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Review Paper

Leveraging Genome Editing to Revive Multi-Ear Traits for Climate Resilient Maize: Systematic Review

Abenezer Abebe Tefera*, Guta Tesema Debele

Ethiopian Institute of Agricultural Research, Holeta Agricultural Research Center, P.O. Box: 31, Holeta, Ethiopia

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Abstract

Maize is central to global food security because of its high yield potential and broad adaptability. However, modern breeding has unintentionally narrowed its genetic base by favoring a single-ear ideotype, thereby reducing resilience to climate stress and limiting yield stability under adverse conditions. As climate variability intensifies, the buffering capacity of multi-ear phenotypes is becoming increasingly important. This review synthesizes evidence demonstrating that reintroducing prolificacy is both promising and challenging, as the trait is polygenic and constrained by physiological trade-offs involving source-sink balance, ear initiation, and developmental synchrony. Recent advances in genome-editing technologies, including CRISPR-Cas systems, base editing, and prime editing, now allow precise modification of key regulators of ear architecture, such as tb1, fea2, RA1/RA2, and hormonal pathways controlling axillary meristem activity and ear number. Emerging insights into synchrony-related genes, including GIF1, RA2, and gibberellin-deactivation loci, further support targeted editing strategies to coordinate ear development and minimize yield penalties. By integrating these molecular tools, breeders can design multi-ear ideotypes that enhance yield stability and climatic adaptation. To accelerate progress, future research should prioritize the systematic identification and validation of promoters and cis-regulatory elements that fine-tune prolificacy pathways, alongside the development of high-throughput phenotyping platforms capable of capturing subtle variation in ear number, developmental synchrony, and resource allocation.

1. Introduction

Maize (*Zea Mays L.*) is valued for its genetic diversity and high yield potential and is a crucial cereal crop supporting global food security, animal feed, and numerous industrial applications (Reddy et al., 2019; Sneha et al., 2025). However, global climate change poses increasing challenges to maize production by affecting yield stability, crop phenology, and geographical suitability. Future resilience will require traits that buffer yield under stress, whereas conventional breeding has largely focused on maximizing productivity under optimal conditions.

The remarkable gains in maize productivity over the past century can be attributed to intensive breeding practices that systematically reshaped plant architecture, culminating in the widespread adoption of a single-ear ideotype (Li et al., 2021; Strable, 2021). Central to this paradigm was strong selection for a single dominant ear, achieved by suppressing axillary branching and reinforcing apical dominance through key regulators such as *teosinte branched1* (*tb1*) (Dong et al., 2019; Prakash et al., 2020). This emphasis on architectural

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*Corresponding author, e-mail: abentef2012@gmail.com

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uniformity and increased kernel number per ear has driven substantial yield improvements.

However, these advances have been accompanied by a significant reduction in functional genetic diversity, particularly for traits such as tillering and multi-ear formation (Adhikari et al., 2022; Chen et al., 2020; Samayoa et al., 2021). These traits, which characterize maize's wild progenitor *teosinte* and many traditional landraces, are increasingly recognized as valuable resources for enhancing crop resilience and yield stability (Yang et al., 2016). Under growing climate variability and resource constraints, the buffering capacity of multi-ear phenotypes is gaining importance, whereas reliance on a single-ear architecture may represent a vulnerability (Azeem et al., 2024; Noru et al., 2024). As noted by Sahoo et al. (2021), such underutilized genetic variation is critical for developing robust maize varieties. Prolificacy can function as a yield-stabilizing mechanism, whereby secondary ears partially compensate for failure of the primary ear, particularly in low-input and subsistence farming systems (Ross et al., 2020). Recent studies further argue that revisiting traits such as multi-ear formation within broader, multi-trait breeding frameworks could enhance breeding efficiency and resilience (Chang et al., 2024; Denic et al., 2007).

Despite its potential benefits, reintroducing prolificacy into elite, high-yielding maize germplasm has proven challenging. The polygenic nature of ear number, strong genotype-by-environment interactions, and inherent physiological trade-offs have constrained the effectiveness of conventional breeding approaches (Parco et al., 2022). Nevertheless, the emergence of precision genome-editing technologies, most notably CRISPR-Cas systems and their derivatives, including base and prime editing, has marked a paradigm shift in crop improvement (Hernandes-Lopes et al., 2023; Wang et al., 2022). These tools enable rapid and targeted genetic modifications, offering new opportunities to overcome barriers imposed by complex trait architecture and environmental variability.

A comprehensive assessment of the evolutionary, genetic, and physiological constraints associated with prolificacy is therefore essential to transform multi-ear traits from breeding challenges into assets for climate resilience. With genome editing serving as a precise and targeted tool, it may now be possible to reintroduce prolificacy into elite maize germplasm without compromising yield stability. Accordingly, the objectives of this review are to: (1) re-examine the evolutionary and breeding history underlying the dominance of the single-ear phenotype and its associated limitations; (2) integrate current knowledge of the genetic and physiological mechanisms controlling ear number, with emphasis on their relevance for restoring prolificacy; and (3) synthesize recent advances in genome-editing technologies and evaluate their potential to overcome long-standing barriers to manipulating multi-ear architecture in elite maize.

2. Materials and Methods

This review was conducted following a systematic literature review approach. A comprehensive and structured search was performed in Google Scholar and SciSpace to identify peer-reviewed studies related to maize prolificacy, ear development, physiological regulation, and recent advances in genome-editing technologies. The search strategy employed predefined combinations of keywords, including maize prolificacy, multi-ear, ear number, source–sink balance, hormonal regulation, genome editing, base editing, and prime editing.

Study selection was carried out in multiple stages. First, titles and abstracts were screened to exclude irrelevant or non-peer-reviewed publications. Subsequently, full-text articles were assessed for eligibility based on predefined inclusion criteria. Studies were included if they (i) focused on maize (*Zea mays L.*), (ii) addressed genetic, physiological, or developmental regulation of ear number or prolificacy, and/or (iii) examined genome-editing approaches relevant to modifying ear architecture or related traits. Articles that did not meet these criteria were excluded. The final set of selected studies was synthesized qualitatively to integrate evidence on the evolutionary background, genetic control, physiological trade-offs, and genome-editing strategies associated with maize prolificacy.

3. Evolutionary and Breeding Shift in Single-Ear Maize

The evolutionary transition from teosinte to modern maize represents one of the most striking examples of crop domestication. Teosinte, the wild progenitor of maize, produces numerous small ears on highly branched lateral shoots, an adaptation well suited to natural reproduction and survival (Iltis & Benz, 2000; Sahoo et al., 2021). Yang et al. (2016) demonstrated that prolificacy, defined here as the capacity to produce multiple ears per plant, confers reproductive success and survival advantages in teosinte through increased ear number.

Domestication, however, involved a major evolutionary trade-off, whereby traits promoting survival across heterogeneous environments were progressively reduced (Sahoo et al., 2021). Although the multi-ear phenotype enhances fitness in wild populations, early domesticators prioritized harvestability, uniformity, and ease of management over prolificacy. Consequently, selection favored genotypes with a single dominant ear, resulting in a fundamental shift in maize plant architecture. Studies by Dong et al. (2019), Gallagher et al. (2023a), and Prakash et al. (2020) showed that this transition toward a single-ear ideotype was driven by strong selection on key domestication genes, notably *teosinte branched1* (*tb1*) and *grassy tillers1* (*gt1*), which regulate axillary branching and apical dominance (Figure 1). This selection reduced ear number while increasing kernel productivity per ear, thereby facilitating domestication and the adoption of modern agricultural systems.

Subsequent research has revealed that tb1 and gt1 operate within a complex regulatory network integrating developmental and hormonal controls. Gaarslev et al. (2021) reported interactions between these loci and auxin and cytokinin signaling pathways, which regulate axillary bud activation and differentiation. While reduced ear number optimized grain yield under intensively managed conditions, it also diminished the adaptive plasticity characteristic of teosinte and many traditional landraces.

Importantly, the genetic potential for prolificacy has not been entirely lost across all maize germplasm. Al-Niemi et al. (2019) and Khatun et al. (2022) reported that certain landraces and open-pollinated varieties retain allelic variation influencing tillering, axillary bud growth, and inflorescence development. Notably, Prakash et al. (2024) and Kapoor et al. (2022) described the Sikkim Primitive landrace from the northeastern Himalayas of India as highly prolific, producing up to seven to nine ears per plant. This landrace represents a valuable genetic resource, harboring alleles for multi-ear development that are largely absent from elite modern germplasm. However, effective utilization of this variation requires advanced genomic approaches to mitigate linkage drag and address the polygenic architecture of prolificacy while maintaining agronomic performance (Kapoor et al., 2022).

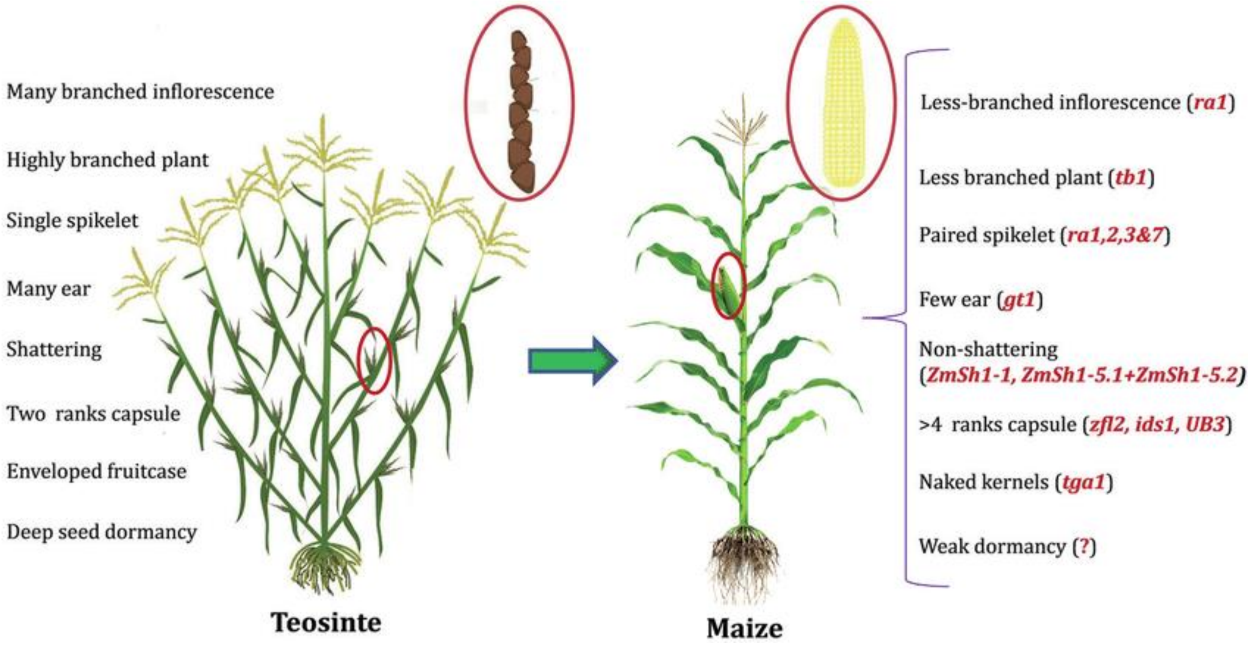

Figure 1: Morphological change during maize domestication and the underlying key genes (Source: Sharma et al., 2021)

Prakash et al. (2021) identified a quantitative trait locus, qProl-SP-8.05, and six candidate genes associated with prolificacy, proposing their introgression into elite maize via marker-assisted selection. Similarly, Babb and Muehlbauer (2003) and Winkelmolen et al. (2024) highlighted allelic variants associated with axillary branching and inflorescence architecture that could be incorporated into breeding programs targeting resilience or niche production systems. Nevertheless, reintroducing prolificacy into elite germplasm through conventional breeding remains challenging due to its polygenic basis, linkage drag, and the narrow genetic base of modern maize (Sanchez et al., 2024). Recent studies suggest that marker-assisted selection and genomic selection can help overcome these limitations and accelerate the integration of prolificacy into elite backgrounds (Kumar et al., 2024; Verma et al., 2024).

Numerous studies have confirmed the potential for enhanced productivity through increased prolificacy (Khatefov et al., 2019; Parco et al., 2022; Ross et al., 2020), and genomic analyses have identified quantitative trait loci, such as qNE7, that regulate ear number and placement (Wang et al., 2023a). However, stable expression of prolificacy remains constrained by physiological factors, including photosynthetic capacity, hormonal regulation, and source-sink balance, as well as agronomic factors such as plant density and soil fertility. Integrating genetic, physiological, and agronomic perspectives is therefore essential for re-optimizing maize plant architecture and exploiting prolificacy for future production systems.

4. Sink−Source Constraints and Agronomic Barriers to Prolificacy

The multi-ear phenotype in maize has the potential to enhance grain yield, particularly under low plant density or stress-prone environments. However, its agronomic expression is constrained by complex physiological and developmental processes. Li et al. (2023a) and Rotili et al. (2022) reported that the presence of multiple ears on a single plant intensifies competition for assimilates, thereby reducing partitioning efficiency to individual ears and lowering reproductive success. These limitations primarily arise from an imbalance between the source (photosynthetic capacity) and the sink (ear number and developmental demand), which can result in ear abortion, reduced kernel size, or asynchronous ear development (Wu et al., 2023). This source–sink imbalance is tightly regulated by interconnected genetic and hormonal networks that determine axillary meristem fate and branching potential.

Key regulatory genes such as teosinte branched1 (tb1), grassy tillers1 (gt1), and Growth-Regulating Factor Interacting Factor 1 (GIF1) suppress tillering and axillary branching, thereby limiting the number of potential sinks (de Neve et al., 2025; Gallagher et al., 2023b; Prakash et al., 2020). In contrast, barren stalk1 (ba1) and the KNOTTED1 (KN1) transcription factor promote axillary meristem initiation and branching, increasing sink capacity (Satterlee et al., 2020; Tai et al., 2025). In addition, the auxin-to-cytokinin ratio plays a critical role in regulating source–sink balance, highlighting the importance of understanding and manipulating hormonal crosstalk through advanced genomic approaches.

Under abiotic stress conditions or at high plant densities, assimilate supply to secondary and tertiary ears becomes increasingly limited, leading to incomplete ear development (Wang et al., 2023b). Consequently, without concurrent enhancement of physiological support systems, such as assimilate production, transport efficiency, and vascular capacity, greater ear number does not necessarily translate into increased grain yield. Liu et al. (2022) and Yan et al. (2022) emphasized that maintaining an optimal source–sink balance is essential for converting increased ear number into realized yield gains.

To address source-sink constraints, recent research has focused on manipulating carbon allocation, vascular development, and sugar transport pathways (Artur, 2023; Shi et al., 2024). For example, upregulation of ZmSWEET13a/b sugar transporters has been shown to improve sucrose partitioning to developing ears (Wang et al., 2024a; Wu et al., 2023). Similarly, Wang et al. (2025) demonstrated that vascular bundle density strongly influences grain yield and identified genes controlling this trait. These findings highlight vascular architecture as a promising molecular breeding target for improving assimilate delivery and optimizing source–sink relationships in prolific maize.

Beyond assimilate competition, disruption of developmental synchrony critically affects prolificacy. Shelton et al. (2024) demonstrated that synchronously flowering inflorescences exhibit higher pollination efficiency and produce nearly twice as many seeds as asynchronously flowering ones. In maize, asynchrony between the primary and secondary ears often results in poor pollination, reduced kernel set, and diminished yield stability (D'Andrea et al., 2022). This asynchrony is not merely developmental but is rooted in hormonal crosstalk, particularly between gibberellins (GA) and ethylene. Ethylene modulates GA metabolism by activating GA-deactivation genes, thereby suppressing cell proliferation in meristems and altering the timing of organ initiation (Qin et al., 2022; Zhang et al., 2020). Azevedo et al. (2025) further showed that such hormonal interactions influence both the timing and intensity of axillary meristem activity, directly affecting branch number, ear vigor, and overall sink strength. Targeted modification of synchrony-related regulators, including GIF1, RA2, and GA-deactivation genes, represents a promising strategy for improving coordinated ear development through genome editing.

Finally, the agronomic expression of prolificacy is highly sensitive to management practices. Plant density, nutrient availability, and genetic background strongly influence ear number, synchrony, and yield stability (D'Andrea et al., 2022; Duan et al., 2023; Ross et al., 2020). These studies collectively emphasize that optimized management of plant density, nutrient supply, and genotype selection remains essential for achieving stable and productive expression of prolificacy across diverse agro-ecological conditions.

5. Genetic and Hormonal Regulation of Prolificacy in Maize

Maize has a genome size of approximately 2.3 gigabases and contains an estimated 60,000 genes. During domestication and subsequent crop improvement, however, only about 2% of these genes were subject to strong selection (Sharma et al., 2021). Consistent with this, Resende et al. (2022) and Xu et al. (2022) reported that approximately 2-4% of maize genes exhibit clear signatures of selection associated with domestication and breeding. These findings highlight the presence of extensive and largely untapped genetic variation, particularly within landraces and wild relatives that could be leveraged to improve key agronomic traits (Sharma et al., 2021). Among these opportunities, manipulation of prolificacy, a complex, polygenic trait regulated by interconnected genetic networks, transcription factors, and hormonal pathways, represents a promising strategy for enhancing yield stability and stress resilience in maize (Duan et al., 2023).

5.1 Key regulatory genes and transcriptional networks

The initiation of axillary meristems, inflorescence determinacy, branch architecture, and the development of multiple ears are governed by a complex interplay of transcription factors and regulatory genes. Teosinte branched1 (tb1) and grassy tillers1 (gt1) act as primary inhibitors of lateral branching by modulating the balance of auxin and cytokinin (Dong et al., 2019). Prakash et al. (2020) and Dong et al. (2019) reported that tb1, through its specific structure and promoter regulatory elements, integrates environmental cues such as light and stress signals to maintain meristem identity and repress lateral branching. Further, loss-of-function mutations in key genes can drastically alter ear development. For example, mutations in barren stalk1 (ba1), a basic helix-loop-helix (bHLH) transcription factor, can result in the complete absence of ears, whereas mutations in fascinated ear2 (fea2) increase ear number per plant (Ai et al., 2023; Yao et al., 2019; Song et al., 2025).

Genes regulating inflorescence determinacy add another layer of complexity. Li et al. (2022) highlighted that Growth-Regulating Factor Interacting Factor 1 (GIF1) is essential for maintaining meristem determinacy; loss of GIF1 results in highly branched ears. GIF1 modulates hormonal biosynthesis and regulates the expression of key meristem identity genes such as RAMOSA2 (RA2) and the signaling gene CLE4a (Li et al., 2022). The RA pathway, including RA1 and RA2, together with genes such as tasselsheath1/4 (tsh1/4) and BARREN INFLORESCENCES2 (Bif2), collectively control branching patterns and floral structure transitions (Kong et al., 2023; Xiao et al., 2022; Xie et al., 2024).

Recent high-resolution transcriptomic and single-cell RNA sequencing studies indicate that members of the YABBY, KNOX, and WOX gene families are dynamically expressed during maize ear primordium initiation, particularly under conditions favorable to prolificacy and multi-ear formation (Shen et al., 2023; Wang et al., 2024b).

5.2 Elucidating genetic architecture via modern genomics

The genetic basis of maize prolificacy has been progressively investigated using quantitative trait locus (QTL) mapping, genome-wide association studies (GWAS), and transcriptomic approaches, leading to the identification of numerous candidate genes and causal variants. Duan et al. (2023) identified novel QTL candidate genes, including qGEN261 and its causal gene ZmEN1, which regulates prolificacy, using multiple mapping methods. Similarly, Kuang et al. (2023) identified genes such as ZmPIN1a and ZmPG1 through GWAS, which are involved in auxin transport and influence plant architecture. Another GWAS-identified gene, Zm00001d045408, was associated with axillary bud development and leaf angle (Kuang et al., 2023). Qian et al. (2023) and Yang et al. (2020) conducted GWAS in tropical maize lines and identified several single-nucleotide polymorphisms (SNPs) linked to increased ear number per node, including variants near the KNOTTED1 and ZmCKX10 genes. These loci influence cytokinin homeostasis and axillary bud fate. Collectively, these findings provide valuable targets for breeding programs aimed at enhancing grain yield and optimizing maize plant architecture.

5.3 Central hormonal crosstalk

The hormonal signaling network, particularly auxin, cytokinin (CK), and strigolactones (SLs), interacts to regulate axillary meristem initiation and axillary bud outgrowth, thereby governing ear number per node and multi-ear development in maize (Aragon-Raygoza & Strable, 2025; Liu et al., 2024). Chaudhry et al. (2024) demonstrated that major phytohormones, including auxin, CK, abscisic acid (ABA), gibberellins (GA), brassinosteroids (BR), jasmonic acid (JA), ethylene, and SLs, play pivotal roles in shaping maize inflorescence architecture and reproductive success (Figure 2).

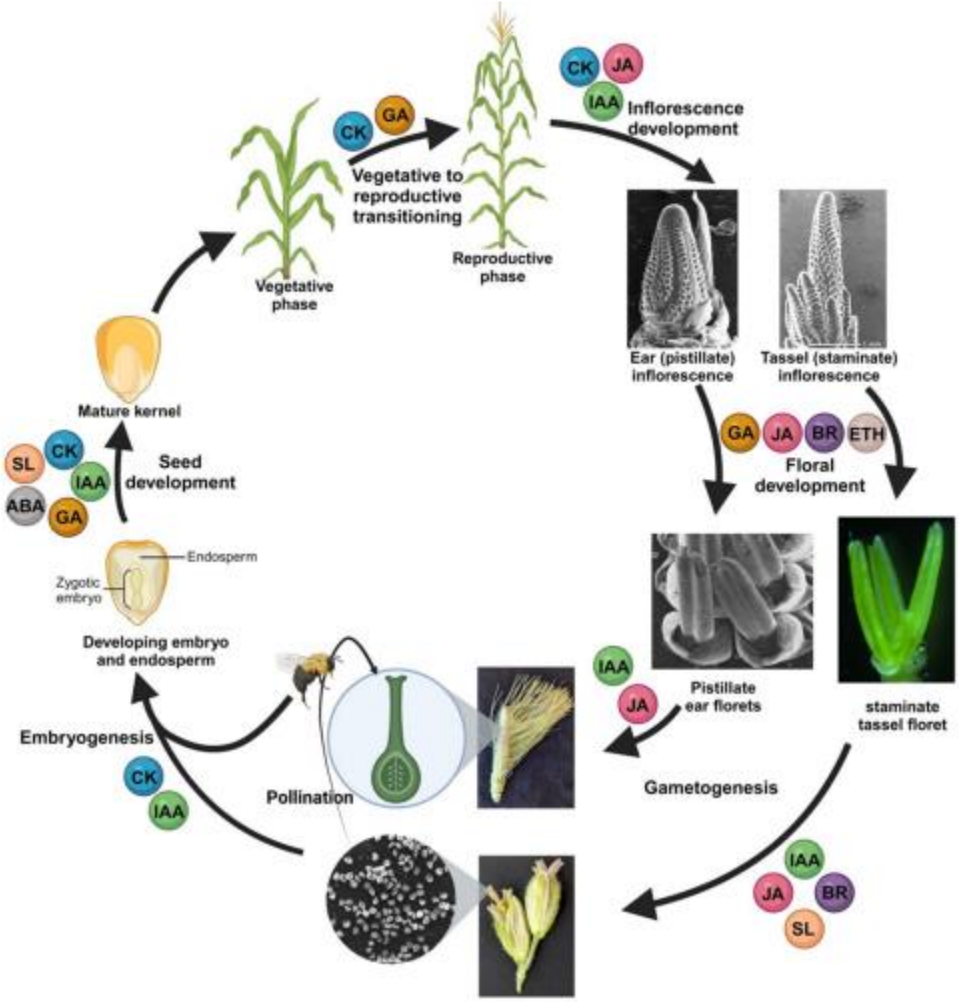

Figure 2: Phytohormones regulation of maize inflorescence development, reproduction, and seed formation (Source: Chaudhry et al., 2024)

In particular, an increased CK-to-auxin ratio favors axillary bud activation and the formation of additional ears, contributing to the maize multi-ear phenotype.

Hormone biosynthesis in maize is controlled by well-characterized genetic pathways. Auxin biosynthesis is primarily regulated by TSB and YUCCA (YUC) gene families, whereas CK biosynthesis is mainly governed by isopentenyl transferase (IPT) genes (Jiang et al., 2022b; Lee et al., 2009). Auxin and CK act in a tightly coordinated manner, and their precise spatial and temporal regulation is essential for normal branching patterns, axillary meristem fate, and ear development. These hormones further interact with other hormonal signals, environmental cues, and transcriptional regulators, underscoring the complexity of the regulatory network underlying prolificacy and plant architecture.

Strigolactones (SLs) function as central negative regulators of axillary bud outgrowth by suppressing lateral branching, thereby limiting ear number per node and overall shoot architecture in maize (Alvi et al., 2022; Dun et al., 2023; Kelly et al., 2023). Dun et al. (2023) and Guan et al. (2023) reported that elevated SL levels strongly inhibit branching and reduce ear number. SL biosynthesis involves key enzymes encoded by genes such as carotenoid cleavage dioxygenase 7 (CCD7), CCD8, more axillary growth1 (MAX1), lateral branching oxidoreductase (LBO), and D27 (Jia et al., 2019; Wani et al., 2021; Yu et al., 2022). While these enzymes are essential for maintaining proper plant architecture, excessive SL accumulation leads to suppressed axillary bud outgrowth. Consequently, targeted hormonal manipulation has emerged as a promising strategy for improving cereal crop yield potential (Wakeman & Bennett, 2023). In this context, CRISPR/Cas9-based genome editing provides a precise approach for modifying hormone-related genes to enhance prolificacy without compromising overall plant performance (Cheng et al., 2022).

Overall, axillary bud development, branching, and ear number in maize are governed by a dynamic hormonal network, with auxin, CK, and SLs forming the core regulatory module, while other hormones fine-tune downstream developmental outcomes (Figure 2). A deeper understanding of the genetic and hormonal control of these processes, combined with advanced genomic and gene-editing tools, will be critical for guiding future maize breeding strategies aimed at optimizing plant architecture and grain yield.

5.4 Genome editing technologies for engineering prolificacy in maize

Efforts to enhance maize prolificacy through conventional breeding have been constrained by the quantitative, polygenic, and environmentally responsive nature of ear number per node and related architectural traits (Parco et al., 2020; Zhou & Xu, 2024). In contrast, recent advances in precise genome-editing technologies enable targeted manipulation of key regulatory genes and pathways governing axillary meristem initiation, axillary bud outgrowth, hormonal homeostasis, and shoot branching, thereby providing new opportunities to engineer prolificacy and optimize maize plant architecture.

5.4.1 CRISPR−Cas-Based Editing of Prolificacy Genes

Clustered regularly interspaced short palindromic repeats (CRISPR/Cas9) technology has emerged as a powerful tool for dissecting and manipulating genes that regulate axillary meristem initiation, axillary bud outgrowth, shoot branching, and hormonal signaling in maize. Several key genes controlling branching and meristem determinacy have been characterized, including teosinte branched1 (tb1), grassy tillers1 (gt1), barren stalk1 (ba1), fascinated ear1 (fea2), and ramosa (RA1/RA2). CRISPR/Cas9-mediated editing of tb1 and gt1 restored basal branching and enhanced prolificacy under controlled conditions (Ai et al., 2023; Wills et al., 2013). Similarly, knockout of fea2, a key regulator of meristem size, resulted in increased ear number per plant (Li et al., 2023b).

The application of genome editing for maize breeding requires finely tuned engineering strategies or allele-dosage optimization, as excessive branching and prolificacy can result in undesirable pleiotropic effects, including increased lodging susceptibility, reduced grain yield, delayed silking, and smaller primary ears (Khaipho-Burch et al., 2023; Zhang et al., 2023). Potential approaches include generating weak or tissue-specific alleles and combining edits with traits that enhance plant standability. Notably, Khaipho-Burch et al. (2023) reported that pleiotropy in maize is relatively limited, with only 1.56–32.3% of genetic intervals showing pleiotropic effects, indicating minimal tradeoff risk and enabling largely independent trait selection. Consistent with this, Wind (2023) emphasized that genome editing can effectively manage trade-offs by precisely targeting genes that enhance desired traits while minimizing adverse effects.

Beyond coding sequences, genome editing is increasingly being applied to non-coding regulatory regions, including cis-regulatory elements (CREs) such as promoters, enhancers, and untranslated regions, allowing fine-tuning of gene expression while preserving protein function (Chen et al., 2023).

Integrating high-throughput genomic approaches, including genome-wide association studies (GWAS) and quantitative trait locus (QTL) mapping, has uncovered multiple loci associated with prolificacy and ear number per node. Several studies have identified overlapping genomic regions near well-established regulators of axillary meristem activity, including KNOTTED1, ZmCKX10, and tb1-adjacent intervals (Li et al., 2020; Li et al., 2023; Wang et al., 2023b). For example, loci near ZmCKX10, which modulates cytokinin degradation, have been consistently detected in both GWAS panels and bi-parental QTL analyses, supporting its conserved role in regulating ear number. Recent GWAS reports identifying ZmEN1, Zm00001d020683, and Zm00001eb365210 further reinforce the central role of branching and axillary bud activation networks in controlling prolificacy (Duan et al., 2023; Li et al., 2023b; Prakash et al., 2024; Wang et al., 2021). Additional QTLs and candidate genes have been reported on chromosomes 1, 5, 7, 8, and 9. CRISPR/Cas9 and multiplex genome-editing platforms, such as BREEDIT, are increasingly employed to validate gene function and accelerate breeding for prolificacy (Rice et al., 2020; Lorenzo et al., 2023). Key genes, their functions, and their relevance to maize prolificacy are summarized in Table 1.

Next-generation CRISPR variants, including CRISPR activation (CRISPRa) and CRISPR interference (CRISPRi), enable precise transcriptional regulation of target genes without inducing double-strand breaks (McLaughlin et al., 2025; Oh & Lee, 2024). Qi et al. (2023) demonstrated that an optimized CRISPRa/dCas9 system can activate target genes in maize with high specificity. Likewise, CRISPRa/i platforms have been used to fine-tune the expression of regulatory loci such as tb1 and gt1 through engineered promoters, enhancing tiller formation and ear initiation while minimizing negative effects such as lodging and kernel abortion (Cai et al., 2023; Karlson et al., 2021; Pandey et al., 2024). This level of transcriptional control facilitates the development of multi-ear maize ideotypes optimized for specific environments and planting densities, thereby reducing trade-offs among branching, photosynthetic efficiency, and grain yield potential.

Table 1: Key maize genes regulating prolificacy, their functions, and evidence

Genus/locus	Primary functions	References
tbl(teosinte branched1)	Represses axillary branching; major domestication gene	Dong *et al.* (2019)
gt1(grassy tillers1)	Suppress tillers and basal ears, mutants increase ear numbers	Gallagher *et al.* (2023b)
KNOTTED1(KN1)	Maintains shoot apical meristem; regulates cytokinin, promotes axillary meristems persistence and ear initiation	Lechon *et al.* (2025)
BA1(barren stalk1)	Required for axillary meristem initiation and its mutants lack ears or regulate ear bearing nodes	Tai *et al.* (2025) and Yao *et al.* (2019)
tsh4(TASSEL SHEATH4)	Activate axillary meristem formations, influences lateral branching and secondary ears	Xiao *et al.* (2022) and Liu *et al.* (2021b)
RA1(RAMOSA1)	Determines branch formation influencing ear-bearing branches, it mutant results highly branched inflorescences	Gallavotti *et al.* (2010)

Multiplex CRISPR editing has also emerged as an effective strategy for manipulating polygenic traits such as prolificacy by simultaneously targeting multiple loci (Abdelrahman et al., 2021; Cheng et al., 2025; Prajapati & Tyagi, 2024). The use of single guide RNA constructs capable of targeting multiple sites simplifies multiplex editing and reduces the risk of off-target effects (Bewg et al., 2022). Gawande et al. (2024) and Gallagher et al. (2023b) demonstrated that simultaneous editing of ZmRA2, ZmBIF2, and ZmCKX10 increased lateral branching, elevated cytokinin accumulation, and promoted secondary ear initiation. Collectively, these findings underscore the potential of coordinated genome-editing strategies to modulate multiple developmental and hormonal pathways governing maize prolificacy.

5.4.2 Base and prime editing in prolificacy Engineering

The limited capacity of conventional CRISPR–Cas systems to introduce precise base-level DNA changes has driven the development of more accurate and stable genome-editing technologies, notably base editing and prime editing (Verma et al., 2023). These approaches have transformed precision genome engineering in maize, particularly for modulating prolificacy and ear number per node through targeted single-nucleotide alterations (Alharbi et al., 2024). Unlike standard CRISPR/Cas9, which induces double-strand breaks (DSBs) that can generate disruptive insertions or deletions, base and prime editors enable precise, programmable nucleotide substitutions without cleaving the DNA backbone. This feature makes them especially suitable for fine-tuning the expression of key regulators of axillary meristem activity, such as tb1, by editing cis-regulatory regions including the prol1.1 promoter, thereby achieving moderate and agronomically desirable levels of prolificacy without the severe architectural perturbations associated with complete gene knockouts (Alharbi et al., 2024).

Cytosine base editors (CBEs) and adenine base editors (ABEs) (Figure 3) catalyze C-to-T and A-to-G transitions, respectively, enabling efficient, DSB-free generation of allelic variants for crop improvement (Kharbikar et al., 2023; Mishra et al., 2022; Zhong et al., 2023).

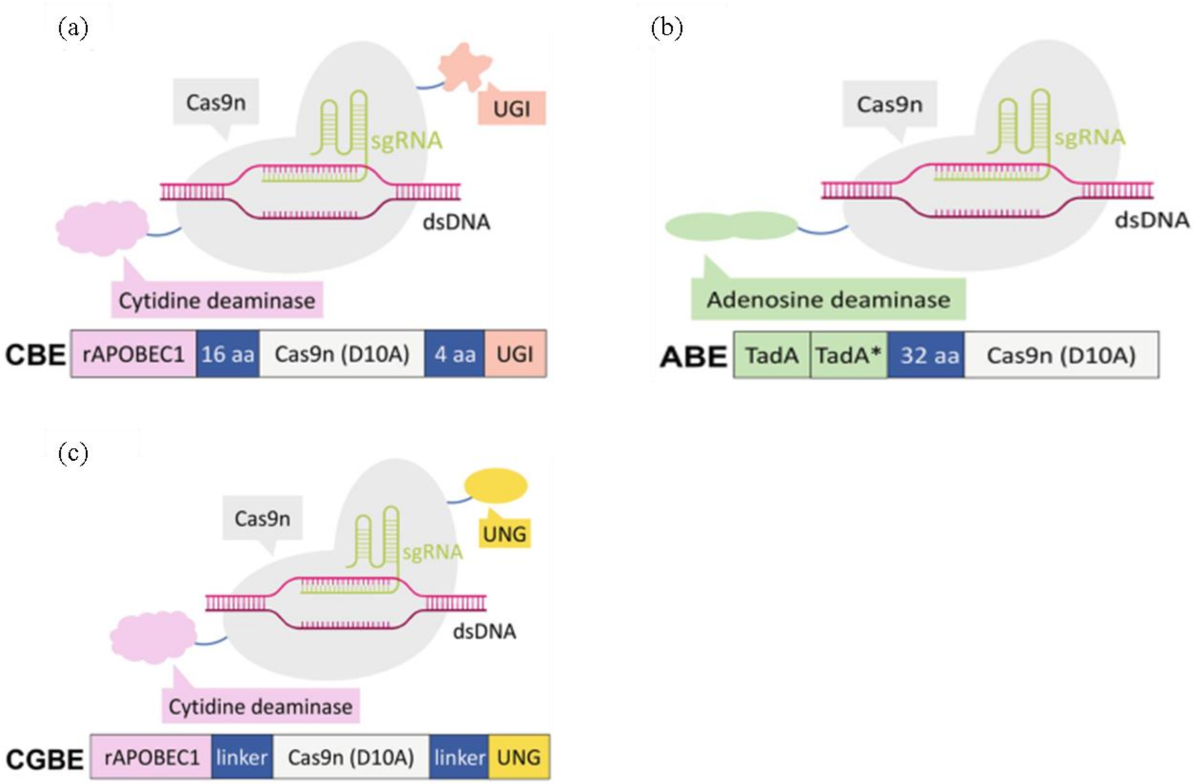

Figure 3: Schematic diagram of base editors and prime editors (a) Cytosine base editors (CBE) mediate C-to-T conversion, (b) Adenine base editors (ABE) mediate A-to-G conversion, (c) Prime Editing (PE) (Kaya, 2023)

These tools are particularly effective for recreating naturally occurring alleles or precisely modifying cis-regulatory elements such as promoters and enhancers. For example, Luo et al. (2022) used a CBE system to edit a key single-nucleotide polymorphism (SNP) in the ZmCKX10 promoter, resulting in increased cytokinin signaling and enhanced prolificacy. Similarly, targeted edits within the prol1.1 region upstream of tb1 have been shown to modulate tb1 expression and partially restore prolificacy in elite genetic backgrounds (Chen et al., 2023; Wills et al., 2013).

Prime editing has also been applied to regulators of meristem determinacy and inflorescence architecture, including RA1/RA2 and fea2, facilitating detailed analyses of gene dosage effects on maize prolificacy (Jiang et al., 2020; Tingting et al., 2023). This approach employs a prime editing guide RNA (pegRNA) and a Cas9 H840A nickase fused to a reverse transcriptase (Verma et al., 2023). Collectively, these studies highlight the unique advantages of base and prime editors for generating subtle, tunable regulatory variants that optimize ear number per node and axillary bud outgrowth while preserving overall plant architecture.

5.4.3 Regulatory element and synthetic promoter engineering

Beyond coding regions, genome-editing technologies are increasingly being applied to cis-regulatory elements (CREs), including promoters, enhancers, and untranslated regions (UTRs), which govern gene expression patterns and developmental outcomes (Liu et al., 2021a). Targeting CREs enables crop-improvement strategies with greater precision, robustness, and fewer unintended effects than conventional approaches focused on coding sequences (Saeed et al., 2022). For example, synthetic promoter engineering of tb1 and gt1 using CRISPR activation (CRISPRa) and CRISPR interference (CRISPRi) platforms has demonstrated conditional and tissue-specific control of axillary bud outgrowth, branching, and ear development (Gondalia et al., 2025; Nayak et al., 2025). Such regulatory fine-tuning is critical for enhancing prolificacy and ear number per node without compromising overall plant architecture or reproductive performance.

Despite substantial progress in elucidating the genetic and hormonal control of multi-ear development, genome-edited maize lines with stable prolific or multi-ear phenotypes have not yet reached commercial release (Dong et al., 2023; Wang et al., 2023b). Nevertheless, the successful commercialization and advanced development of genome-edited maize for other agronomically important traits, such as waxy starch, drought tolerance, male sterility, and disease resistance, demonstrate both the technical feasibility of genome editing and its acceptance within existing regulatory frameworks. Notably, Corteva's CRISPR/Cas9-derived high-amylopectin (waxy) maize has been approved in the United States, Argentina, Brazil, and Chile (Yassitepe et al., 2021). In addition, promoter-edited ARGOS8 lines with enhanced drought tolerance and CRISPR-based male-sterility systems have progressed through development pipelines (Figure 4) (Hernandes-Lopes et al., 2023; Rasheed et al., 2023). Collectively, these genome-editing successes illustrate the capacity of the technology to generate stable, agronomically valuable traits with clear regulatory pathways, thereby paving the way for the future commercialization of prolific or multi-ear maize.

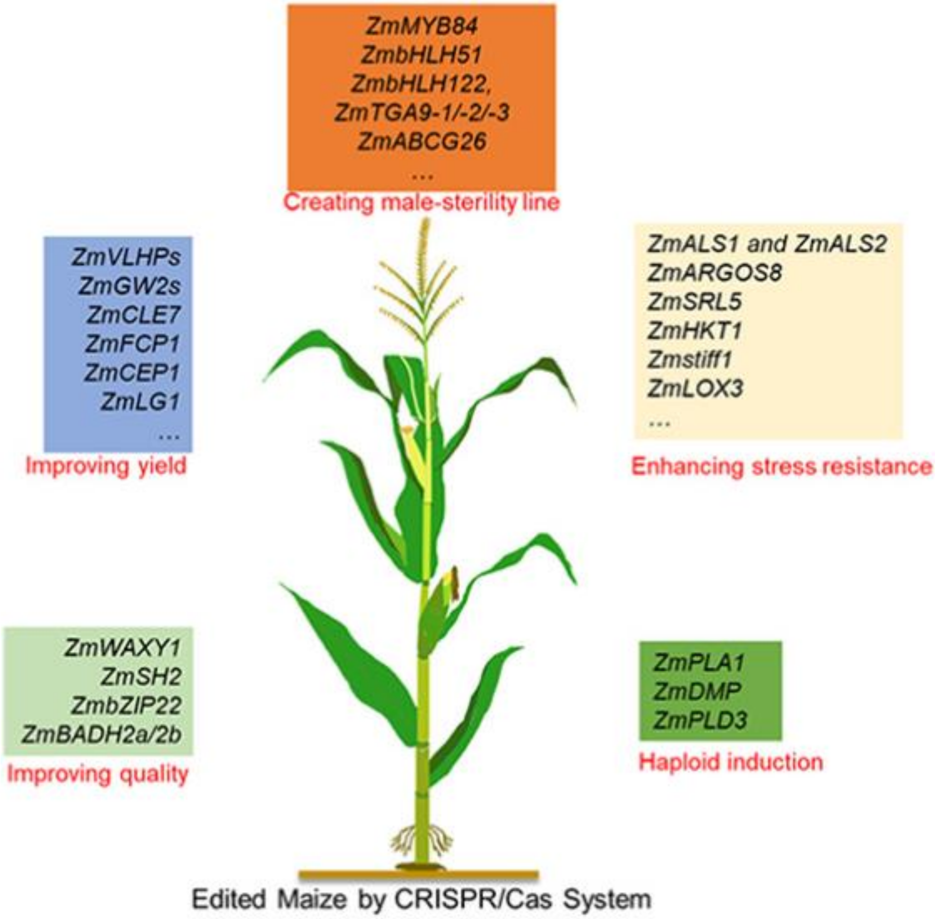

Figure 4: CRISPR/Cas genome editing system in maize for various targeted traits (source: Jiang et al., 2022a)

6. Conclusion

Multi-ear traits in maize are critical for enhancing yield stability, environmental adaptation, and stress tolerance across diverse production conditions. Although modern breeding has emphasized single-ear ideotypes to maximize productivity, this approach has narrowed functional genetic diversity and reduced adaptive flexibility. Revisiting prolificacy offers a pathway to restore yield-buffering capacity; however, successful implementation requires careful management of physiological and genetic trade-offs involving ear number, resource allocation, and reproductive efficiency. In addition, stabilizing the performance of prolific maize varieties across environments while maintaining an optimal source–sink balance remains a significant challenge.

Recent advances in genome-editing technologies, including CRISPR-Cas systems, base editing, and prime editing, now make it feasible to precisely manipulate key regulators such as tb1, gt1, fea2, RA1/RA2, and hormone-related pathways. Strategies such as multiplex editing and fine-tuning of cis-regulatory elements offer opportunities to enhance maize prolificacy without compromising plant vigor or grain quality.

To achieve precise and durable gene control, future research should prioritize the identification and validation of promoters and other cis-regulatory elements, alongside the development of high-throughput phenotyping platforms to evaluate edited lines across diverse environments and management practices. In parallel, socio-economic studies are needed to assess the adoption potential of multi-ear varieties, particularly among smallholder farming systems. By addressing these challenges, the theoretical promise of genome-edited prolificacy can be translated into tangible climate resilience, sustainable yield gains, and broader adoption in global maize production.

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