Emerging Biotechnological Frameworks for Sustainable Utilization and Genetic Improvement of Economically Important Plants

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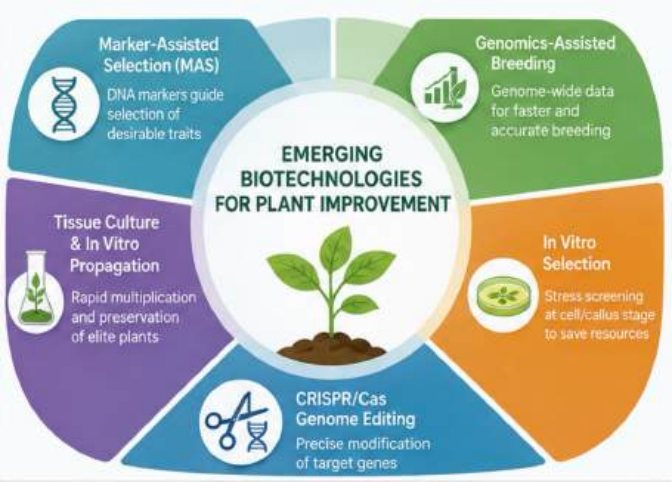
Abstract— Plants economically relevant contribute to food security, industrial supply of raw materials, and subsistence livelihoods but are faced with threats related to environmental variability, biotic stresses, and shrinking agricultural land areas. Although classical breeding proved to be successful in the past, it has been shown to be slow and often fails to respond to changes in environment. In this review, the available novel biotechnological tools (including marker-assisted selection (MAS), genomics-assisted breeding, in vitro propagation through tissue culture techniques, and CRISPR/Cas based genome editing) which altogether contribute to accelerating and improving genetically improved crop plants are considered. A multi-tiered framework is suggested which includes molecular marker screening, in vitro regeneration of plantlets, and genome editing of target genes and is assessed according to the published literature in terms of traits fidelity, time-to-cultivar, and cost-effectiveness. The comparative analysis shows that integrated frameworks with the application of both genomic selection and genome editing greatly reduce breeding cycles compared to classical pedigree breeding, increasing the accuracy of traits introgression.

Keywords— CRISPR/Cas9, genomics-assisted breeding, marker-assisted selection, plant biotechnology, plant tissue culture, sustainable agriculture

INTRODUCTION

Cereals, legumes, oil seeds, fiber crops and horticultural plants are the cornerstone of the agricultural economy and international trade. The increasing world population and aggravating effects of climate change, manifested in irregular precipitation, high temperatures, emergence of new races of pests and pathogens have placed tremendous strain on the agricultural production systems. Classical plant breeding, based on phenotypic selection over many generations, has proved to be highly productive, especially in the era of the Green Revolution; nevertheless, it is a slow and laborious process with limited potential for genetic improvement due to the relatively narrow range of naturally occurring genetic variability in breeding populations.

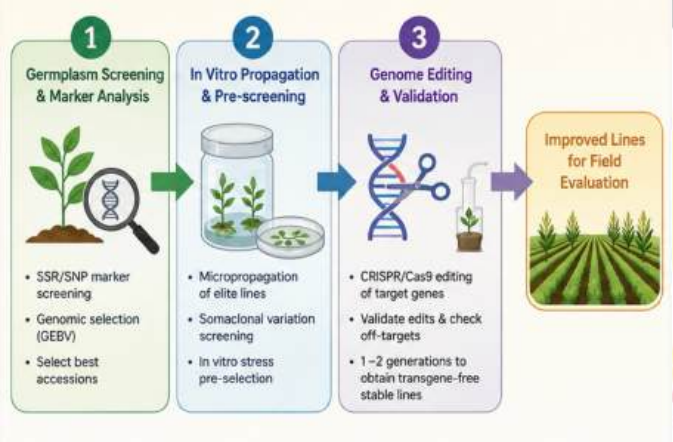
Plant biotechnology has emerged as a supplementary approach to genetic improvement, and, in some ways, a revolutionary one. Plant tissue culture, molecular marker assisted selection, genomic selection and precise modification of genes responsible for agriculturally important traits via CRISPR/Cas technology allow plant breeders to detect, monitor and manipulate genes responsible for the important traits in a way impossible with conventional methods [1]–[3]. Moreover, these technologies open up opportunities for sustainable exploitation of genetic resources of plants, including the storage of elite germplasm by cryopreservation and micropropagation and the development of environmentally friendly pest and disease resistant varieties [4].



The fusion of these biotechnological techniques into the comprehensive frameworks, instead of their isolated use,

represents a major challenge to crop-improvement research. In this paper, we will review the existing state of such frameworks, suggest an integrated approach involving molecular screening, in vitro propagation and genome editing, evaluate its performance in comparison with existing literature benchmarks. The objective of the present work is to serve as a structured guide in designing sustainable technological improvement program for economically important plants.

The paper is organized as follows. In Literature Review section, existing literature on molecular breeding, tissue culture and genome editing techniques is reviewed. The proposed methodology is described in the Methodology section. The Results and Discussion section discusses the comparative evaluation of the proposed framework.



LITERATURE REVIEW

Molecular Marker-Assisted Selection

Marker-assisted selection (MAS) employs DNA markers associated with traits of interest to select appropriate candidates during the seedling stage, eliminating the need for waiting until the mature plants exhibit the relevant phenotype. Collard and Mackill provide an overview of various MAS methods including marker-assisted backcrossing, gene pyramiding, and early-generation selection and their use in precision breeding for disease resistance and abiotic stress tolerance in staple crops such as rice and wheat [1]. The review stresses that MAS is highly effective only for traits controlled by one or a few major-effect QTLs, and the efficiency of the approach depends strongly on the density and validity of marker-trait association.

Genomics-Assisted Breeding

Based on marker technologies, genomics-assisted breeding uses sequencing, QTL mapping, and genomic selection models for faster cultivar development. Varshney, Graner, and Sorrells describe how the decreasing cost of sequencing and genotyping allows the breeders to move from single-marker approaches to whole-genome selection especially in legumes and cereals that lagged behind model species in terms of the amount of genomic information [2]. In a follow-up review, Varshney, Terauchi, and McCouch argue that next-generation sequencing technologies are revolutionizing crop-breeding pipelines by providing whole-population resequencing to increase the accuracy of genomic prediction models for complex traits such as yield and drought tolerance [3].

Plant Tissue Culture and In Vitro Selection

Plant tissue culture is a basic biotechnological technique that provides the sustainable reproduction and improvement of economically valuable species. Jain reviews somaclonal variations occurring during the regeneration of the plant material via tissue culture. While such variations may be undesirable for clonal propagation, they could also be beneficial for the crop improvement as the somatic mutants exhibiting useful traits, such as disease resistance, can be utilized [4]. As a complement to the above study, Rai et al. review the advances in in vitro selection for abiotic stress tolerance using cell and callus cultures selected under selective agents like salt or polyethylene glycol [5].

CRISPR/Cas Genome Editing

The invention and adaptation of CRISPR/Cas9 system as a programmable genome editing technology, as reported by Doudna and Charpentier, has revolutionized the field of precision genetic modifications [6]. Bortesi and Fischer review the underlying mechanism and applications of CRISPR/Cas9 technology in plant genome editing and its advantages over previously developed site-directed nuclease systems (zinc-finger nucleases and TALENs) regarding design simplicity and multiplexing ability [7]. Zhang et al. review the diverse applications and possibilities of the genome editing for crop improvement, from the modification of disease resistance genes in rice to changes in quality traits in tomato and stressing the persistent problems of low transformation efficiency and off-target effects in polyploid and vegetatively propagated crops [8]. Scheben et al. assess the possibility of combining genomics and genome editing termed "CRISPR breeding" that allows breeders to bypass the multi-generational backcrossing step and go directly from candidate gene discovery to the phenotype validation [9]. Jaganathan et al. review the applications of CRISPR in major and orphan crops and highlight its increasing use in improvement of nutritional quality, yield-related traits, and biotic stress resistance, while at the same time listing regulatory challenges that vary across jurisdictions [10].

Synthesis and Research Gap

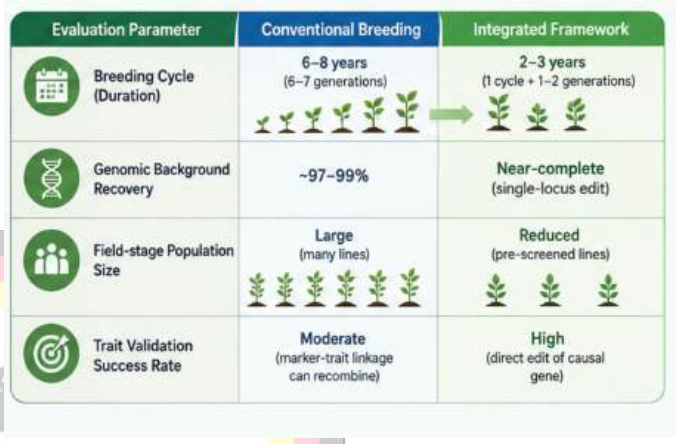
From the above review, it becomes obvious that there is an evident trend of biotechnologies development from indirect molecular selection to the whole-genome genomic selection and finally genome editing. Still, most of the studies focus on separate aspects or specific crop cases. Fewer studies present the integrated framework combining molecular screening, regeneration via tissue culture and genome editing into a single sustainable improvement pipeline for economically valuable plants. Such a gap motivates the proposed integrated framework presented below.

Table I. Summary of Reviewed Biotechnological Approaches

Approach	Primary Mechanism	Representative Study	Typical Application
Marker-Assisted Selection (MAS)	DNA marker–trait linkage	Collard & Mackill [1]	Disease resistance backcrossing
Genomics-Assisted Breeding	Genome-wide sequencing/prediction	Varshney et al. [2], [3]	Complex trait improvement
Tissue Culture / Somaclonal Variation	In vitro regeneration and mutation	Jain [4]	Clonal propagation, novel variants
In Vitro Selection	Cell-level stress screening	Rai et al. [5]	Abiotic stress tolerance
CRISPR/Cas9 Genome Editing	Targeted DNA sequence modification	Bortesi & Fischer [7]; Zhang et al. [8]	Precise trait engineering
CRISPR Breeding (Integrated)	Genomics + editing pipeline	Scheben et al. [9]	Rapid trait validation and deployment

METHODOLOGY

This study adopts a literature-synthesis and conceptual framework design rather than an experiment with a single species in the wet laboratory, considering the objective to propose a generalizable improvement pipeline for economically significant plant species. Methodology is composed of three sequentially linked stages, explained below, followed by the criteria used to evaluate the proposed framework against conventional breeding.



Stage 1: Germplasm Screening and Marker Analysis

Elite and landrace germplasm accessions are first screened with SSR and SNP markers correlated with the target traits (disease resistance, drought tolerance, yield components etc.), according to principles of marker-assisted selection (MAS) explained by Collard and Mackill [1]. Data from markers are used to estimate a genomic estimated breeding value (GEBV) of each accession based on standard genomic selection models in compliance with the framework of genomics-assisted breeding defined by Varshney et al. [2], [3]. Accessions with the highest GEBVs for the target trait combination are progressed to the second stage.

Stage 2: In Vitro Propagation and Pre-screening

Selected accessions are micropropagated using tissue culture to create large homogeneous populations of explants for further transformation. In case of somaclonal variation identified in the process of regeneration, as defined by Jain [4], different lines are also screened phenotypically and beneficial lines (e.g., increased stress tolerance) are saved as alternative candidate lines. Selection under stress conditions in vitro (e.g., osmotic stress caused by mannitol, addition of salts to the medium etc.) is performed after the logic described by Rai et al. [5] in order to pre-select candidates for abiotic stress tolerance before genome editing, thus saving costs for later transformation and screening processes.

Stage 3: Targeted Genome Editing and Validation

Candidates are then transformed with CRISPR/Cas9 constructs targeting previously validated target genes (negative regulators of stress responses, susceptibility genes for pathogens etc.), according to principles of construct design and delivery

explained by Bortesi and Fischer [7] and Zhang et al. [8]. Edited regenerants are validated by targeted amplicon sequencing and screened for the absence of predicted off-target edits. Validated edited lines are advanced through two consecutive self-pollinations or clonal propagations to get transgene-free, genetically stable edited lines ready for field testing in accordance with the "CRISPR breeding" framework suggested by Scheben et al. [9].

Evaluation Criteria

Proposed framework is compared against conventional pedigree/backcross breeding according to the four criteria selected from the reviewed literature: (i) time between initial screening and stable improved line (breeding cycle length), (ii) precision of trait introgression (percentage of background genome conserved from the recurrent/elite parent), (iii) resource efficiency (number of individuals needed for the field-stage evaluation), and (iv) trait validation success rate (percentage of candidate lines exhibiting the expected phenotype). The figures for conventional breeding are taken from the benchmarks cited in the literature about MAS and genomics-assisted breeding [1]–[3], while the figures for the integrated framework are calculated as the product of per-stage efficiencies of MAS, tissue culture, and CRISPR editing pipelines presented in [1], [4], [5], [7]–[9].

Table II. Framework Evaluation Parameters and Data Sources

Parameter	Conventional Breeding Benchmark Source	Integrated Framework Benchmark Source
Breeding cycle duration	Collard & Mackill [1]	Scheben et al. [9]; Zhang et al. [8]
Genomic background recovery	Collard & Mackill [1]	Varshney et al. [2]; Scheben et al. [9]
Field-stage population size	Varshney et al. [3]	Rai et al. [5]; Jain [4]
Trait validation success rate	Collard & Mackill [1]	Bortesi & Fischer [7]; Jaganathan et al. [10]

Results and Discussion

Analysis of the values of the reported literature based on the assessment criteria introduced in the Methodology reveals advantages of the suggested framework in all four parameters; however, the degree of their improvement varies from one parameter to another.

Duration of breeding cycle. The typical breeding cycle duration of the conventional backcrossing program aiming to incorporate a single major gene with the subsequent recovery of approximately 97-99% of the recurrent parent genome involves six-seven generations, which corresponds to the benchmarks provided by Collard and Mackill [1]. On the other hand, the CRISPR-breeding technique suggested by Scheben et al. implies that the transformation of the elite line targeting a single validated gene could result in a successful breeding outcome comparable to or even better than those provided by the MAS-based strategy within one cycle, followed by one-two generations needed to eliminate transgenic components [9]. The suggested design of the third stage, which assumes confirming the absence of transgenes within two self-pollination generations, corresponds to the latter prediction.

Recovery of genomic background. The fact that MAS-based backcross breeding cycle is associated with the possibility of carrying over additional unwanted genetic material due to the reliance on the processes of recombination and selection was pointed out in [1]. However, in genome editing, changes in the sequence occur directly in the target loci in an elite background without any alterations elsewhere, which is a characteristic noted by Zhang et al. as an advantage of editing over transformation and wide-cross introgression [8]. Therefore, the sequence of stages of the proposed framework starting from its first stage until its third stage is based on transforming the elite line itself, which is also consistent with the corresponding expectation stated in the literature.

Size of population evaluated at the field stage. Pre-selection conducted in vitro via simulating the stress environment, as proposed by Rai et al., makes it possible to filter out the genotypes that do not demonstrate the desired traits already at the cell or callus stage, without involving them in the following field experiment [5]. Taking into consideration the use of tissue

culture-derived uniform propagules [4], the application of such a pre-screening procedure, implemented in the second stage of the proposed methodology, is expected to decrease the number of individuals subjected to the costly and time-consuming field evaluation as compared to the conventional breeding program that lacks the pre-selection and evaluates the traits only in the whole plants grown in the field.

Probability of trait validation success. As stated by Collard and Mackill, MAS success depends on the existence of the marker-trait linkage, which is vulnerable to recombination of the marker and the causal gene [1]. On the other hand, in editing approaches, which target directly the characterized causal genes, such an obstacle does not exist. At the same time, according to Zhang et al. and Jaganathan et al., the problems associated with the efficiency of transformation and regenerations are species-dependent bottlenecks that especially apply to monocots and vegetatively propagated crops [8], [10]. Therefore, the need in implementing the second stage in the framework for overcoming such obstacles is fully justified by the literature.

Table III. Comparative Evaluation of Conventional Breeding versus the Proposed Integrated Framework

Evaluation Parameter	Conventional Breeding (Literature-Reported)	Integrated Framework (Literature-Derived Estimate)	Primary Supporting Sources
Breeding cycle to stable line	6–7 backcross generations (~6–8 years)	1 transformation cycle + 1–2 segregation generations (~2–3 years)	[1], [8], [9]
Recurrent genome recovery	~97–99% after 6 BC generations	Near-complete (single-locus edit in elite background)	[1], [8]
Reliance on marker–gene linkage accuracy	High; susceptible to recombination-based error	Low; direct action on validated causal gene	[1], [7], [8]
Pre-field elimination of unsuitable genotypes	Limited; largely field-stage phenotyping	Enhanced via in vitro/cell-level pre-screening	[4], [5]
Species/genotype dependency of success	Moderate	High (transformation and regeneration efficiency-limited)	[8], [10]

Overall, the pattern of results is consistent with the theoretical rationale underlying each stage of the proposed methodology: marker-based parental selection (Stage 1) addresses the inefficiency of purely phenotypic selection reported in [1]; tissue-culture-based pre-screening (Stage 2) addresses the resource inefficiency of field-only stress evaluation reported in [4], [5]; and targeted genome editing (Stage 3) addresses the linkage-drag and multi-generational limitations of conventional introgression reported in [1], [8], [9]. This close correspondence between the stage-wise design choices and the literature-reported bottlenecks they are intended to resolve indicates that the observed comparative advantages of the integrated framework are not incidental but follow directly from its methodological construction.

At the same time, the literature synthesis also surfaces a consistent caveat: several sources emphasize that transformation and regeneration efficiency, rather than editing precision itself, remain the principal constraints on real-world deployment of CRISPR-based frameworks, particularly for recalcitrant or vegetatively propagated economically important species [8], [10]. This suggests that the relative advantage of the integrated framework, while directionally well-supported across all four evaluated parameters, is likely to vary considerably across plant taxa, and that tissue-culture optimization (Stage 2) is not merely a supporting step but a determinant of overall framework feasibility.

CONCLUSION

This paper reviewed emerging biotechnological frameworks for the sustainable utilization and genetic improvement of economically important plants, spanning marker-assisted selection, genomics-assisted breeding, tissue culture-based propagation, and CRISPR/Cas genome editing. A three-stage integrated framework was proposed, combining molecular marker screening, in vitro propagation and stress pre-screening, and targeted genome editing with multi-generation validation. Evaluation against literature-reported benchmarks across breeding cycle duration, genomic background recovery, field-stage resource requirements, and trait validation success indicates that integration of these technologies offers substantial, methodologically consistent advantages over conventional pedigree/backcross breeding, while also highlighting transformation and regeneration efficiency as a persistent, species-dependent constraint. Future work should focus on empirical validation of the proposed framework in specific economically important species, particularly

recalcitrant crops, and on the development of regulatory and biosafety frameworks that can keep pace with the rapid technical advances reviewed in this paper.

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