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# Smart Breeding for Climate Resilient Agriculture

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## Abstract

Human society is at a turning point of its time as climate change is becoming more and more real and inevitable. From rising temperature, which undermines the food production, to melting glaciers, causing disastrous flooding and erosion, the global repercussions of climate change are unprecedented. Plant breeding has always played a pivotal role in human history by revolutionizing agriculture to feed the ever-growing population. It can rescue humankind from imminent threats to agriculture posed by weather fluctuations, rapidly evolving pests and limiting resources. Unlocking the repository of genetic diversity and extensive utilization of wild germplasm invariably is imperative to every crop improvement program. But recent advancements in genomics, high throughput phenomics, sequencing and breeding methodologies along with state-of-the-art genome-editing tools in integration with artificial intelligence open up new doors for accelerated climate-resilient crop improvement. Therefore, holistic smart breeding approaches can be promising way out to tackle climate change and develop better-adapted crop varieties.

**Keywords:** climate resilience, genetic diversity, genomics, artificial intelligence, food security, phenomics, smart breeding

## 1. Introduction

Land is shrinking but world population is increasing in a rapid phase, so, modern agricultural practice is struggling to meet the level of primary productivity required to feed approximately 10 billion people by 2050 [1]. From last few decades the adverse effects of climate change and higher CO<sub>2</sub> concentrations, the consequence of expected impacts on the water-use efficiency of dryland as well as irrigated crop production, potential effects on biosecurity, production, and quality of product through increased the frequency of introduced various abiotic (heat, salinity and drought) and biotic stresses (pests and diseases). In addition, climate change is also expected to cause losses of biodiversity, mainly in more marginal environments. Drought alone is expected to reduce crop productivity in half of the global arable land and it's estimated around 50% in the next five decades [2]. It has been predicted that, on average, global yields of major economic important crops will be reduced by the unfavorable climatic conditions in wheat (6.0%), rice (3.2%), maize (7.4%) and soybean (3.1%) for every degree celsius increase in global mean temperature [3].

Climate resilience is an ability of the plant/crop to survive and recover from the effects of climate change. Some important practices that may help to adapt the climate change are soil organic carbon build up or carbon sequestration, in-situ moisture conservation, residue incorporation instead of burning, water harvesting and recycling for supplemental irrigation, growing biotic and abiotic

resistance/tolerant varieties, location specific agronomic and nutrient management and breeding for multiple traits of interest including quality.

Plant Breeding has always played a pivotal role in human history from revolutionizing agriculture to feed the ever-growing population. The key role of plant breeding in agriculture is to develop a genetically superior genotype/variety, which is suitable for a specific as well as general cultivation of particular environment towards higher production [4]. Realizing the importance of genomic resources to expedite the breeding programs, huge amount of genetic data related to genes and QTLs (Quantitative Trait Loci) are generated after the advent of molecular biology and biotechnology [5]. The progress in precise phenotyping and genotyping offers tremendous opportunities to develop crop varieties that are suit for better changing the climatic conditions, which ameliorate in boosting the plant breeding activities for developing climate resilient varieties/cultivars [6]. Hence, development of climate resilient varieties utilizing Smart breeding tools to ensures the food security in adverse climatic conditions.

## 2. Effect of climate change on agriculture and food production

The effect of climate on agriculture is related to variability's in local climates rather than in global climate patterns. The changes in the rainfall patterns, temperature, CO<sub>2</sub> level and greenhouse gases resulting in the frequency and severity of extreme events such as flooding, drought, hail, and hurricanes etc. are major hindrance in achieving the food security for ever increasing population [7].

According to Intergovernmental Panel on Climate Change (IPCC), global temperature may be rise from 1.7 to 4.8°C during the twenty-first century and precipitation pattern will also be altered [8]. In recent times, it has been reported that the Yangtze river basin in China has become hotter and it is expected that the

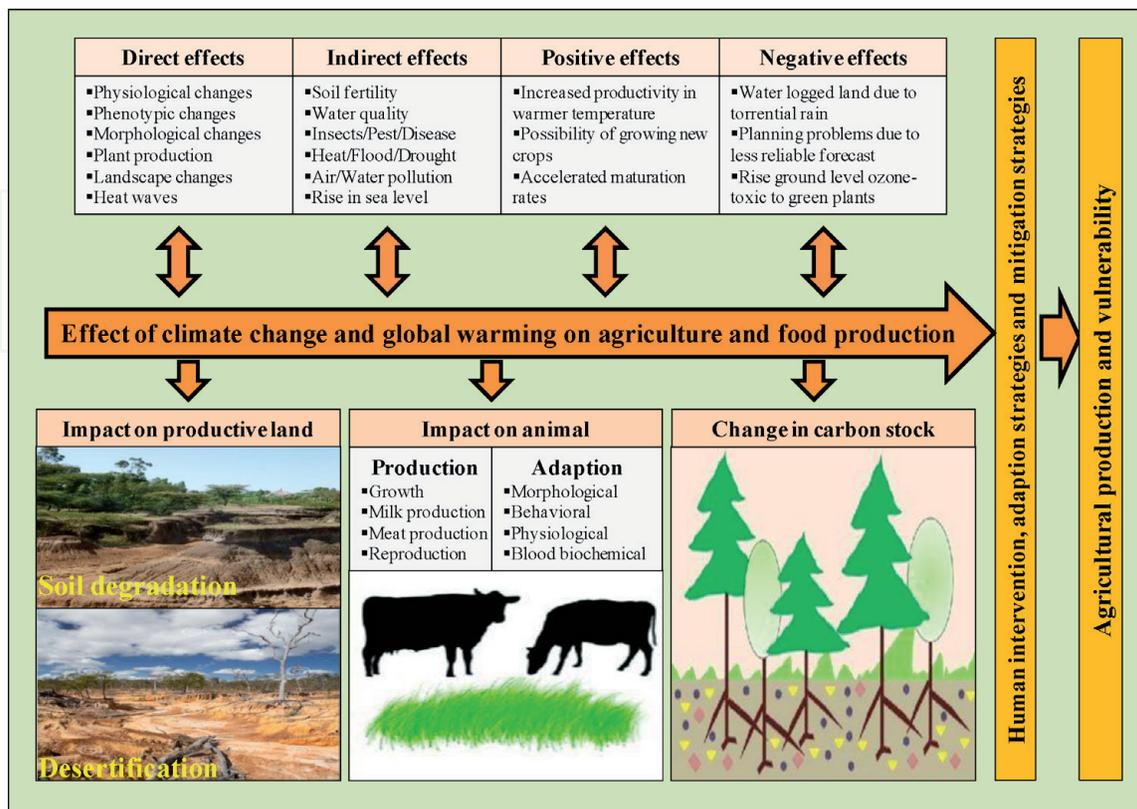


Figure 1. Adverse effects of climate change on agriculture and food production.

temperature will increase up to 2°C by 2050 relative to 1950 [9], and also reduce the rice (41%) and maize (50%) production by the end of the 21st century. This shift in climate will affect the environment, including the soil ecology and thus has the potential to threaten food security through its adverse effects on soil properties and processes [10]. Additionally, the direct and indirect effects of climatic change would lead to alter the nutrient and their bioavailability in soils (**Figure 1**). The effect of climate changes on biotic and abiotic stresses have already reduced the global agricultural production from 1 to 5% during the past three decades [11].

### **3. Technological approaches towards climate resilient agriculture**

Some important practices that assist to adapt the climate changes for crop production including (i) Building resilience in soil (tillage management, avoid bare soil, fertilizer application after mandatory soil testing, increase soil carbon through organic manure, green manuring, crop rotation or intercropping with legume sequester carbon and biochar), (ii) Adapted cultivars and cropping systems (crop diversification, shallow-deep root and legume-cereal cropping system, improved early/short duration cultivars for tolerant against drought, heat and submergence capturing optimum yields despite climatic stresses), (iii) Rainwater harvesting and recycling (inter-row water harvesting, inter-plot water harvesting, in farm ponds and reservoirs and recycling), (iv) Farm machinery (chisel and para plow to opening the furrows which conserves rain water, laser leveler helps in increasing nutrient as well as water use efficiency), (v) Crop contingency plans (livestock and fishery interventions), (vi) Weather based agro advisories (automatic weather stations establishment at experimental farms and mini-weather observatories records for real time weather parameters such as rainfall, temperature and wind speed, which customized through agro advisories and improve weather literacy among the farmers).

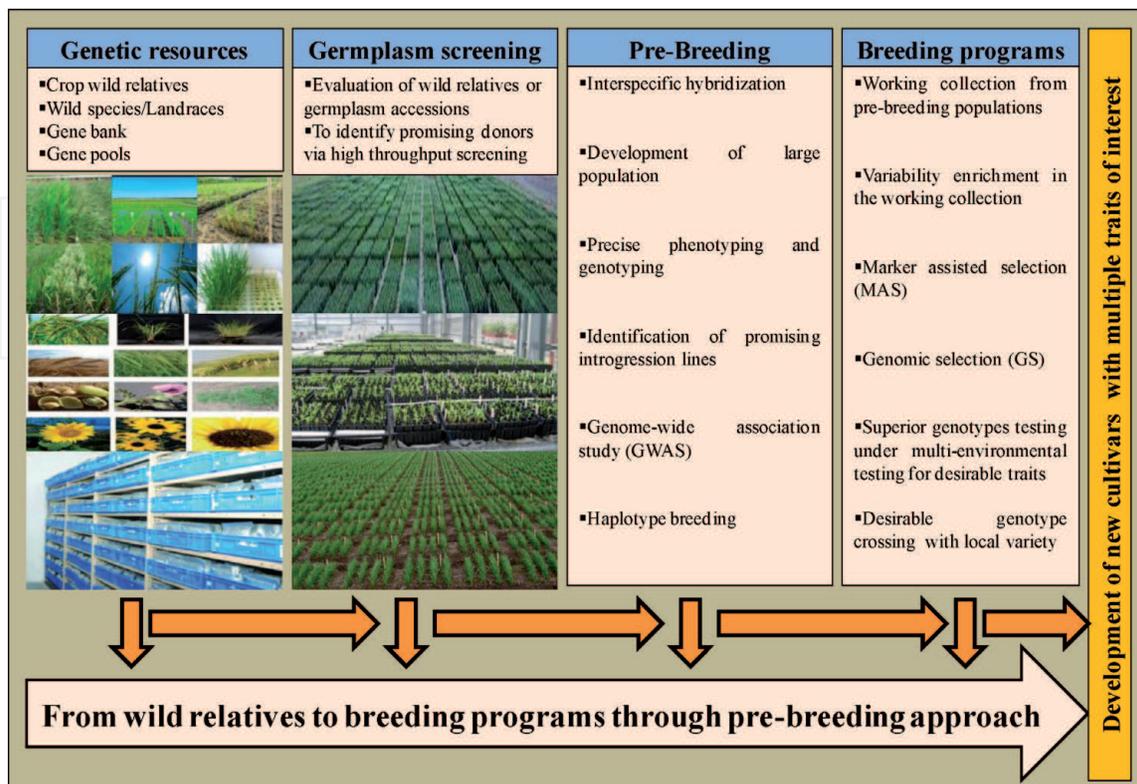
Plant breeding procedures have been constantly evolving to meet the increasing food demand. The art of plant breeding has been practiced in various forms since the start of human civilization. In conventional plant breeding, development of a new cultivar take around 10–14 years and may even exceed this period based on the plant habit, reproductive cycle and complexity of traits involved. The rapid climate change necessitates the development of varieties in a shorter period to tackle with the unpredictable weather parameters. The concept of Smart breeding is an integration of conventional breeding strategies with advanced molecular, genomic and phenomic tools to efficiently and effectively breed the resilient crop cultivars with enhanced yield potential. New breeding approaches such as rapid generation advancement, doubled haploid (DH), marker assisted back crossing (MABC), marker assisted recurrent selection (MARS), genomic selection (GS) etc. have been used to help shorten the breeding cycle along with efficient screening for specific biotic and abiotic stresses. Biotechnology-based breeding technologies (marker-assisted breeding and genetic modifications) will be essential to assist and accelerate genetic gain, but their application requires additional investment in the understanding, genetic characterization and phenotyping for complex adaptive traits to be exploited for climate resilient breeding.

### **4. Pre-breeding and crop wild relatives (CWR): exploring untapped hub of genetic diversity**

Climate change leading to severe weather fluctuations would also lead to evolution of plant diseases and pests, exposing crops to higher biotic pressure in addition

to abiotic stresses. To make crop adaptation feasible in the era of changing climate, there is indispensable need to breed the crop plants with diverse genetic backgrounds. In order to feed the mushrooming population, there is urgent need to use crop wild relatives for developing broader spectrum varieties to tackle various biotic and abiotic stresses. During the era of domestication, selection preferences lead to modern crops with narrow genetic background, resulting in limitation of environmental adaptation and breeding capacity using modern germplasm [12]. Wild relatives and ancestral species relatively possess broader adaptation to environment and climates ultimately higher potential in crop improvement.

Prebreeding activity is a bridge for linking the desirable traits of CWR to the modern cultivar development by providing breeders with wild genetic diversity in a more immediately usable form [13, 14]. Pre-breeding is an opportunity to introgression of desirable genes, from wild species (primary, secondary and tertiary gene pools) into elite breeding lines/cultivars/genotypes, to overcome the linkage drag (**Figure 2**). Almost all cultivated crop species were originally domesticated from wild plants by humans, due to domestication inherently reduced the genetic variation [15]. The genetic potential of wild relatives has been reported in different crops like rice, wheat, maize, potato, tomato, cotton, tobacco, sugarcane, chickpea and pigeonpea [16–21]. Genomics strategies have been widely utilized in staple crops for transferring major genes (i.e. disease resistance) from wild germplasm to elite cultivars [22]. It is well documented that application of molecular mapping and sequencing to could be useful to unlock the genetic potential of CWR [23]. So, crop wild relatives (CWRs) are good reservoir of untapped genetic diversity, which may not exist in the cultivated gene pool that can be used to improve the numerous trait of interest including resistance/tolerance against diseases, insect-pests, drought, salinity, cold, heat and good agronomic adaption with quality improvement.



**Figure 2.** Untapped genetic resources/ CWRs towards the germplasm enhancement.

#### 4.1 Exotic introgression into elite varieties

Wild species are used mainly for the introgression of disease and insect resistance into crops although drought, cold, heat and salinity tolerance have also been addressed in some staple crops. This is because most pathogens have faster adaptation to climate rendering cultivars vulnerable to novel deadly diseases [24]. The use of interspecific or intergeneric hybridization for disease resistance introgression is conventional one. Another potential technique to enhance genetic diversity and facilitate crop vigor with adaptation to different environmental niches is creating the polyploidy crops mimicking natural evolution through hybridization [25]. Enriched genes for biotic and abiotic stress resistance of CWR can be studied using comparative pool sequencing of genome assemblies, elucidating the potential genomic segments responsible for adaptation to different ecological niches. These have been explored in wild relatives of many crops including chickpea, barley and maize [26–29].

To address the diversity within species, pan-genomics based on entire gene repository of a species can reveal the genetic variations such as structure variants (SVs) and single nucleotide polymorphism (SNPs) abundantly found in plants. One such example under SVs is presence/absence variants (PAVs) of Elicitor response (ELR) gene between wild and cultivated potato leads to resistance/susceptibility response to late blight disease [30]. Larger pan-genomes including both wild relatives and cultivars can acquire glut of dispensable genes resulting in phenotypic variations; thereby easing out with characterization of the trait associated genomic variants [31]. To tackle the deadly rust diseases in wheat in the context of changing climate, several pan-genomic R genes have been successfully identified and cloned from wild diploid wheat *Aegilops tauschii* [32].

#### 4.2 De novo domestication for future crops

Considering the risks of introducing foreign alleles into cultivars, other potential technique for developing climate-friendly crops is de novo domestication [33]. As most staple crops are grown majorly in the regions other than where they were originally domesticated with different climatic regimes. Nevertheless, their wild relatives and landraces exhibit better adaptation to local climate in the native regions. In the scenario of climatic change, there is chance to leverage this opportunity to use those underutilized or orphan crops e.g. rise in *Sinapis alba* (white mustard) acreage replacing the *B. napus* in Europe for biofuel production [34]. A pipeline strategy has been proposed for domestication of wild germplasm in some orphan crops such as quinoa [35]. In addition to direct planting of non-domesticated crop plants, relatively advance methodology of CRISPR/Cas9 boosts the wild germplasm domestication by editing of domesticated genes e.g. editing in wild tomatoes (*Solanum pimpinellifolium*) and ground cherry (*Physalis pruinosa*) mainly focused on flower improvement, plant architecture improvement, fruit size, fruit number and nutritional content [36–38]. It is evident from such a few successful introgressions of domesticated genes that use of wild germplasm in regular plant breeding is quite promising in countering the effects of climate change on agriculture and hence, food security.

#### 4.3 Introgressiomics approach for adaptation to climate change

The actual potential of the CWR in plant breeding largely remains under-exploited due to linkage drag and frequent breeding barriers with the crops.

Introgressiomics approach allows mass scale development of plant material and populations with introgression lines from CWR into the genetic background of crops [39]. This pre-emptive breeding technique could be focused or unfocused depending upon the objective. Besides genetic analysis of traits present in CWR, MAS driven generation of chromosome substitution lines (CSL), introgression lines (IL) or MAGIC populations allow the development of genetically characterized elite material. Genomic tools like high throughput molecular markers facilitate the characterization and development of Introgressiomics populations, which can be easily incorporated into major breeding programs for coping with the accelerating environmental challenges.

#### **4.4 Some other techniques for CWR use**

After the introgression into domesticated background from CWR, populations such as backcross populations (BC), recombinant inbred lines (RILs), doubled haploids (DH), near isogenic lines (NILs), multiparent advance generation intercross (MAGIC) populations as well as nested association mapping (NAM) populations are developed to study the introgressed gene(s). After mapping their locations on to the genome and its genotypic validation with molecular markers, they are further deployed using Marker assisted selection (MAS). Systematic screening of the huge number of progenies with MAS enhances the efficiency of breeding program (van de Weil 2010). Desirable recombinants can be developed at early generations using larger populations e.g. using marker-assisted backcrossing (MABC), an important QTL was introduced into a new lowland rice background in just 2 rounds of backcrossing [40].

Genomic scans can also reveal candidate domestication and improvement loci as well as post-domestication introgression using CWR [41, 42] to be further harnessed in the scenario of climatic challenges. In case of CWR, high throughput sequencing offers a cheap and rapid way to deploy thousands to millions of markers for mapping purposes [43]. Reduced representation techniques as genotyping by sequencing (GBS) or even nimble exom capture have been exploited to this effect in several CWR species already [42, 44, 45]. These technologies offer rapid marker density as required for rapid fine mapping and can saturate mapping populations in terms of capturing all of the recombinants.

The availability of a reference genome sequence in CWR during recent times greatly boosts the use of high-throughput sequence data. Some large scale genomic sequencing and re-sequencing programs are well underway [27, 46] often with reduced representation methods. Whole genome shotgun sequencing (WGS) techniques can also be utilized to characterize CWR germplasm for climate resilience breeding in major staple crops. E.g. Rice having smaller genome size (430 mb) long with its wild relatives has been re-sequenced using WGS [47–49]. Already sequenced germplasm collections including Chickpea [50], Rice [48], Soybean [51] and Wheat [52] etc. will provide insights into these diverse gene pools to be exploited in combating various biotic and abiotic challenges during this era of climate change. More recently, a massive scale genomic study of almost 80000 accessions from CIMMYT and ICARDA unraveled unprecedented amount of genetic diversity in 29 wheat species comprising cultivated wheats, CWRs and landraces to be exploited in wheat improvement for range of climate related plant traits [53].

Potentially revolutionary technology in modern plant breeding like genome editing has enabled scientists to alter genome of any organism with unprecedented precision without involvement of any foreign DNA [54]. CWR and their sequence information may serve as a reference library for all kind of diversity. This information on allelic diversity and its phenotype is a vital requirement for many genome

editing approaches. In fact, these approaches will allow the use of this information from more distantly related, cross-incompatible CWR and domesticated species to be further utilized in crop improvement [55, 56].

## **5. Biotechnology: a toolkit for climate resilient agriculture**

Considering the various direct and indirect impacts of climate change on food production and agriculture along with rapid deterioration of arable land and perplexity of rainfall patterns, all these factors triggering various abiotic stresses such as drought, heat stress and biotic stresses like pest and disease attacks, the sophisticated techniques laden biotechnology toolkit has potential to address these immense challenges of developing the stress tolerant food crop cultivars in this hour of need [57]. With population growing at rapid rate under threatening scenario of climate change, it is high time to shift resilience from conventional breeding along with fertilizers and pesticides to genomics-assisted crop improvement techniques in order to achieve more sustainable and efficient yield gains [58].

### **5.1 Marker assisted breeding**

Recent advances in biotechnology tools have the potential to understand the function of genes/QTLs that govern the economic traits, and applying this information's to Smart breeding programs, leading to crop improvement. The advent of molecular markers such as Restriction fragment length polymorphism (RFLP), Rapid Amplified Polymorphic DNA (RAPD), Simple Sequence repeat (SSR), Kompetitive allele specific PCR (KASP), Cleaved amplified polymorphic sequence (CAPS) and especially Single Nucleotide polymorphism (SNP) have revolutionerized the field of plant genetics and facilitated molecular crop breeding [59].

The ultimate goal of crop breeding to develop super-varieties by assembling multiple desirable traits, such as yield related, superior quality, tolerance/resistance against biotic and abiotic stress and good environmental adaption. It is very challenging, difficult and time consuming to combine all traits in single genotypes by traditional breeding, so some alternates need to be compiling all important traits, into single varieties, can be done through marker assisted selection (MAS), which have become an integral component of genotypes/germplasm improvement. The potential benefits of using molecular markers linked to the genes/QTLs of interest in breeding programmes, which have shifted from phenotype-based (traditional breeding) to a combination of phenotype and genotype-based selection, are of great importance to the Smart breeding programme [60].

Breeding programme combine, with MAS strategies have major advantages compared to traditional phenotype-dependent breeding in terms of convenience and efficiency for transferring the genes/QTLs of interest to the plant genome [61]. Selection can be done selectively with the genotypes of molecular markers linked to the target traits, selection in off-season nurseries (reduce breeding cycle), making the technique more cost effective to grow for more generations per year (speed breeding), reduction of required population size because many lines can be discarded in earlier breeding generations after MAS. The most effective and usefulness of MAS approaches, for traits of simple inheritance (qualitative traits controlled by one or a few genes) have been well proven in many important crops [62].

Basically, two major MAS strategies are usually applied in breeding programme, (i) backcrossing for favorable alleles into elite germplasm, i.e. marker-assisted-backcrossing (MABC) and (ii) stacking multiple genes of different sources into elite breeding lines, i.e. marker-assisted gene pyramiding (MAGP). The success of MAS

has depends to search the important QTLs for complex traits (controlled by minor genes), which account for a large proportion of phenotypic variation (major QTLs). Successful applications of MABC and MAGP for improving yield or yield component traits by using well characterized major QTLs/genes in important crops [63]. Successful implementation of MAS breeding in broad range of crops including barley, beans, cassava, chickpea, cowpea, groundnut, maize, potato, rice, sorghum, and wheat [64]. Genetic markers associated with agronomic traits can be introgressed into elite crop genetic backgrounds via marker assisted breeding (MAB). It allows stacking of desirable traits into elite varieties to make them better adapted to climatic changes.

## **5.2 DNA sequencing and advent of genomics assisted breeding**

With plummeting cost and greater accessibility of high throughput genome sequencing technology, the breadth of genomic data is expanding rapidly. In order to capture diversity of specific gene families within a large group, DNA samples can preferentially be enriched before sequencing. This approach can be adopted to define genetic variation in disease resistance gene repositories in Solanaceae and Triticeae (RNA seq) [65] and gluten gene families I bread wheat (GlutEn Seq) [66].

Sanger sequencing to study plant genomes is unfeasible due to low throughput and high sequencing costs. In 2005, Roche released its revolutionary 454 pyrosequencing platform [67]. Subsequently, several sequencing platforms such as developed by Illumina, ABI, Life technologies, PacBio, Oxford Nanopore and Complete genomics were released commercially, changing the scenario of genome sequencing. Depending on chemistry, second generation sequencing (SGS) approaches are classified as ligation based approaches and synthesis based approaches [68]. To rectify the problems of assembling repetitive genomic regions, long read sequencing offers solution by producing reads spanning the repeat regions [69].

Rapid cost reduction in genome wide genotyping allows large scale assessment of crop species diversity to capture climate related traits. It leverages cheaper sequencing to identify up to millions of SNPs in plant population [70]. High SNP density approach like whole genome resequencing (WGR) & low SNP density approach like reduced representation sequencing (RRS) are majorly used approaches. However, high density genotyping assay “SNP chips” enable large scale genotyping using SNP specific oligonucleotide probes rather than direct sequencing.

The variants identified by genotyping by sequencing (GBS) can be used for conventional QTL analysis and modern approach like genome wide association studies (GWAS). GWAS exploits the past recombinations in a diverse association panels to identify genes lined to phenotypic traits [71]. SNP genotyping have been widely used in many crops including wheat [72] and Maize [73]. Extensive use of GWAS is resulting in our enhanced understanding of genetics of important climate specific traits viz. drought and heat tolerance. In light of reducing sequencing cost and expensive validation of candidate genes, use of WGR to further enhance resolution of mapping studies is likely to become routine task in future [70].

The availability of reference genome assembly rewards us with information about gene content, ability to associate the traits with specific genes with subsequent insights into related biophysical and biochemical roles of gene(s) in the expression of that particular trait [74]. Resequencing of diverse crop cultivars reveals the gene content variation and DNA sequence differences between allelic variants, while sequencing of expressed gene products provides information on where and when genes are functioning. Such information when integrated within breeding pipelines, offers promise to accelerate the development of climate smart crop varieties.

The recent explosion in genomic data is rapidly triggering a fundamental shift to genomic based breeding [75]. The ability to identify and genotype umpteen SNPs at ever reducing costs facilitated expansion of MAS in breeding to plethora of traits and across wider range of crops [76]. A major outcome of availability of high throughput genome wide markers is a move towards population based trait association and breeding i.e. NAM or MAGIC populations to ultimately enhance the trait mapping resolution by greatly increasing the number of recombinations in the population. After identification and validation of the candidate genes, there achieved the deeper understanding of biological mechanism underlying the trait, which can subsequently be improved through MAB or genetic alterations. Furthermore, precise understanding of the molecular basis of traits enables the engineering of novel alleles or mining of potentially desirable alleles from CWR, facilitating further enhancement of the trait.

## 6. Genome editing: a revolutionary tool in breeders' toolkit

Genome editing has enabled breeders to precisely add or delete any DNA sequence in the genome and has shown enormous potential to revolutionize the crop improvement in this very decade [70, 77]. Some approaches like transcription activator-like effector nucleases (TALENs) and zinc finger nucleases (ZFNs) have been in the game for more than 2 decades. However, type II clustered regularly interspaced short palindromic repeat (CRISPR)/CRISPR-associated protein (Cas) system from *Streptococcus pyogenes* [78] developed in last decade has been most versatile tool in breeder's toolkit to introduce desirable or novel traits and accelerate development of climate smart crop varieties.

Usually, a custom-made guide RNA (gRNA) along with Cas9 nuclease is delivered into plant protoplast, where Cas9 produces double strand break (DSB) 3 bp upstream of the NGG motif (protospacer adjacent motif-PAM sequence) [78]. Cellular repair machinery through non-homologous end joining (NHEJ) can lead to frameshift mutation causing a knock-out. Otherwise, a donor DNA template can be provided for precise genetic knock-in through homologous recombination (HR). CRISPR/Cas9 was initially used to disrupt genes related to disease susceptibility in crops such as OsERF922 gene disruption in rice for blast resistance [79] and loss of function in susceptibility gene TaMLO for powdery mildew resistance in wheat [80]. Genome editing has also been used to tackle some abiotic stresses in staple crops like a promoter of a gene AGROS8 was replaced with a stronger one to impart drought tolerance in maize [81].

Due to changing climates, it may be quite beneficial for the farmers to have early maturing varieties, which enables plants to complete crucial developmental periods before the onset of a stress. It has been achieved by disrupting a flower repressing gene SP5G to develop early maturing tomato varieties [82]. For instance, developing climate rice to grow in diverse climates, generally desirable traits are cold, heat and drought tolerance at seedling and reproductive stages [83]. Secondary characters like root and flag leaf traits can be useful to generate cultivars with improved drought and heat tolerance [84]. Here, CRISPR tools could prove to be of great value for exploration of the candidate genes from CWR (*O. officinalis*, *O. nivara* and *O. glaberrima*) for abiotic stress resistance [85].

Genome editing has also huge potential to accelerate the domestication of novel crops from CWR or minor crops with valuable traits for coping with extreme climatic events. This would allow the editing of key genes for domestication in potential new crops for rapid enhancement of currently limited gene pools to maximize the use of germplasm adapted to climate change. Also, multiplexing of CRISPR

systems for simultaneous editing of multiple genetic loci can boost the speed and efficiency manifolds. However, there are a number of shortcomings in this approach including off target effects [86], low efficiency of HR, restrictive PAM sequences and regulatory concerns, which paved the way for advent of more sophisticated technologies like DNA free genome editing, base editing and prime editing.

### **6.1 DNA free genome editing (DFGE)**

Conventional genome editing using recombinant DNA (rDNA) leads to random host genome integration and can generate undesirable genetic changes or DNA damage [87], along with concerns over genetically modified organism (GMO) regulations with introduction of foreign DNA [88]. DFGE takes care of such critical issues along with reduced risk of off-targets. Initially, it was successfully deployed in rice and tobacco with transfection of protoplast with CRISPR-Cas9 ribonucleo-protein (RNP) [89]. Also, a particle bombardment mediated DFGE approach has been developed in wheat and maize [90, 91].

### **6.2 Base editing**

It is evident that a single base change can cause variation in the elite traits [92], so there required an efficient technique to cause precise and efficient point mutations in plants. CRISPR-Cas9 driven base editing is new approach which accurately transform one DNA base to another without repair template [93]. E.g. Cytidine deaminases convert cytosine (C) to uracil (U), which is treated as thymine (T) in subsequent DNA repair and replication, thus creating C•G to T•A substitution. It has been utilized in wheat, maize and tomato [94] and can be quite useful for gene functional analysis and therefore can assist breeding for better stress adapted varieties.

### **6.3 Prime editing**

Another latest milestone in this genome engineering era called prime editing allows introduction of all known 12 base to base conversions in addition to mutations such as insertions and deletions using prime editing guide RNA (pegRNA) [95]. This promising approach opening up numerous possibilities for effectively targeting and modifying desirable genome sequences to accelerate functional genomics and introduction of genes for adaptation to diverse climates can boost breeding for climate smart crop varieties in near future [96].

In this rejuvenated plant mutagenesis breeding era, genome editing can be used in functional genomics for the identification of candidate genes for climate related agronomic, physiological and phenological traits, which can be exploited for crop improvement in adaptation to changing climate. Despite having enormous potential and real world applications of genome editing technologies, the regulatory and ethical concerns may limit it, as happened in a few European countries. In the nutshell, genome editing in complementation with conventional plant breeding can be adopted to develop and deploy climate smart crop varieties in the farmers' fields.

## **7. Phenomics and artificial intelligence (AI): supplementing the genetic gains**

Advances in phenomics and genomics have generated unprecedented amount of new data, enabling breeders to continuously pushing the crop yields on positive side [97]. Despite success in techniques like genomic selection (GS) in cereals and

legumes, lack of predictive accuracy for many complex traits (yield) have revealed their inability to adequately model all relevant factors inherent to such traits due to complexity of the interactions between genetic and environmental components of phenotypic variation [98]. Several mapping studies have shown that such complex traits are controlled by minor genes (polygenes) with small but cumulative effect, hence go undetected while analyzing them in smaller population size.

Relationship between genotype and phenotype is not always linear and small changes on one hierarchical level may have bigger impact on other levels. Many statistical models therefore fail to accurately delineate the non-linear relationships. Additionally, epistatic interactions are hard to detect while mapping genotype to phenotype with linear models due to low power and sheer computational demand [99]. With continuously falling cost of genome sequencing, advent of innovative genetic assays to explore missing heritability and genetic regulation, breeders have access to wide range of high-throughput sensors and imaging techniques for spectrum of traits and field conditions.

Omics technologies (genomics, transcriptomics, proteomics, metabolomics, phenomics, epigenomics and microbiomics) together with approaches to gather information about climate and field environment conditions have become routine in breeding programs now a days. However, ability to accurately predict & select best lines for the specific environment relies on our ability to model these immensely complex systems from web of genomic and phenomic data at hand e.g. multiomics big data. Integrating with phenomics and genomics, AI technologies by assisting with big data, can boost up the development of climate resilient crop varieties with enhanced yield potential and stability and improved tolerance to expected simultaneous environmental stresses (abiotic and biotic).

## **7.1 Field phenomics**

Accelerated plant breeding for climate resilience is critically dependent upon high resolution, high throughput, field level phenotyping that can effectively screen among better performing breeding lines within larger population across multiple environments [100]. With advent of novel sensors (unmanned air vehicle-UAV), high resolution imagery and new platforms for wide range of traits and conditions, phenomics has been elevating the collection of more phenotypic data over the past decade [101, 102]. High throughput phenotyping (HTP) allows the screening for plant architectural traits and early detection of desirable genotypes. It enables accurate, automated and repeatable measurements for agronomic traits (seedling vigor, flowering time, flower counts, biomass and grain yield, height and leaf erectness, canopy structure) as well as physiological traits (photosynthesis, disease and stress tolerance). HTP methods such as RGB imaging, 3-D scanning, thermal and hyper spectral sensing and fluorescence imaging have been successfully utilized to identify, quantify and monitor plant diseases [103].

By coupling GWAS with high throughput phenotyping facilities, phenomics can be adopted as novel tool for studying plant genetics and genomic characterization enhancing the crop breeding efficiency in era of climate change [104]. Recently, deep learning (DL) has been extensively used to analyze and interpret more phenomic big data, especially for advancing plant image analysis and environmental stress phenotyping [105].

## **7.2 Next gen based GS**

Genomic selection as been extensively used breeding approach for climate resilience in agriculture in last decade, especially for complex polygenic traits. It

involves prediction models developed by estimating the combined effect of all existing markers simultaneously on a desirable phenotype. Highly accurate prediction can result into enhanced levels of yields by shortening the breeding cycles. Omics layers (gene expression, metabolite concentration and epistatic signals) can be better predictors of phenotype than SNPs alone due to their molecular proximity to the phenotype. Many such omics layers that explain trait variation have not been made available to the statistical models lowering down its efficacy. Several approaches such as mixed effect linear models and Bayesian models to select only most important predictive SNPs are majorly used.

From the prospective of breeding, by accessing the rich set of omics and environmental data lying between plant genotype and its phenotype, superior and refined impact can be achieved on desirable phenotype. Next gen AI holds promise for GS as acquisition of large scale genomics and phenomics data in addition to molecular layers between them such as transcriptomics, proteomics and epigenomics will facilitate a period, where AI models can identify and explain the complex biological interactions [99].

Next gen AI will surely require knowledge and rationality of breeders as well as farmers to evaluate the efficacy of outcomes. In coming times, agriculture will rely on Next Gen AI methods for making decisions and recommendations from big data (highly heterogeneous and complex) that are representative of environment and system biology based understanding of the behavioral response of plants.

## 8. Speed breeding: an acceleration to crop improvement

The current pace of yield increase in staple crops like wheat, rice and maize is insufficient to meet the future demand in the wake of climate change [106]. A major limiting factor in plant breeding is the longer generation times of the crops, typically allowing 1–2 generations in a year. Several ‘speeding breeding’ protocols, using extended photoperiods and controlled temperatures have enabled breeders to harvest up to 6 generations per year by reducing the generation time by more than half [107]. Such protocols have been reported in several important crops such as spring wheat (*Triticum aestivum*) [108], barley (*Hordeum vulgare*) [109], chickpea (*Cicer arietinum*), rice (*Oryza sativa*) [110] and canola (*Brassica napus*).

Speed breeding can potentially accelerate the discovery and use of allelic diversity in landraces as well as in CWR to be further used in developing climate resilient crop varieties. One such example is recent discovery of new sources of leaf rust resistance after screening of the Vavilov wheat collection using speed breeding along with gene specific molecular markers [111].

Interestingly, speed breeding can also be integrated with advanced technique like gene editing to precisely alter the plant genes for better coping with various biotic and abiotic stresses in threatening climatic changes. In traditional CRISPR gene editing, the sgRNA directs Cas9 enzymes to cut target sequence. ‘CRISPR-ready’ genotypes containing heterologous Cas9 gene can be created. For instance, a transformant harboring a Cas9 transgene can be used a donor to create a stock of elite inbred lines using speed marker-assisted backcrossing. Such an integrated system like ExpressEdit could circumvent the bottlenecks of in vitro manipulation of plant materials also making gene editing fast-tracking [1]. Integration of both the techniques without tissue culture/foreign DNA requires handful of technological breakthroughs with the desirable outcomes being allelic modification, these would

bypass genetically modified organism (GMO) label. It has been widely reported that single or multiplex edits can be obtained [112] and could be implemented with some tissue culture free techniques like CRISPR-Cas9 ribonucleoprotein (RNP) complexes in wheat [91] and maize [90].

Crop species	Target trait/Improved trait	Technology/Technique used	Reference
Rice	Submergence tolerance	MAB	[116]
Rice	Grain number, dense erect panicles and larger grain size	CRISPR/Cas9	[117]
Rice	Maintenance of heterosis	CRISPR/Cas9	[118, 119]
Wheat	Heat tolerance	GWAS	[120]
Wheat	Leaf rust, fusarium head blight and stripe rust resistance	Speed breeding	[121–124]
Wheat	Powdery mildew-resistant	CRISPR/Cas9	[80]
Finger millet	Salt tolerance	RNA sequencing	[125]
Sorghum	Low and high nitrogen conditions	RNA sequencing	[126]
Sugarcane	Drought and chilling resistance	CRISPR/Cas9	[127]
Maize	Kernel row number	RNA sequencing	[128]
Maize	High amylopectin content	CRISPR/Cas9	[129]
Cotton	Salt and drought tolerance	GWAS	[130]
Soybean	Salt and drought tolerance	CRISPR/Cas9	[131, 132]
Soybean	Salt tolerance	RNA sequencing	[133]
Chickpea	Drought, salinity, cold and heavy metal stress resistance	RNA sequencing	[134]
Lentil	Seedling drought stress resistance	RNA sequencing	[135]
Tomato	High temperature stress responsiveness	GWAS	[136]
Tomato	Powdery mildew-resistant	CRISPR/Cas9	[137]
Tomato	Longer internodes and lighter green leaves with smoother margins	TALEN	[138]
Tomato	Short (hairy) roots with stunted meristematic, altered branching and increased yield	CRISPR/Cas9	[139, 140]
Tomato	Fruits never turn red, altered firmness	CRISPR/Cas9	[141]
Broccoli	Dwarf phenotype	CRISPR/Cas9	[142]
Watermelon	Albino phenotype	CRISPR/Cas9	[143]
Potato	Reduced steroidal glycoalkaloids in leaves and Undetectable level of reducing sugar in tubers	TALEN	[144, 145]
Mushroom	Reduced browning	CRISPR/Cas9	[146]
Banana	Cold and salt resistance	CRISPR/Cas9	[147]
Coconut	Root wilt disease	CRISPR/Cas9	[148]
Papaya	Drought, heat and cold resistance	CRISPR/Cas9	[149]
Apple	Albino phenotype and Blight resistance	CRISPR/Cas9	[150, 151]

**Table 1.**  
 Utilization of smart breeding tools and techniques for crop improvement.

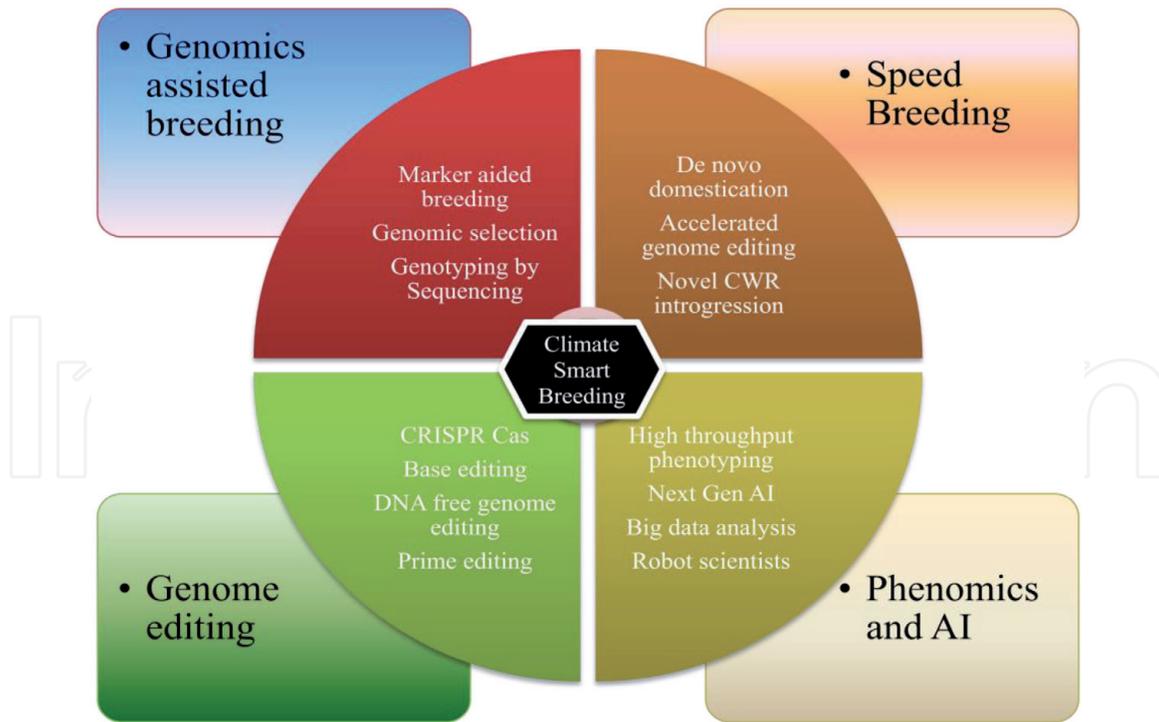
Genomic selection (GS) unlike MAS uses genome-wide DNA markers in order to predict the genetic gain of breeding individuals for complex traits such as yield [113]. The effect of large number of genetic variants for such a complex traits is captured through linkage disequilibrium (LD) with the genome-wide markers (SNPs), effects of which are determined in large training populations (lines in which marker genotype and trait are measured). Since speed breeding can substantially lowers down the generation periods, it can maximize the benefits by applying genomic selection at every generation to select parents for next generation. Modern genotyping techniques such as rAmpSeq may considerably reduce the genotyping cost for genomic selection [114]. When combined with speed breeding protocol, the approach for stacking of best haplotypes (ones with desirable resistance alleles/desirable edits) could be used rapidly to develop new cultivars [1] with improved performance across multiple traits like coping with adverse climatic variations or any pathogen/insect attack.

Re-domestication of crop plants for capturing the desirable alleles for climate resilience can be sped up by linking it with speed breeding. Re-creation of the polyploids such as groundnut (*Arachis hypogea*) and banana (*Musa spp.*) can be benefitted by such approach. Speed breeding could accelerate re-domestication at multiple selection steps after crossing of diploids followed by colchicine application [115]. Ultimately, it will provide access to novel plant traits for developing cultivars of these crops exhibiting disease resistance and stress adaptation. Also, Gene editing and targeted mutagenesis coupled with speed breeding could prove to be more efficient to create healthier foods by biofortification. For instance, the increased content of vitamin B9 in rice and antinutritional glucosinolates from *Brassica* seeds etc. [1].

Combining all these tools with speed breeding approach would provide rapid access to desirable alleles and novel variation present in CWR and would accelerate the breeding pipelines to develop more climate resilient varieties (**Table 1**).

## 9. Future prospects

In the face of ongoing and projected climate change, including higher temperatures and more erratic climate events across extensive regions over the globe, breeding of crop plants with enhanced yield potential and improved resilience to such environments is crucial for global food security. Improved plant varieties that can withstand diseases and pests with efficient use of fewer resources, exhibiting stable yields amidst stressful climate in near future could only help to achieve the goal of climate resilient agriculture. In order to be able to make contribution in climatic resilience, research attention is indispensable for currently underutilized crop species. The concept of smart breeding largely depends upon generating large breeding populations, efficient high throughput phenotyping, big data management tools and downstream molecular techniques to tackle the vulnerability of crop plants to changing climate (**Figure 3**). The efficient preservation and conservation of plant genetic resources is also a pre requisite for climate smart breeding. Strategies for capturing the novel variation may include the state of the art tools such as gene editing to directly introduce novel alleles found in wild plants into domesticated crop varieties. Generating new crop cultivars with the capability to tolerate multiple stresses can be achieved with increasing information on their basal physiological and genetic mechanisms. The technological improvements in phenotypic and genotypic analysis, as well as the biotechnological and digital revolution could definitely pave the way for developing and deployment of climate smart varieties in coming times.



**Figure 3.**  
*Compilation of state-of-the-art genomic, phenomic and computational tools comprising smart breeding approach for climatic resilience in agriculture.*

### Conflict of interest

The authors declare they have no conflict of interest.

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