the discovery and cloning of R genes from a plant diversity panel.

This new technique, consist of high-throughput sequencing of DNA with state-of-the-art bioinformatics. By using association genetics, researchers identify associations between genomic loci and disease-resistant traits across plants population; and through sequence capture marking of specific genomic regions encoding resistant genes. This is a cost-effective alternative approach to whole-genome sequencing. The researchers scanned the diversified accessions of Aegilopstauschii, a wild progenitor of bread wheat and cloned the four-stem rust-resistant genes in the time of six months with minimum cost and by passed the 10-15 years. Contrary to other genome association research studies, the AgRenSeq method is independent to the reference genome and involved in direct identification of the Nucleotide-Binding/Leucine-Rich Repeat (NLR) that confer resistance instead of identification of a genomic region which encode several paralogs for subsequent confirmation of candidate gene.

Moreover, AgRenSeq is not only limited to genetic variability and recombination which exists in the bi-parental population but can be used to scan pan-genome sequence variability in diversified germplasm collection to isolate unidentified R genes. So, by this technique notably no extra crossing over or generation of mutations is needed to clone R gene. Hence it become possible to isolate R genes from those accessions of wild plant species which do not possess any superior agronomic traits and only phenotyping of enrichment sequenced diversity panel is required.

Dr. Wulff the project leader demonstrated that AgRenSeq is a robust technique for the rapid cloning of resistance genes from a diversified panel of a wild crop relative. In the case of an epidemic, the generated library can be phenotyped across the diverse panel to isolate the resistant genes. By combining speed cloning and speed breeding elite resistant crop varieties can be developed within few years. Therefore, this method has massive value for crop improvement and offers new fundamental insight into the structure and evolution of functional R gene architecture on species level. A Project co-leader Brian Steffenson said "AgRenSeq is an important step forward that will assist and speed up the development of more resilient crop plants varieties other than wheat. "With a large pool of resistance genes in hand, we can breed new varieties with broad-based, multi-gene resistance that will minimize diseases losses.

Breeders will be able to use the results of AgRenSeq to breed crops that are more resistant to pests and diseases. These crops will require less pesticide application bringing down production costs, and hopefully the price of food products made from these crops, thus benefiting the consumer.

The speed cloning is a great innovation, which plant breeders and scientists have long wanted. Beside speedily clone the R genes this technique can be used to clone genes for others traits improvement including yield, nutrition profile and climate tolerant crops to make them accessible to the world's grower.

Genome Editing Emerging as an Advance Tool for Crop Improvement

Genetic diversity or variations is a major factor for improving traits in plants. Traditional plant breeding was really booming in developing new varieties. However, in the modern era due to crop domestication, genetic diversity turns out to be poor for breeding and now it is a limiting factor to develop the elite germplasms through conventional approach [11,12].Genome editing has come out as a new alternative unconventional modern approach to traditional breeding with higher mutagenic capability.

Among other genome editing approaches ZFN and TALEN, recently the CRISPR (Clustered Regularly Interspaced Short Palindromic Repeat) system allows the potentially new projection to generate genetic diversity for breeding in a unique way. CRISPR/Cas provides incomparable levels of hold on mutation thus permitting instant pyramiding of many valuable characters into elite genotype in a single generation [13]. Moreover, the linkage drag does not introduce in genome-edited elite varieties. The benefits of gene editing could be achieved by the integration of these tools into speed breeding programs. Plant Genome editing emerged the only couple of years back; it is an easy and robust method. CRISPR has a multiple applications in agricultural research areas; emerging new opportunities for the development of novel plant varieties in a shorter time by deleting the deleterious characters or adding of important CRISPR system may offers a promising opportunities to edit genes in their native situation for the improvement of traits of agronomic value such as high yield, enhanced tolerance to biotic and abiotic stresses.

A wide range of non-coding regulatory elements such as promoters and enhancers can be edited for the precise improvement of enviable traits [14]. A number of recent researches have proven the potential of CRISPR/Cas9 to produce a wide range of diversity in alleles at specific loci. CRISPR based genetic modification is a relatively novel approach, regardless of its novelty, it is effectively adapted to a broad range of crop plants for improvement of yield, quality, nutritional values, resistance to herbicides, biotic and abiotic stresses. First, we provide a brief overview of genetically edit crops for enhancing disease resistance against many pathogens. It is well known reality that plants are invaded by a variety of pathogens causing considerable losses in quality and yield of crops [15]. Genome editing has been extensively used to increase the resistance against pathogens [16]. In the upcoming years Gene-Edited (GE) disease-resistant crops will become a standard tool in plant breeding. Genomes of different crops such as wheat, maize,

rice, etc. have been successfully edited for developing disease-resistant varieties. For example, in wheat resistant against powdery mildew is developed by Wang Y et al. and Zang Y et al. by induction of mutation in Mildew Resistance Locus (MLO) and knocking out of TaEDR1 gene respectively [17,18]. To develop a resistance in tomato against powdery mildew a tomato SlMLO1 gene was edited by CRISPR-Cas9 [19].

In rice, the resistance against bacterial blight was enhanced by the editing of OsSWEET11, OsSWEET13 and OsSWEET14 genes [20,21]. Similarly, another attempt was made in which OsERF922 target gene was edited against rice blast caused by *Magnaporthe oryzae* [22]. The crops which are modified for disease resistance by CRISPR cas9 system are listed in Table 1. In addition to developing diseases-resistance crops through genome editing, it is tremendously important to improve the yield which is a quantitative trait under the control of multiple genes to ensure the food security. To overcome the shortcomings of conventional breeding editing the yield-related genomic loci is a promising approach. In rice, the four yield-related genes such as dense erect panicle (DEP1), grain number (Gn1a), grain size (GS3), and ideal plant architecture IPA1 have been knocked out. The T2 mutated generation showed enhanced yielding features [23-32].

Crop plant	Gene editor	Edited gene	Causal organism	Target trait	References
Wheat	CRISPR/Cas9	EDR1	*Blumeria graminis f. sp. tritici*	Powdery mildew resistance	[18]
Rice	CRISPR/Cas9	OsERF922	*Magnaporthe oryzae*	Enhanced rice blast resistance	[22]
Rice	CRISPR/Cas9	OsSWEET13	*Xanthomonas oryzae*	Bacterial blight resistance	[21]
Rice	CRISPR/Cas9	OsSWEET11	*Xanthomonas oryzae*	Bacterial blight resistance	[20]
		OsSWEET14			
Tomato	CRISPR/Cas9	SlMLO1	*Oidium neolycopersici*	Powdery mildew resistance	[19]
Tomato	CRISPR/Cas9	SlDMR6-1	*Xanthomonas, Pseudomonas syringae*, and *Phytophthora capsici.*	downy mildew resistance	[24]
Grapefruit	CRISPR/Cas9	CsLOB1 promoter	*Xanthomonas citri subsp. citri*	Alleviated citrus canker	[25]
Orange	CRISPR/Cas9	CsLOB1 promoter	*Xanthomonas citri subsp. citri*	Citrus canker resistance	[26]
Grapefruit	CRISPR/Cas9	CsLOB1	*Xanthomonas citri subsp. citri*	Citrus canker resistance	[27]
Cucumber	CRISPR/Cas9	eIF4E	Cucumber Vein Yellowing Virus (CVYV), Zucchini Yellow Mosaic Virus (ZYMV) and Papaya Ring Spot Mosaic Virus-W (PRSV - W)]	Virus resistance	[28]
Tobacco	CRISPR/Cas9	BeYDV (short intergenic region)	Bean yellow dwarf virus	Leaf thickening, chlorosis, curling	[29]
Tobacco	CRISPR/Cas9	TYLCV-IR (Intergenic Regions), RCA regions	Tomato yellow leaf curl virus, Beet curly top virus	Leaf curl disease	[30]
Arabidopsis thaliana	CRISPR/Cas9	eIF(iso)4E (eIF transcription factor)	Potyvirus (TuMV)	Turnip mosaic virus disease	[31]
Cotton	CRISPR/Cas9	CLCuD IR and Rep	Begomo virus	Cotton leaf curl disease	[32]

Table 1: List of edited genes by CRISPR/Cas9 system for disease resistance in different plants.

Likewise, CRISPR-Cas9 based multiplex genome editing system was used to concurrently knocked out the three genes (GW2, GW5 and TGW6) which negatively regulate the grain weight, the resulting edited plants produced grains with increased weight [33]. For more tillering a study was conducted by Miao J; they disrupted the LAZY1 gene by CRISPR-Cas9 system and T1 mutated rice plant with more tillers were generated [34]. Large-sized tomato fruit has been developed by targeting the cis-regulatory element of CLAVATA-WUSCHEL; the genes involved in stem cell circuit and direct the meristem size. By editing these circuit genes in tomato number of locules are produced resulting in bigger sized fruit [35]. In tomato plant expression of SELF-PRUNING 5G (SP5G) was enhanced during long days causes early flowering resulting in early yield. These findings facilitated the extension of tomato cultivation beyond its origin [36].

In addition to developing genetically edited disease-resistant plants and improving yield-related characteristics several other traits have been manipulated by the CRISPR-Cas9 system.

Presently malnutrition is one of the most serious issues regarding human health and researcher's interest in developing crops with improved quality is dramatically increasing since a couple of years back. Genome editing has huge potential for enhancement of nutritional properties, for example, the quality of potato was enhanced by knocking out of granule-bound starch synthase GBSS gene. The resultant mutants with no GBSS enzyme activity were phenotyped for starch quality; the amylopectin contents were higher in the starch than the amylose. In a similar effort, Dupont Pioneer generated the waxy corn by disruption of the Wx1 gene through the CRISPR-Cas9 system.

Contrary, the rice quality was improved by editing the starch-branching enzymes (OsSBEI and OsSBEII), genes to reduce the quantity of amylopectin and to increase the amylose content [37]. In wheat to generate the mutants with low gluten the functions of α-gliadin genes were knocked out and in tomato to increase the lycopene contents, multiple genes involved in carotenoid biosynthesis pathway have been manipulated [38,39]. In rice edited eight quality and yield-related concurrently [40].

Presence of weeds in fields negatively affects the crops yield because of competition for resources. For the management of weeds chemical and transgenic approaches have been used. At present, genome editing tools are being employed to produce herbicides resistant crops. Such as herbicides resistant rice varieties have been developed by site-specific replacement and insertion in the endogenous EPSPS gene [41]. Similarly, potato, maize, soybean, flax and cassava plants have been genetically edited for developing herbicide resistance [42-46].

Abiotic stress tolerance is an intricate trait and due to this complexity, fewer numbers of edited crops in this field have so far been developed as compared to disease-resistant crops. Shi et al. manipulated the maize ARGOS8 gene by CRISPR-Cas9 to enhance the drought stress tolerance under scarce water condition [5]. Likewise, the OST2 gene of model Arabidopsis plant has been mutated resulting in changing the pattern of stomata closure to cope with the environmental drought condition thus conferring the drought tolerance to plants. Table 2 describes the use of CRISPR-Cas9 tool for improving significant traits in crop plants.

Since 2013, first documentation of the use of CRISPR/Cas9 of plant genome editing, there came many advance variants of CRISPR/Cas system such as CRISPR/dCas9, CRISPR/Cas12 (Cpf1), CRISPR/Cas13 (SHERLOCK), ribonucleoproteins (RNP) and base editors. All these modern editors are being employed to improve the agricultural crop more efficiently and have revolutionized the functional genomics. In this review we will not provide the detail studies about the use of these modern variants. Moreover, initially, first-generation gene-editing applications rely on one or two non-elite genotypes which are *in vitro* transformed and regenerated. But newly developed techniques facilitate more effective transformation even for elite genotypes [47,48]. Gene editing still have prolonged tissue culture requirements as well as specialized labs with physical containment appropriate for genetic manipulation using CRISPR system [49]. However, now the Express Edit system is available that edit the gene directly in the speed breeding scheme could avoid the bottleneck of *in vitro* transformation and regeneration of plants.

Crop plant	Gene editor	Edited gene	Target trait	References
Wheat	CRISPR/Cas9	Ta GW2	Grain weight	[50]
Maize	CRISPR/Cas9	ZmLIG1, ZmM26, Zm	-	[51]
Maize	CRISPR/Cas9	Wx1	High amylopectin content	Pioneer,2018
Maize	CRISPR/Cas9	ZmIPK	Reduce phytate	[52]
Maize	CRISPR/Cas9	TMS5	Thermosensitive male-sterile	[53]
Maize	CRISPR/Cas9	ARGOS8	Drought stress tolerance	[5]
Oryza sativa	CRISPR/Cas9	LAZY1	Tiller-spreading	[34]
Oryza sativa	CRISPR/Cas9	Gn1a, GS3, DEP1, IPA1	Enhanced grain number, larger grain size dense erect panicles	[23]
Maize	CRISPR/Cas9	ALS	Herbicide resistance	[51]
Oryza sativa	CRISPR/Cas9	SBEIIb	High amylose content	[37]
Oryza sativa	CRISPR/Cas9	ALS	Herbicide resistance	[54]
Oryza sativa	CRISPR/Cas9	EPSPS	Herbicide resistance	[23]
Camelina sativa	CRISPR/Cas9	FAD2	Decreased polyunsaturated fatty acids	[55]
Potato	CRISPR/Cas9	GBSS	High amylopectin content	[56]
Potato	CRISPR/Cas9	ALS	Herbicide resistance	[57]
Tomato	CRISPR/Cas9	SP5G	Earlier harvest time	[36]
Tomato	CRISPR/Cas9	SlAGL6	Parthenocarpy	[58]
Tomato	CRISPR/Cas9	SP, SP5G, CLV3, WUS, GGP1	Tomato domestication	[38]
Soybean	CRISPR/Cas9	ALS	Herbicide resistance	[44]
Flax	CRISPR/Cas9	EPSPS	Herbicide resistance	[45]

Cassava	CRISPR/Cas9	EPSPS	Herbicide resistance	[46]

Table 2: List of edited genes by CRISPR/Cas9 system for improving different traits in plants.

Speed Breeding: A New Avenue for Crop Improvement

Since ancient times multiple techniques, practices and approaches have been developed to enhance the crop productivity. In spite of advanced and efficient molecular techniques new pathways are still needed to meet the future food demands. The time-consuming crop improvement by traditional breeding is attributed to the prolong generation times. The "speed breeding" method is a short way to reduce the generation time and speeds up breeding and research programs. To grow plants in space, NASA has developed the procedure of speed breeding afterward advancements in this technique were brought by UK and Australian Scientists of John Innes Center and Queensland University respectively. Speed breeding technology of NASA has motivated the Amy Watson and their colleagues to carry out an experiment with Dr. Lee Hickey on speed breeding techniques [12]. They ascertained that generation time of major crops like wheat, chickpea, canola and barley can be considerably reduced by adaptation of this technology (Figure 1). Six generations per annum can be achieved for spring, durum wheat (*Triticum aestivum*, T. durum), barley (*Hordeum vulgare*), chickpea (*Cicer arietinum*) and pea (*Pisum sativum*) and 4 generations of canola crop (*Brassica napus*), through speed breeding which normally can be grown up to 2-3 generations in glasshouse conditions. For speed breeding purpose plants were grown in completely closed chambers with controlled environmental conditions which speed up the growth and development of plan.

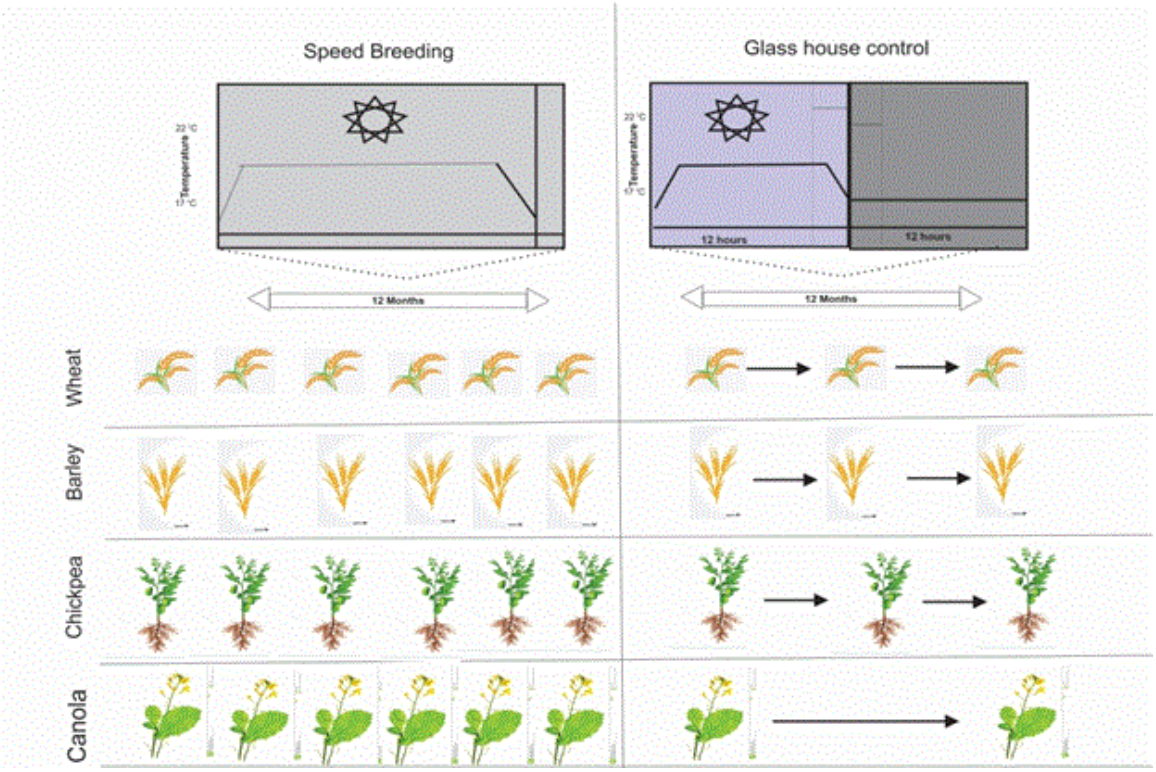

Figure 1: A Schematic representation of Speed breeding by Watson et al. accelerates the generation time of major crop plants for research and breeding. Comparison of glasshouse control condition with filed variable photoperiod (10-16 hours), in natural condition 2-3 generations of wheat, barley, chickpea and canola can be grown per annum (right), by speed breeding annually 4-6 generations of these crops to be achieved(left). These values are representative of relatively rapid cycling cultivars of each crop.

Speed breeding approach has already been effectively practiced to hasten breeding aims for pea (*P.sativum*), amaranth (*Amaranthus spp.*) and peanut (*Arachis hypogaea*) [59-61]. Other crop plants such as radish, sunflower and pepper give better response to prolong photoperiod and it is likely to minimize their generation time through speed breeding technique [62]. Advancements in genomic techniques and resources along with reduced sequencing expenditures have facilitated the plant scientist to switch their attention from model plants to crops. Even with such advances, prolong generation times of many crops enforce a barrier. Different researchers have developed speed breeding protocols for different crops to hasten the generation time [12]. Contrasting to double haploid methodology, in which to generate the homozygous lines; haploid embryos are rescued for chromosomes doubling speed breeding, applies to diverse germplasm and there is no requirement of *in vitro* culturing in particular labs [63]. In speed breeding technique to reduce the generation time quality and

intensity of light, photoperiod and controlled temperature are used to speed up the photosynthesis and flower setting rate, along with early seed production. Specialized protocols are on hand for plants which necessitate specific environmental conditions for flower setting, for instance, vernalization or short days. By applying these techniques cereals can be grown at high densities for example 1,000 plants/m^2 with limited space and cost [64]. Combining speed breeding with state-of-the-art technologies will strengthen the efforts to fulfill the feeding requirement of the growing population [65]. There is impressive potential for amalgamation of speed breeding with these modern technologies and resources including, speed cloning, genomic selection, high throughput genotyping, marker-assisted selection, CRISPR gene editing, etc. to make the crop improvement faster.

Therefore, by combining the speed cloning, gene editing and speed breeding techniques generation time can be bypass from 10-15years to 5-6 years. This rapid crop improvement program of 5-6 years include three major techniques firstly by speed cloning within 6 months desirable genes can be cloned from a diverse population, secondly, manipulation of crop genomes can be done by different genome editing approaches like express edit and at third phase through speed breeding techniques within 1 year 4-6 generation can be generated, finally field trial takes 1-3 years (Figure 2).

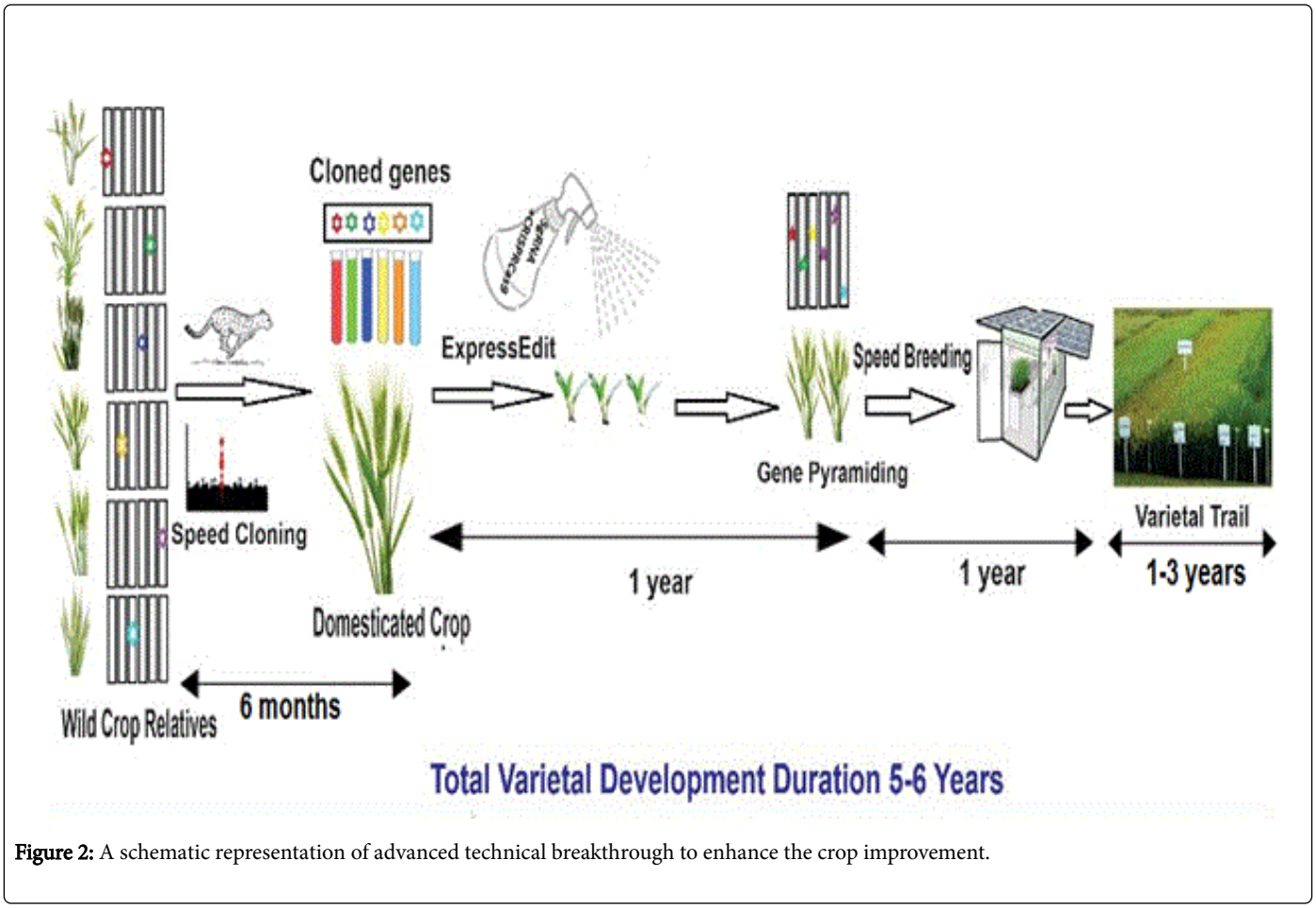

Figure 2: A schematic representation of advanced technical breakthrough to enhance the crop improvement.

Speed breeding technology is capable to accelerate the advancement in different plant research areas including crossing, generation of mapping populations and phenotyping of plants for specific traits [12]. Additionally, backcrossing and traits pyramiding can be accelerated by speed breeding [65].

Conclusion

Over the past couple of decades, conventional breeding that relies on diversified plant populations has enormous contributions in agriculture. However, the variations in a population are mostly consequents of spontaneous mutations or chemically induced mutations or physical irradiation which are generally rare and occur randomly. Additionally, in elite varieties variation for complex traits might not be possible by labor extensive breeding program. Therefore, advanced technologies can generate rapid precise targeted manipulation in crops. In this review, we have discussed the technical advancements and their integration in breeding programs to reduce the generation time of 10-15 years to 5-6 years. To get the generation in short time the integration of advanced techniques of speed cloning and genome editing leading to speed breeding are best choice. In gene editing approach CRISPR/Cas system is an accurate, rapid and versatile tool which can be employed to targeting any part of the genome of an organism by altering the sequence of gRNA. It will useful in annotating a huge quantity of sequence data which will further facilitate in discovering the genomes of the different crops by overcoming the limitations of searching diversified plant populations and conventional breeding methods. In consequence, these speed

cloning and CRISPR/Cas system being easier, safer and precise techniques have to be integrated into the crop improvement programs to get benefits of the presently existing enormous genome sequence data to generate improved crop varieties to meet the demands of a future growing population.

Acknowledgments

The authors are grateful to National Institute for Genomics and Advanced Biotechnology (NIGAB), NARC, Islamabad, Pakistan for technical and financial support.

Conflict of Interest

All authors declare that they have no conflict of interest.

References

1. Hunter MC, Smith RG, Schipanski ME, Atwood LW, Mortensen DA (2017) Agriculture in 2050: Recalibrating targets for sustainable intensification. Bioscience 67: 386-391.
2. Lidder P, Sonnino A (2012) Biotechnologies for the management of genetic resources for food and agriculture. In: Adv Genet 78: 1-167.
3. Collard BC, MackillDJ (2007) Marker-assisted selection: An approach for precision plant breeding in the twenty-first century. Philos Trans R Soc B: Biol Sci 363: 557-572.
4. Desta ZA, Ortiz R (2014) Genomic selection: genome-wide prediction in plant improvement. Trends plant sci 19: 592-601.
5. Shi J, Gao H, Wang H, Lafitte HR, Archibald RL, et al. (2017) ARGOS 8 variants generated by CRISPR-Cas9 improve maize grain yield under field drought stress conditions. Plant Biotechnol J 15: 207-216.
6. Christou A, Manganaris GA, Papadopoulos I, Fotopoulos V (2013) Hydrogen sulfide induces systemic tolerance to salinity and non-ionic osmotic stress in strawberry plants through modification of reactive species biosynthesis and transcriptional regulation of multiple defence pathways. J Exp Bot 64: 1953-1966.
7. Steuernagel B, Periyannan SK, Hernández-Pinzón I, Witek K, Rouse MN, et al. (2016) Rapid cloning of disease-resistance genes in plants using mutagenesis and sequence capture. Nat Biotechnol 34: 652.
8. Sánchez-Martín J, Steuernagel B, Ghosh S, Herren G, Hurni S, et al. (2016) Rapid gene isolation in barley and wheat by mutant chromosome sequencing. Genome Boil 17: 221.
9. Thind AK, Wicker T, Šimková H, Fossati D, Moullet O, et al. (2017) Rapid cloning of genes in hexaploid wheat using cultivar-specific long-range chromosome assembly. Nat Biotecnol 35: 793.
10. Rahman A, Hallgrímsdóttir I, Eisen M, Pachter L (2018) Association mapping from sequencing reads using k-mers. Elife7:e32920.
11. Shi J, Lai J (2015) Patterns of genomic changes with crop domestication and breeding. Curropin Plant Boil 24: 47-53.
12. Watson A, Ghosh S, Williams MJ, Cuddy WS, Simmonds J, et al. (2018) Speed breeding is a powerful tool to accelerate crop research and breeding. Nat Plants 4: 23.
13. Zhang F, Wen Y, Guo X (2014) CRISPR/Cas9 for genome editing: progress, implications and challenges. Hum Mol Genet 23: R40-R46
14. Wolter F, Schindele P, Puchta H (2019) Plant breeding at the speed of light: The power of CRISPR/Cas to generate directed genetic diversity at multiple sites. BMC Plant Boil 19: 176.
15. Savary S, Ficke A, Aubertot JN, Hollier C (2012) Crop losses due to diseases and their implications for global food production losses and food security. Springer.
16. Baltes NJ, Hummel AW, Konecna E, Cegan R, Bruns AN, et al. (2015) Conferring resistance to geminiviruses with the CRISPR–Cas prokaryotic immune system. Nat Plants 1: 15145.
17. Wang Y, Cheng X, Shan Q, Zhang Y, Liu J, et al. (2014) Simultaneous editing of three homoeoalleles in hexaploid bread wheat confers heritable resistance to powdery mildew. Nat Biotechnol 32: 947.
18. Zhang Y, Bai Y, Wu G, Zou S, Chen Y, et al. (2017) Simultaneous modification of three homoeologs of Ta EDR 1 by genome editing enhances powdery mildew resistance in wheat. Plant J 91: 714-724.
19. Nekrasov V, Wang C, Win J, Lanz C, Weigel D (2017) Rapid generation of a transgene-free powdery mildew resistant tomato by genome deletion. Sci Rep 7: 482.
20. Jiang W, Zhou H, Bi H, Fromm M, Yang B (2013) Demonstration of CRISPR/Cas9/sgRNA-mediated targeted gene modification in Arabidopsis, tobacco, sorghum and rice. Nucleic Acids Res 41: e188-e188.
21. Zhou J, Peng Z, Long J, Sosso D, Liu B, et al. (2015) Gene targeting by the TAL effector PthXo2 reveals cryptic resistance gene for bacterial blight of rice. Plant J 82: 632-643.
22. Wang F, Wang C, Liu P, Lei C, Hao W, et al. (2016) Enhanced rice blast resistance by CRISPR/Cas9-targeted mutagenesis of the ERF transcription factor gene OsERF922. PloS one 11: e0154027.
23. Li M, Li X, Zhou Z, Wu P, Fang M, et al. (2016) Reassessment of the four yield-related genes Gn1a, DEP1, GS3, and IPA1 in rice using a CRISPR/Cas9 system. Front Plant Sci 7: 377.
24. Thomazella DP, Brail Q, Dahlbeck D, Staskawicz B (2016) CRISPR-Cas9 mediated mutagenesis of a DMR6 ortholog in tomato confers broad-spectrum disease resistance. Bio Rxiv pp: 064824.
25. Jia H, Zhang C, Pervaiz T, Zhao P, Liu Z, et al. (2016) Jasmonic acid involves in grape fruit ripening and resistant against Botrytis cinerea. Functintegr Genomics 16: 79-94.
26. Peng A, Chen S, Lei T, Xu L, He Y, et al. (2017) Engineering canker-resistant plants through CRISPR/Cas9 - targeted editing of the susceptibility gene Cs LOB 1 promoter in citrus. Plant Biotechnol J 15: 1509-1519.
27. Jia H, Zhang Y, Orbović V, Xu J, White FF, et al. (2017) Genome editing of the disease susceptibility gene Cs LOB 1 in citrus confers resistance to citrus canker. Plant Biotechnol J 15: 817-823.
28. Chandrasekaran J, Brumin M, Wolf D, Leibman D, Klap C, et al. (2016) Development of broad virus resistance in non-transgenic cucumber using CRISPR/Cas9 technology. Mol Plant Pathol 17: 1140-1153.
29. Baltes NJ, Gil-Humanes J, Cermak T, Atkins PA, Voytas DF (2014) DNA replicons for plant genome engineering. Plant Cell 26: 151-163.
30. Ali Z, Abulfaraj A, Idris A, Ali S, Tashkandi M (2015) CRISPR/Cas9-mediated viral interference in plants. Genome Boil 16: 238.
31. Pyott DE, Sheehan E, Molnar A (2016) Engineering of CRISPR/Cas9-mediated potyvirus resistance in transgene-free Arabidopsis plants. Mol Plant Pathol 17: 1276-1288.
32. Iqbal Z, Sattar MN, Shafiq M (2016) CRISPR/Cas9: A tool to circumscribe cotton leaf curl disease. Front Plant Sci 7: 475.
33. Xu R, Yang Y, Qin R, Li H, Qiu C, et al. (2017) Rapid improvement of grain weight *via* highly efficient CRISPR/Cas9-mediated multiplex genome editing in rice. J Genet Genomics 43: 529.
34. Miao J, Guo D, Zhang J, Huang Q, Qin G, et al. (2013) Targeted mutagenesis in rice using CRISPR-Cas system. Cell Res 23: 1233.
35. Rodríguez-Leal D, Lemmon ZH, Man J, Bartlett ME, Lippman ZB (2017) Engineering quantitative trait variation for crop improvement by genome editing. Cell 171: 470-480.
36. Soyk S, Müller NA, Park SJ, Schmalenbach I, Jiang K, et al. (2017) Variation in the flowering gene SELF PRUNING 5G promotes day-neutrality and early yield in tomato. Nat Genet 49: 162.
37. Sun Y, Jiao G, Liu Z, Zhang X, Li J, et al. (2017) Generation of high-amylose rice through CRISPR/Cas9-mediated targeted mutagenesis of starch branching enzymes. Front Plant Sci 8: 298.
38. Li T, Yang X, Yu Y, Si X, Zhai X, et al. (2018) Domestication of wild tomato is accelerated by genome editing. Nat Biotechnol.
39. Li X, Wang Y, Chen S, Tian H, Fu D, et al. (2018) Lycopene is enriched in tomato fruit by CRISPR/Cas9-mediated multiplex genome editing. Front Plant Sci 9: 559.

40. Shen L, Wang C, Fu Y, Wang J, Liu Q, et al. (2018) QTL editing confers opposing yield performance in different rice varieties. J Integr Plant Boil 60: 89-93.
41. Sun Y, Jiao G, Liu Z, Zhang X, Li J, et al. (2017) Generation of high-amylose rice through CRISPR/Cas9-mediated targeted mutagenesis of starch branching enzymes. Front Plant Sci 8: 298.
42. Butler NM, Baltes NJ, Voytas DF, Douches DS (2016) Gemini virus-mediated genome editing in potato (Solanum tuberosum L.) using sequence-specific nucleases. Front Plant Sci 7: 1045.
43. Svitashev S, Young JK, Schwartz C, Gao H, Falco SC (2015) Targeted mutagenesis, precise gene editing, and site-specific gene insertion in maize using Cas9 and guide RNA. Plant Physiol 169: 931-945.
44. Li Z, Liu ZB, Xing A, Moon BP, Koellhoffer JP, et al. (2015) Cas9-guide RNA directed genome editing in soybean. Plant Physiol 169: 960-970.
45. Sauer NJ, Narváez-Vásquez J, Mozoruk J, Miller RB, Warburg ZJ, et al. (2016) Oligonucleotide-mediated genome editing provides precision and function to engineered nucleases and antibiotics in plants. Plant Physiol 170: 1917-1928.
46. Hummel AW, Chauhan RD, Cermak T, Mutka AM, Vijayaraghavan A, et al. (2018) Allele exchange at the EPSPS locus confers glyphosate tolerance in cassava. Plant Biotechnol J 16: 1275-1282.
47. Lowe K, Wu E, Wang N, Hoerster G, Hastings C, et al. (2016) Morphogenic regulators Baby boom and Wuschel improve monocot transformation. Plant Cell 28: 1998-2015.
48. Richardson T, Thistleton J, Higgins T, Howitt C, Ayliffe M (2014) Efficient Agrobacterium transformation of elite wheat germplasm without selection. Plant Cell Tiss Org 119: 647-659.
49. Doudna JA, Charpentier E (2014) The new frontier of genome engineering with CRISPR-Cas9. Science 346: 1258096.
50. Liang Z, Chen K, Li T, Zhang Y, Wang Y, et al. (2017) Efficient DNA-free genome editing of bread wheat using CRISPR/Cas9 ribonucleoprotein complexes. Nat Commun 8: 14261.
51. Svitashev S, Schwartz C, Lenderts B, Young JK, Cigan AM (2016) Genome editing in maize directed by CRISPR–Cas9 ribonucleoprotein complexes. Nat Commun 7: 13274.
52. Liang Z, Zhang K, Chen K, Gao C (2014) Targeted mutagenesis in Zea mays using TALENs and the CRISPR/Cas system. J Genet Genomics 41: 63-68.
53. Li J, Zhang H, Si X, Tian Y, Chen K, et al. (2017) Generation of thermosensitive male-sterile maize by targeted knockout of the ZmTMS5 gene. J Genet 44: 465.
54. Endo M, Mikami M, Toki S (2016) Biallelic gene targeting in rice. Plant Physiol 170: 667-677.
55. Jiang WZ, Henry IM, Lynagh PG, Comai L, Cahoon EB (2017) Significant enhancement of fatty acid composition in seeds of the allohexaploid, Camelina sativa, using CRISPR/Cas9 gene editing. Plant Biotechnol J 15: 648-657.
56. Andersson M, Turesson H, Nicolia A, Fält A-S, Samuelsson M (2017) Efficient targeted multiallelic mutagenesis in tetraploid potato (Solanum tuberosum) by transient CRISPR-Cas9 expression in protoplasts. Plant Cell Rep 36: 117-128.
57. Butler NM, BaltesNJ, Voytas DF, Douches DSJFips (2016) Gemini virus-mediated genome editing in potato (Solanum tuberosum L.) using sequence-specific nucleases. Front Plant Sci 7: 1045.
58. Klap C, Yeshayahou E, Bolger AM, Arazi T, Gupta SK, et al. (2017) Tomato facultative parthenocarpy results from Sl AGAMOUS-LIKE 6 loss of function. Plant Biotechnol J 15: 634-647.
59. Mobini SH, Warkentin TD (2016) A simple and efficient method of *in vivo* rapid generation technology in pea (Pisum sativum L.). *In Vitro* Cellular Dev Biol-Plant 52: 530-536.
60. Stetter MG, Zeitler L, Steinhaus A, Kroener K, Biljecki M (2016) Crossing methods and cultivation conditions for rapid production of segregating populations in three grain amaranth species. Front Plant Sci 7: 816.
61. O'Connor D, Wright G, Dieters M, George D, Hunter M, et al. (2013) Development and application of speed breeding technologies in a commercial peanut breeding program. Peanut Sci 40: 107-114.
62. Sysoeva MI, Markovskaya EF, Shibaeva TG (2010) Plants under continuous light: A review. Plant Stress 4: 5-17.
63. Laurie DA, Bennett M (1988) The production of haploid wheat plants from wheat x maize crosses. TheorAppl Genet 76: 393-397.
64. Ghosh S, Watson A, Gonzalez-Navarro OE, Ramirez-Gonzalez RH, Yanes L, et al. (2018) Speed breeding in growth chambers and glasshouses for crop breeding and model plant research. Nat Protoc 13: 2944.
65. Hickey LT, Germán SE, Pereyra SA, Diaz JE, Ziems LA, et al. (2017) Speed breeding for multiple disease resistance in barley. Euphytica 213: 64.