Review

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Review

Advances in Micronutrients Signaling, Transport, and Integration for Optimizing Cotton Yield

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Abstract

Micronutrients, particularly boron (B), iron (Fe), manganese (Mn), and zinc (Zn), are pivotal for cotton (*Gossypium* spp.) growth, reproductive success, and fiber quality, yet their roles are often overshadowed by macronutrient-focused fertility programs. This review synthesizes recent advancements in understanding the physiological, molecular, and agronomic roles of B, Fe, Mn, and Zn in cotton production, with a focus on their signaling integration and impact on nutrient use efficiency (NUE). Drawing from peer-reviewed literature, experimental data, and regional surveys, we highlight how these micronutrients regulate critical processes such as photosynthesis, cell wall integrity, hormone signaling, and stress responses, directly influencing root development, boll retention, and fiber quality. Deficiencies, exacerbated by soil pH, redox conditions, and nutrient interactions, contribute to significant yield gaps, even when macronutrients like nitrogen (N), phosphorus (P), potassium (K), and sulfur (S) are adequately supplied. Key genes, including BOR1, IRT1, NRAMP1, and GhZIP3, mediate micronutrient uptake and homeostasis, offering targets for breeding high-yield, nutrient-efficient cotton varieties. Advanced phenotyping using unmanned aerial vehicles (UAVs) and single-cell RNA sequencing (scRNA-seq) provide novel avenues for identifying nutrient-efficient genotypes and regulatory networks. The review also explores synergistic interactions between micronutrients and macronutrients to influence growth and yield of cotton. Future research directions include leveraging microRNAs, CRISPR-based gene editing, and precision nutrient management to enhance B, Fe, Mn, and Zn use efficiency, addressing environmental challenges while closing persistent yield gaps in sustainable cotton production systems.

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1. Introduction

Although present in plants in only trace quantities, micronutrients are indispensable for sustaining the metabolic processes that drive cotton growth, reproduction, and fiber development. Out of nearly 90 elements occurring naturally in soils, only a limited number are classified as essential, and among them, micronutrients such as Fe, Zn, Mn, B, copper (Cu), molybdenum (Mo), and chlorine (Cl) play particularly critical roles. Despite their minute concentration requirements compared with macronutrients, these elements regulate key physiological functions including enzyme activation, photosynthesis, and reproductive development making them fundamental for realizing cotton's genetic yield and quality potential [1,2]. Unlike macronutrients, which are supplied in large quantities, micronutrients often exist in soils at sufficient total concentrations but with limited bioavailability due to pH, organic matter, redox potential, and interactions with other nutrients [3].

Among these, Fe stands out as particularly critical for cotton development, yet its management has long been overshadowed by emphasis on N, P, K, and S [3,4]. Iron plays a fundamental role in

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photosynthesis, chlorophyll biosynthesis, electron transport, and enzyme activation, directly influencing boll retention, flowering, and fiber development [5–7]. Deficiencies manifest as interveinal chlorosis, stunted growth, and poor boll formation, conditions increasingly reported in calcareous, sodic, and alkaline soils across major cotton-growing regions [8]. Although Fe and other micronutrients are abundant in the earth's crust their solubility and plant availability decline sharply over time especially in alkaline conditions, resulting in hidden deficiencies or hidden hunger that are often overlooked in cotton fertility programs [9,10].

Other micronutrients such as Zn, B, and Mn also exert critical influences on cotton physiology, each contributing to distinct but interconnected processes that regulate plant growth and yield formation. Zinc for example is fundamental to auxin biosynthesis, protein metabolism, and membrane integrity, and it activates a wide array of enzymes involved in carbohydrate and nucleic acid metabolism. In cotton, adequate Zn supply promotes balanced vegetative growth, proper leaf expansion, and reproductive development, while deficiencies result in shortened internodes, small, deformed leaves, delayed flowering, and reduced boll set [11]

Boron, though required in very small quantities, is indispensable for cell wall stability, sugar transport, and reproductive processes. In cotton, B deficiency disrupts pollen germination and tube elongation, leading to poor fertilization, floral deformities, and premature boll shedding [12,13]. Its role in maintaining membrane function and assimilate partitioning is especially critical during the flowering and boll-filling stages, when demand is high. Manganese, on the other hand, is a cofactor in numerous enzymatic systems and plays a pivotal role in photosystem II function, aiding in water-splitting and oxygen evolution. It also contributes to lignin biosynthesis, disease resistance, and the detoxification of reactive oxygen species [14,15]. Cotton grown in Mn-deficient soils exhibits interveinal chlorosis, leaf speckling, and impaired photosynthetic activity, which collectively reduce biomass accumulation and reproductive success.

Despite their importance, these micronutrients have historically received limited attention compared with macronutrients. Fertilizer recommendations, research priorities, and extension programs worldwide have largely focused on N-P-K and, more recently, S, leaving critical gaps in understanding the role of these micronutrients in sustaining cotton yields, and profitability. This imbalance in nutrient management has likely contributed to unexplained yield gaps, with emerging evidence suggesting that micronutrient deficiencies can constrain cotton productivity even when macronutrient levels are optimal. Given the global significance of cotton as both an economic and industrial crop, it is imperative to reevaluate the current advances, mechanisms and the contribution of B, Fe, Mn, and Zn in cotton growth and yield formation. This review examines their roles in cotton growth, physiology, soil-plant dynamics, and agronomic performance across diverse environments, while also identifying the opportunities and limitations of current research in the understanding of these elements. By synthesizing recent findings, this work highlights how unbalanced nutrient management perpetuates hidden yield losses and the need for integrated approaches that incorporate micronutrient nutrition into sustainable cotton production systems.

2. Effects of Micronutrients of B, Fe, Mn, and Zn in Cotton Growth Phenotypic Traits

Cotton growth and yield depend not only on adequate macronutrients but also on the balanced supply of micronutrients such as B, Fe, Mn, and Zn [16]. Each plays a unique physiological role that directly influences growth traits such as vegetation, nodes, height of the plant, flowering, flower retention, boll retention, and fiber development. Boron is essential for cell wall formation, membrane integrity, and reproductive structures formation, particularly during pollination, pollen germination and tube growth, which ultimately ensures successful boll formation, and boll setting growth stages [12,17]. Iron regulates chlorophyll biosynthesis, photosynthetic electron transport, and respiration, making it indispensable for leaf expansion and energy metabolism [5,18]. Research shows that when Iron is applied to cotton, it enhances its tolerance to ionic stress from metals such as Lead and downregulates the effect of macronutrient deficiency [19]. Manganese functions as a cofactor for

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enzymes in photosystem II and is critical for lignin biosynthesis and oxidative stress regulation. Zinc contributes to auxin synthesis, enzyme activation, and reproductive organ development. Optimal supply of these nutrients sustains both vegetative vigor and reproductive success, while their deficiencies impose severe limitations on cotton physiology and yield.

Figure 1. Showing the visible symptoms of Zn deficiency in cotton. Image courtesy of Krishi Bazaar.

2.1. Plant Growth and Development

Cotton's growth phases from seedling, root development, vegetative, and reproductive growth and development are highly sensitive to the availability of all nutrients including the micronutrients especially B, Fe, Mn, and Zn. The deficiencies in these nutrients exist synergistically and antagonistically and often interacting to exacerbate plant stress. For example, B deficiency impairs pollen tube growth during flowering, affecting flower fertilization. This negatively affects the square, boll numbers, sizes of individual boll, and micronaires in cotton lint. These come up due to the resultant distorted flowers, boll shedding, and poor fiber initiation. For the case of Iron, its deficiency, commonly expressed as interveinal chlorosis of young leaves, reduces chlorophyll content, photosynthetic efficiency, and ultimately boll retention [20]. Manganese shortages impair enzyme activity and photosynthetic water-splitting, causing leaf speckling, reduced vigor, and susceptibility to oxidative stress. Zn deficiency leads to shortened internodes, stunted growth, smaller leaves, and poor boll development.

These micronutrient deficiencies not only reduce crop performance directly but also diminish the efficiency of applied macronutrients such as N and P. For instance, Fe and Zn are essential for nitrate reductase activity and protein synthesis [21], meaning that their absence reduces the efficiency of N fertilization. This assertion is in line with several studies that observed yield reduction when these micronutrients are deficient compared to fertilized plots [22–24]. Similarly, B deficiency interferes with carbohydrate transport and reproductive growth, lowering the effectiveness of P and K applications [25,26]. Interactions among micronutrients further complicate cotton nutrition; for example, excess Zn can induce Fe deficiency in soybeans, while high soil pH reduces the availability of both Fe and Mn [26,27]. Such imbalances frequently result in unexplained yield gaps, where macronutrient inputs are adequate, but cotton fails to reach its genetic potential. The emerging evidence demonstrates the importance of integrating B, Fe, Mn, and Zn management into fertility programs to ensure efficient nutrient use, resilience under stress, and sustainable yield gains in cotton production systems.

2.2. Effects of Micronutrients on Cotton Physiological Stress Indicators

Boron plays a crucial role in pollen germination and carbohydrate translocation, key processes for reproductive development and assimilate partitioning in cotton [28]. Increased B availability

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promotes square retention, boll set, and efficient utilization of nitrogen and potassium, enhancing photosynthetic rates and chlorophyll content through improved nutrient mobilization from leaves to developing bolls [29]. Low B levels disrupt cell wall integrity and accelerate square abscission, leading to reduced leaf area and photosynthetic capacity, while excessive B can induce toxicity that lowers chlorophyll levels and alters antioxidant enzyme activities [30,31]. Adequate B supplementation, often via foliar application, delays leaf senescence and improves yields by sustaining photosynthetic activity [32].

Iron is integral to chlorophyll biosynthesis and electron transport in photosynthesis, with deficiencies severely disrupting chloroplast structure and function in cotton. Increased Fe availability supports chlorophyll production and maintains photosynthetic efficiency, promoting leaf expansion and overall plant vigor. Low Fe levels, common in high pH calcareous soils, accelerate chlorosis through reduced chlorophyll synthesis and impaired photosystem activity, leading to yellowing of young leaves and decreased net photosynthetic rates. Adequate Fe supplementation enhances enzyme activities involved in respiration and nitrogen assimilation, ensuring sustained crop development and higher biomass accumulation (Rout and Sahoo 2015). This is as a result GhbHLH121 gene expression which is upregulated under of Under Fe-deficient conditions cotton growing conditions. The gene GhbHLH121 forms heterodimers with GhbHLH104 or GhbHLH115 both independently activating the downstream expression of the three genes GhbHLH38, GhFIT, and GhPYE [20].

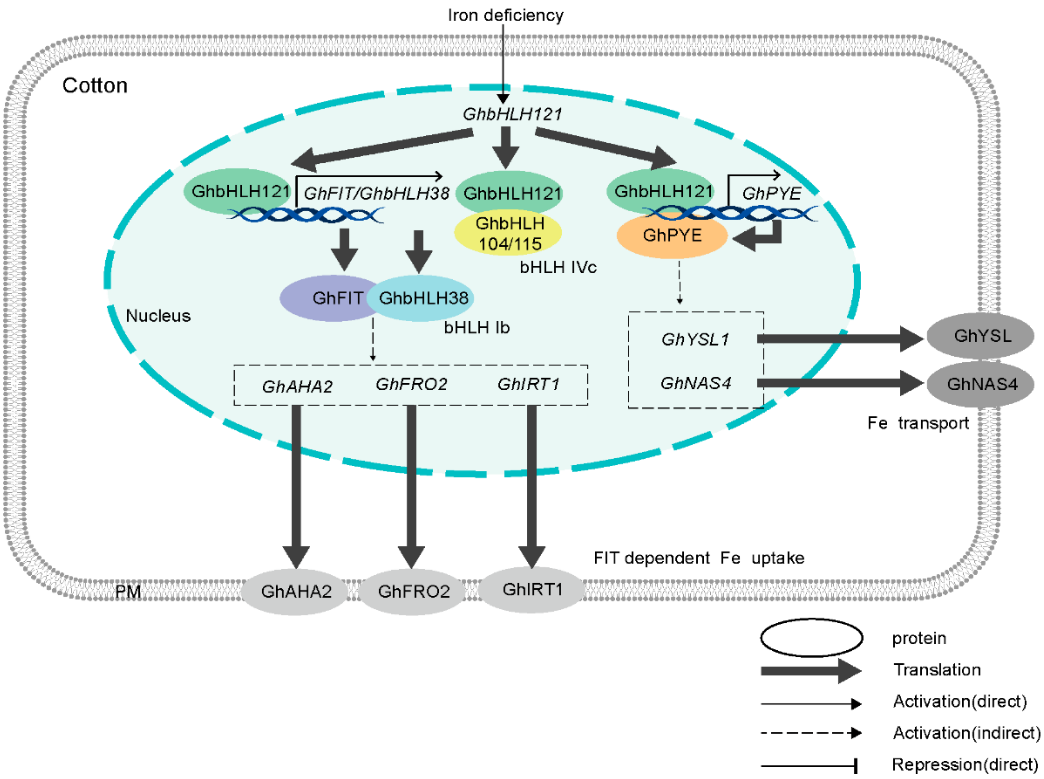

Figure 2. A working model of GhbHLH121-mediated regulation under iron deficiency in cotton [20].

Manganese functions as a cofactor in photosynthetic enzymes, particularly in photosystem II, and supports chlorophyll synthesis and nitrogen metabolism in cotton [33,34]. Increased Mn availability promotes chlorophyll content and stomatal conductance, enhancing the net photosynthetic rate and carbohydrate accumulation. Low Mn levels disrupt oxygen evolution in photosynthesis and reduce enzyme activities such as superoxide dismutase, leading to interveinal chlorosis and accelerated leaf senescence [34]. Excessive Mn can induce toxicity, lowering chlorophyll

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levels and inhibiting growth, but foliar application at optimal rates increases chlorophyll by up to 17% and improves yield quality on calcareous soils [33,35,36].

Zinc is essential for enzyme activation in carbon fixation and protein synthesis, playing a key role in photosynthesis and hormone regulation in cotton. Increased Zn availability boosts carbonic anhydrase activity and chlorophyll concentration, with critical leaf levels around 13-14 μg/g dry weight required for maximum photosynthetic rates and chlorophyll synthesis [37]. Low Zn levels accelerate chlorophyll degradation and reduce stomatal conductance, disrupting cell proliferation and leading to stunted growth and lower leaf area. Adequate Zn supplementation, particularly in combination with B, enhances antioxidant enzyme activities and improves yields by maintaining sustained photosynthetic performance [38,39].

3. Impact of Micronutrients on Cotton Yield and Quality Traits

3.1. Nutrient Dynamics and Cotton Yield

Cotton production hinges on two key elements: the availability and timing of nutrient delivery to the plant, and the plant's ability to store and utilize these nutrients effectively [40]. The first factor ties closely to the plant's ability to photosynthesize efficiently and the activity within its root zone, where nutrients are absorbed. The second factor relates to the development of reproductive structures, such as flowers, buds, and bolls, and the efficient movement of energy-rich compounds produced during photosynthesis to these areas [1,13,40].

3.1.1. Boron's Role in Cotton Development

Boron is a critical player in achieving high cotton yields, as it helps strike a delicate balance between vegetative growth and the formation of fibers. When boron levels are just right, it supports robust boll and fiber development, leading to heavier bolls and better fiber with optimum micronaires and seed production [29,39,41]. However, too much B can throw this balance off, causing toxicity that manifests as fewer bolls, stunted fiber growth, and weaker plant tissues, which often results in increased boll shedding and lower overall yield due to poor light energy use [42]. On the flip side, too little boron hampers plant growth, reduces the weight of individual bolls, and impairs seed development, leading to thicker boll shells and heavier stalks that compromise fiber quality [13]. Research highlights that adequate boron fosters healthy plant growth, boosts dry matter accumulation, and increases the number of mature seeds, while excessive boron can delay crop maturity and diminish fiber quality.

3.1.2. Iron's Influence on Growth and Productivity of Cotton

Iron deficiency can significantly stunt cotton plants, leading to shorter plants with smaller leaves compared to those with sufficient iron [43]. While iron doesn't directly impact lint percentage, its application boosts the number and weight of bolls, ultimately increasing the cotton yield of seed. This improvement largely comes from reduced shedding of buds and bolls and better nutrient delivery to developing bolls. When soil iron levels are low during the reproductive phase, plants may prematurely shift nutrients from leaves to bolls, which lowers photosynthetic efficiency and cuts into yields [13,44].

3.1.3. Manganese's Contribution to Yield and Fiber Quality

Manganese is vital for maximizing cotton yield and enhancing fiber quality. Applying manganese through foliar sprays or seed treatments promotes boll formation and strengthens fiber properties [1]. A lack of manganese can cause yellowing between leaf veins (interveinal chlorosis), lower boll retention, and reduced seed cotton yields. In contrast, sufficient manganese increases boll weight and improves fiber strength. However, excessive manganese can lead to toxicity, marked by bronzed leaves and reduced yields [45]. Balanced manganese application, often paired with other micronutrients, supports higher lint percentages and overall productivity [46]. Zinc nutrition plays a critical role in cotton yield formation, as optimal Zn levels promote boll development and fiber maturation. Zinc deficiency results in stunted growth, fewer bolls per plant, and reduced fiber length and strength, leading to significant yield losses. Adequate Zn application, particularly foliar sprays, increases seed cotton yield by enhancing boll set and weight, while also improving fiber quality parameters such as micronaire and uniformity. However, excessive Zn can interact negatively with other nutrients like B, potentially reducing uptake and affecting yield, though combined applications often mitigate such effects and boost overall efficiency [47].

4. Physiological and Molecular Mechanisms of B, Fe, Mn, and Zn Utilization in Cotton

Micronutrients like B, Fe, Mn, and Zn are like tiny sparks that ignite cotton growth, drive higher yields and supporting global food security. However, heavy reliance on micronutrient fertilizers can harm the environment, deplete soils and cause contamination [48]. To grow more cotton with less environmental cost, improving micronutrient use efficiency (MUE), the plant's ability to make the most of these nutrients, is critical [49]. While smarter farming practices help, the real game-changer lies in breeding cotton varieties that naturally excel at using these nutrients efficiently [18,50,51]. Over the past 30 years, selective breeding has made great strides in enhancing how cotton plants handle B, Fe, Mn, and Zn [20,52]. Looking ahead, boosting nutrient redistribution within the plant will be key to unlocking even greater yields.

When boron runs low in the soil, cotton plants increase boron uptake efficiency (BUE) through root extensions to absorb more and fine-tuning internal processes to make better use of what's available. These adaptations to boron uptake are driven by active root systems and optimized cellular functions that help maintain a healthy boron balance in the soil systems, especially with varieties bred for high BUE [3,26,50,53,54]. Similarly, when iron is deficient in the soil, cotton plants release phytosiderophores, chemical "magnets" that help to chelate iron from the soil and boost the activity of iron transporters to pull it into the plant [20]. Below is a table (Table 1) summarizing iron uptake strategies in various crops, including upland cotton (dicotyledonous plant), employing iron utilization. The commonest strategy is reduction-based, where it acidifies the rhizosphere, reducing Fe^{3+} to Fe^{2+}, and transporting it via specific transporters like IRT1. Cotton for example uses genes like GhbHLH121 to regulate responses to iron deficiency, like Arabidopsis (*Arabidopsis thaliana* (L.) Heynh). The alternative pathway is chelation.

Table 1. Strategies and Key Mechanisms of Iron Uptake in Different Crops, Including Cotton.

Crop	Strategy	How It Works	Key Components/Enzymes/Hormones/Pathways	Reference
Arabidopsis thaliana (L.) Heynh	Reduction	Mediates proton release, reduces Fe^{3+} to Fe^{2+}, and transports Fe^{2+} into roots.	FRO2, IRT1 (transporter); FIT, PYE, BTS pathways; Auxin and ethylene hormones enhance gene expression.	[43,55]
Rice (*Oryza sativa* (L)	Chelation	Secretes phytosiderophores to chelate, Fe^{3+}, forming complexes transported into roots.	Phytosiderophores (e.g., mugineic acid), YSL transporters; OsIRO2 and OsIDEF1 regulate responses.	[56,57]
Cotton	Reduction	Responds to deficiency by reducing Fe^{3+} at root surface and uptaking Fe^{2+}; involves rhizosphere acidification	GhbHLH121 (transcription factor), FRO-like reductases, IRT-like transporters; FIT-like and bHLH pathways;	[20,58,59]

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		and gene upregulation for homeostasis.	Reactive oxygen species signaling.	
Barley	Chelation	Prefers chelation but can show hybrid responses; secretes phytosiderophores to mobilize Fe3+.	HvYS1 (transporter), phytosiderophores; IDEF1/2 transcription factors regulate under deficiency.	[60]
Maize	Chelation under high pH soils	Releases mugineic acid family phytosiderophores (MA's) to bind and uptake Fe3+ complexes.	ZmYS1 (transporter), DMA (deoxymugineic acid); bHLH and NAC transcription factors in response pathways.	[56,57,61]

For manganese and zinc shortages, cotton plants increase the production of metal tolerance proteins and ZIP transporters, which act like gatekeepers to regulate the uptake and balance of these nutrients, ensuring the plant thrives despite limited supply [33].

Boron is a cornerstone of cotton's growth that strengthens cell walls and assists the plant in transporting sugars around the plant. Enzymes like pectin methylesterases and expansins work together to keep cell walls sturdy enhancing resistance to lodging [12,41,50]. Boron helps in maintaining cell wall structure and function through its involvement in the cross-linking of rhamnogalacturonan II (RG-II), a process mediated by enzymes such as UDP-glycosyltransferases that strengthen cell wall integrity. Concurrently, xyloglucan endotransglucosylases contribute to cell wall remodeling during growth and development [41,62]. Beyond structural functions, boron also modulates signaling pathways, where reactive oxygen species (ROS) generated by oxidases act as key regulators of developmental processes and stress adaptation in cotton [54,63].

Iron underpins energy metabolism by driving photosynthesis and respiration, with ferric reductases converting Fe^{3+} to the more soluble Fe^{2+} under deficiency conditions to enhance uptake [6,21,60,64]. Within the plant, enzymes such as ferredoxin and heme oxygenase sustain iron mobilization and availability, ensuring continued photosynthetic efficiency and growth despite limited supply [51]. Manganese is equally critical, functioning as a cofactor in photosystem II to maintain efficient light harvesting and energy conversion. Its role extends to stress defense, where manganese superoxide dismutase (MnSOD) mitigates reactive oxygen species damage, while incorporation into metalloproteins supports boll development and overall growth [33,34]. Zinc contributes to both metabolic regulation and genetic stability, serving as a cofactor for enzymes such as carbonic anhydrase and alcohol dehydrogenase, which facilitate CO_2 fixation and energy metabolism. Under deficiency, alkaline phosphatase and zinc finger proteins become central to mobilizing zinc, regulating gene expression, and sustaining DNA replication and protein synthesis processes essential for fiber quality and seed development [21,22,65].

5. B, Fe, Mn, and Zn Efficiency Genes and Hormonal Regulatory Mechanisms in Plants

Boron B uptake in plants is primarily mediated by the boron transporter (BOR) and nodulin 26-like intrinsic protein (NIP) families, with functional variation observed among species. In cotton (Gossypium), BOR family members have been identified to contribute to B export and tolerance. Specific transporters such as BOR1 and NIP5;1 are directly involved in B uptake, while others regulate B translocation and distribution [5,20,63,66]. Similarly, Fe, Mn, and Zn homeostasis in cotton is mediated by zinc-regulated/iron-regulated transporter-like proteins (ZIP) and natural resistance-associated macrophage proteins (NRAMP) [21,67]. Across plant species related to cotton, 14, 22, and 18 ZIP genes have been identified for Fe, Mn, and Zn transport, respectively, with collinearity analyses highlighting structural and functional conservation among these transporters [21].

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Reactive oxygen species (ROS) further regulate micronutrient balance, with endogenous ROS accumulation enhancing tolerance under low-micronutrient conditions. Adaptive responses to micronutrient stress also include modifications in root morphology, exudation of organic acids, enhanced membrane and intracellular transport, and induction of high-affinity transporter genes, all of which facilitate improved uptake and utilization [66]. In Arabidopsis for example, transcription factors such as FIT, and bHLH regulate Fe deficiency when activated by MYB proteins including MYB30 and MYB121 to enhance its distribution in the plants [68]. Homologous genes in cotton, including GhbHLH121, GhZIP, and GhNRAMP, have been identified as key regulators of micronutrient acquisition and utilization, underscoring conserved regulatory mechanisms across species [20,42,55].

5.1. Hormonal Regulation of B, Fe, Mn, and Zn

Plant hormones act as master regulators of cotton growth and development, coordinating diverse physiological responses to environmental and nutritional signals. Among them, nine major groups, auxin, cytokinins (CTKs), abscisic acid (ABA), ethylene (ETH), gibberellins (GAs), brassinosteroids (BRs), jasmonic acid (JAs), salicylic acid (SA), and strigolactones (SL), play central roles in mediating cotton's adaptation to the availability of B, Fe, Mn, and Zn [69–73]. ABA has been shown to regulate Fe-driven lateral root formation, with ABA-dependent signaling pathways promoting root development and enhancing Fe redistribution from roots to shoots in both cotton and model systems like *Arabidopsis thaliana* (L.) Heynh [71]. CTKs serve as long-distance messengers of nutrient status, relaying Zn and Fe utilization signals via the xylem. Genes such as AtIPT3 and AtIPT5, which govern CTK biosynthesis under Zn and Fe stress in Arabidopsis, appear to function through similar pathways in cotton roots which influences nutrient uptake [72].

The interplay between Fe and GAs has been widely reported, with growth regulator factor 4 (GRF4) identified as a key player in Fe uptake and biomass partitioning in rice; comparable functions are proposed for cotton [73]. BR signaling kinase 3 (BSK3), essential for root elongation under Fe deficiency in Arabidopsis, points to potential BR–Fe crosstalk in shaping cotton root architecture under nutrient stress [35,73]. Likewise, the application of ethephon, an ETH-releasing compound, improves Zn utilization efficiency by enhancing enzyme activity linked to Zn metabolism, thereby increasing Zn and protein concentrations in cotton tissues. Peptide hormones such as CEPD1 and CEPD2, which stimulate IRT1 expression and promote Fe uptake in Arabidopsis, may also have analogous functions in cotton nutrient transport. Boron availability strongly interacts with hormonal pathways regulating root growth and cell wall dynamics. Under B deficiency, SA, ABA, and JA converge on WRKY75, a transcription factor that modulates auxin-mediated lateral root elongation and density, ultimately affecting cell wall stability and root morphology in cotton [69]. In similar way, excess Mn disrupts IAA balance, accelerating IAA oxidation and altering root development, underscoring the crosstalk between hormones and micronutrient signaling [33–35], and more details in Table 2.

Table 2. Key Genes and Hormonal Pathways Regulating **B, Fe, Mn, and Zn** Uptake and Utilization in Cotton and Model Plants.

Nutrient	Gene	Hormone	Pathway Controlled	Plant	Reference
B	BOR1	Auxin (IAA)	Root development and B uptake; regulates B transport under deficiency, interacting with auxin signaling for cell wall integrity	*Arabidopsis thaliana* (L.) Heynh, Cotton	[74,75]
B	NIP5;1	ABA, JA	Stress response and embryogenesis; B deficiency triggers ABA-mediated stress pathways for somatic embryogenesis	*Arabidopsis thaliana* (L.) Heynh	[75,76]

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Fe	IRT1	Ethylene, ABA	Fe uptake and homeostasis; ethylene upregulates IRT1 expression under Fe deficiency, ABA promotes reutilization	*Arabidopsis thaliana* (L.) Heynh, Rice (*Oryza sativa* L), Cotton	[76,77]
Fe	FIT (FER-like)	Auxin, BR	Transcriptional regulation of Fe acquisition; auxin and BR coordinate root elongation and Fe transporter activation	*Arabidopsis thaliana* (L.) Heynh, Cotton	[76,77]
Fe	GhbH LH121	GA	Fe deficiency response regulates upstream Fe uptake genes like FIT in response to GA signaling	Cotton	[20]
Mn	NRA MP1	IAA	Mn homeostasis and toxicity response; Mn excess disrupts IAA oxidation, affecting root growth	*Arabidopsis thaliana* (L.) Heynh, Cotton	[67,78]
Zn	ZIP family (e.g., ZIP2, ZIP4)	Auxin	Zn uptake and hormone biosynthesis; Zn regulates auxin production for root branching and cell elongation	Arabidopsis, Cotton	[21]
Zn	HMA2 /HMA 4	ABA	Zn transport and stress tolerance; ABA-mediated membrane stability under Zn deficiency	*Arabidopsis thaliana* (L.) Heynh	[79]
Zn	GhZIP 3	CTK	Zn signaling from roots to shoots; CTK biosynthesis influences Zn utilization	Cotton	

5.2. B, Fe, Mn, and Zn Signal Integration

The coordinated utilization of B, Fe, Mn, and Zn is essential for sustaining plant growth and maximizing crop productivity. While the signaling pathways of these micronutrients have been extensively studied individually, their interactions remain poorly understood [3,48,80]. Emerging evidence indicates that the integration of B, Fe, Mn, and Zn uptake represents an evolutionary adaptation that enables plants to maintain a balanced nutrient profile [36,43]. Significant progress has been achieved in identifying the key regulatory components governing B–Fe–Mn–Zn interactions in model species such as Arabidopsis and rice [80]. Although research in cotton is limited, available molecular insights suggest promising opportunities to enhance boron use efficiency, iron use efficiency (IUE), manganese use efficiency (MUE), and zinc use efficiency (ZUE) [20,21,34,35,46]. Current findings reveal a complex network that integrates B, Fe, Mn, and Zn signaling pathways, offering a clearer understanding of their interdependence (Table 3).

Table 3. Key Transport Mechanisms and Interactions of B, Fe, Mn, and Zn in Plants.

Nutrient Interaction	Key Components/Signals	Plant	Reference
B-Zn	B transporters (BOR1/NIP5;1) interact with Zn transporters (ZIP family), affecting metabolic processes and uptake of other nutrients like Cu, Fe, Mn	*Arabidopsis thaliana* (L.) Heynh, Cotton	[79]
Fe-Zn	Shared transporters (IRT1, ZIP2/4); FIT transcription factor regulates Fe acquisition with Zn crosstalk; SPX proteins mediate homeostasis	*Arabidopsis thaliana* (L.) Heynh, Rice (*Oryza sativa* L.)	[35,73,81]
Fe-Mn	NRAMP transporters (NRAMP1); Crosstalk in redox homeostasis and toxicity response; MTP11 involved in Mn transport influenced by Fe signals	*Arabidopsis thaliana* (L.) Heynh, Rice (*Oryza sativa* L.)	[35,73]

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Zn-Mn	HMA2/HMA4 transporters; Interaction in stress tolerance and uptake; Overlap in gene regulatory networks (GRNs) under deficiency	Arabidopsis, Sorghum	[79]
B-Fe	BOR1 and IRT1 interaction; Crosstalk in cell wall integrity and Fe deficiency response	*Arabidopsis thaliana* (L.) Heynh	[74]
Multi (Fe-Zn-Mn-Cu)	bHLH transcription factors (e.g., GhbHLH121); Crosstalk via ubiquitin ligases and redox signals	Cotton, *Arabidopsis thaliana* (L.) Heynh	[20,59,68]

5.3. B–Fe–Mn–Zn Signaling Integration in Root Development

The availability of B, Fe, Mn, and Zn influences root architecture, allowing plants to adapt to diverse environmental conditions. Local B supply promotes lateral root elongation [75], while excess Mn induces short, highly branched roots in cotton, altering nutrient uptake [47]. Boron deficiency can have a two-way effect on plant growth, where by it can either promotes or suppresses taproot growth, with inhibition linked to blue light–induced Fe redox reactions near roots that generate reactive oxygen species (ROS), particularly under combined B and Fe deficiency in calcareous soils [42,50,82].

B–Zn interactions further regulate root nutrient dynamics. Adequate B enhances Mn and Fe accumulation while maintaining balanced Mn/Fe ratios, supporting root health. In contrast, B deficiency upregulates ACS11, increasing ethylene biosynthesis, auxin-mediated inhibition of root elongation, and ROS production. Zn supplementation, including ZnO nanoparticles, alleviates B toxicity by improving root biomass, reducing ROS, and enhancing Fe and Mn transporter activity [83]. At the molecular level, the Arabidopsis transcription factor (AtNIGT1) integrates B and Zn signals, with homologs likely functioning in cotton [74,83]. B induces AtNIGT1 via BOR1, while Zn deficiency reduces its stability [84]. This dual regulation modulates taproot growth, with AtNIGT1 suppressing taproot elongation under B deficiency but only in the presence of Zn, coordinating with Fe and Mn signals to maintain root structure. The BOR1-AtNIGT1 module thus links B and Zn cues to downstream regulation of root development [84]. In cotton, B deficiency also enhances Cu, Mn, and Fe accumulation to sustain root function [26]. High planting density reduces root growth due to competition, but B supplementation and growth regulators such as mepiquat chloride improve Mn and Zn uptake, enhancing root morphology [85].

5.4. B-Fe-Mn-Zn Signaling Integration to Regulate Cotton Boll Development

Boron, iron, manganese, and zinc availability plays a pivotal role in boll formation and maturation in cotton, influencing reproductive structures and yield. Adequate boron supply enhances boll weight and reduces boll shedding, while manganese excess can disrupt iron homeostasis, leading to reduced boll nutrient content, as seen in studies where high Mn/Fe ratios impair fiber quality [3,21,22]. Low Zn conditions impair boll development by limiting enzyme activities, with deficiencies exacerbating iron and manganese imbalances that affect cell wall integrity in bolls, particularly under calcareous soils where Zn availability is low [3,11].

In cotton, the interaction between B and Zn affects Fe and Mn content in bolls, with B promoting the transport of Fe and Mn to reproductive tissues, as evidenced by increased boll micronutrient levels under combined B-Zn application [3,47]. Boron deficiency inhibits pollen tube growth and boll setting, but zinc supplementation can mitigate this by stabilizing membrane functions and hormone signaling, leading to higher boll retention and seed quality [21]. Transcription factors like GhbHLH121 integrate Fe and Zn signals to regulate boll gene expression under deficiency, coordinating with boron transporters for nutrient allocation and influencing fiber elongation [20,66]. Optimal B-Zn ratios ensure balanced Mn/Fe uptake, preventing oxidative stress in bolls and improving fiber quality, with foliar applications of Zn, Fe, and B significantly boosting boll yield and micronutrient concentrations.

5.5. B-Fe-Mn-Zn Signaling Integration to Regulate Cotton Response to Stress

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Boron, iron, manganese, and zinc are integral to cotton’s stress response mechanisms, modulating antioxidant defenses and nutrient homeostasis under abiotic stresses like toxicity or deficiency [12,19,86,87]. Boron toxicity elevates ROS levels, but zinc alleviates this by enhancing SOD, POD, and CAT activities, reducing H_2O_2 and MDA in roots and leaves, as demonstrated in cotton seedlings where ZnO nanoparticles improve tolerance [65,69,88]. Manganese and iron interactions under stress influence redox balance, with excess Mn disrupting Fe uptake and exacerbating oxidative damage, particularly in acidic soils where Mn toxicity is prevalent in cotton fields [35].

In cotton, ZnO nanoparticles upregulate ABC transporter genes and photosynthesis pathways to counter boron stress, integrating signals for nutrient redistribution and stress tolerance [65,86]. Boron interacts with Zn to modulate hormone signaling (e.g., jasmonic acid), enhancing resilience to toxicity, with foliar Zn reducing B-induced growth inhibition. Under calcareous soil stress, balanced B-Zn-Fe-Mn ratios improve membrane integrity and reduce susceptibility to environmental cues like waterlogging or salinity, as nano-Zn applications mitigate salt stress by maintaining ionic homeostasis [23]. Heat stress responses are alleviated by micronutrient sprays, including Mn and Zn, which upregulate defense genes and reduce oxidative damage in cotton [1]. More details are available on Table 4.

Table 4. Integrated Roles of Boron, Iron, Manganese, and Zinc Signaling in Regulating Gene Expression and Stress Responses in Cotton and Related Crops.

Nutrient	Gene	Environmental Change	Response	Plant	Reference
B	BOR1/Bot1 ortholog	Boron deficiency/toxicity	Inhibits root cell elongation via ethylene/auxin/ROS pathway; upregulates transporters for tolerance	*Arabidopsis thaliana* (L.) Heynh	[12,83]
B	ACS11	Low boron availability	Upregulates ethylene biosynthesis, reducing root growth and increasing ROS	Tomatoes (*Solanum lycopersicum* L), Cotton	[50,86]
Zn	ZIP family (e.g., ZIP transporters)	Zinc deficiency under salinity, and toxicity	Enhances Zn uptake, stabilizes membranes, mitigates B toxicity by reducing ROS	Cotton	[65,86]
Fe	IRT1/FIT homologs	Iron deficiency with high Mn	Coordinates Fe acquisition, prevents Mn-induced oxidative stress in roots and bolls	Cotton, *Arabidopsis thaliana* (L.) Heynh	
Mn	NRAMP1/MTP11	Manganese excess/toxicity	Regulates Mn transport and sequestration, disrupts Fe homeostasis leading to reduced boll development	Peanuts *Arachis hypogaea* L, Stylosanthes ’ Soybean (*Glycine max* (L.) Merr)	[35,36,81]
B-Zn	ABC transporters	Boron toxicity with Zn supplementation	Upregulates nutrient redistribution, enhances antioxidant enzymes (SOD, POD, CAT) for stress tolerance	Cotton	[65]
Fe-Mn	GhbHLH121	Combined Fe-Mn deficiency in bolls	Integrates signals for fiber quality, regulates cell wall genes under nutrient stress	Cotton	[4,65]

B-Fe	Cell wall-related genes (e.g., expansins)	Boron deficiency roots	Down-regulates expression, impairs root elongation and boll cell integrity	Cotton, *Arabidopsis thaliana* (L.) Heynh	[4,54]

5.6. B–Fe–Mn–Zn Signaling Integration Regulating Nutrient Uptake

The AtNIGT1s family (AtNIGT1.1–1.4) plays a central role in nutrient regulation by repressing B-starvation–induced genes (e.g., *BOR1*, *NIP5;1*) and controlling Zn-starvation–responsive genes (*ZIP2/4*, *HMA2/4*), thereby enhancing Zn utilization. AtNIGT1 expression is induced by high B and Zn deficiency and regulated by transcription factors AtbHLH121 and AtMYB13, linking B and Zn signaling pathways [41,79,89]. Boron directly promotes Zn utilization [3,47]. In rice and cotton, the B transporter *BOR1* activates Zn-starvation induced (ZSI) genes by interacting with SPX4-like proteins, leading to SPX4 degradation, MYB translocation into the nucleus, and induction of Zn-responsive genes[42,53,74]. This BOR1–SPX4–MYB axis exemplifies B–Zn crosstalk, where boron signaling co-activates both B- and Zn-responsive pathways.

The bHLH transcription factors also contribute to B signaling via cytoplasmic–nuclear shuttling, while SPX4 coordinates MYB13 (Zn signaling) and bHLH121 (B signaling), forming a unified BOR1–SPX4–bHLH121–MYB13 cascade that integrates B and Zn responses [20,66]. BOR1 and SPX4 are regulated through ubiquitination by plasma membrane–localized E3 ubiquitin ligases. Under toxic B conditions, BOR1 is targeted for degradation, preventing excess B accumulation while linking B perception to Zn signaling [65,69,88]. This BOR1–SPX module transduces B signals to core transcription factors, enabling synergistic regulation of B- and Zn-responsive genes [90]. Downstream, MYB, bHLH, and NIGT1 sustain B–Zn homeostasis under fluctuating environments [91].

Beyond Zn, B also influences Mn utilization by regulating Mn concentration and assimilation. Boron reduces free Mn levels, represses *NRAMP1*, and activates *MTP11*, while stimulating Mn superoxide dismutase activity. This demonstrates B's role in linking Mn and Zn regulation [92]. In cotton, B–Fe–Mn–Zn integration extends to macronutrient uptake. B enhances N and K absorption but reduces P and Fe translocation under high B. Zn promotes N and K uptake while antagonizing P and Fe, affecting S metabolism through enzyme activation [3,15]. Although the molecular basis of B–Fe–Mn–Zn signaling in cotton remains limited and unclear, evidence from model plants shows these micronutrients function both independently and interactively, coordinating with macronutrient (N, P, K, S) uptake (Table 5).

Table 5. Interactions of Boron, Iron, Manganese, and Zinc with Macronutrient Uptake and Their Effects on Cotton Growth and Fiber Quality.

Micro	Macronutrient influenced	Growth and yield trait	Key Findings	Reference
B	N, P, K, S	Vegetation, Flowering, Pods, Fiber Quality	B enhances N and P metabolism, promotes K translocation; deficiency reduces pod set and fiber strength by disrupting S-involved enzymes.	[3,12,29]
Fe	N, P, K, S	Vegetation, Nodes, Fiber Quality	Fe deficiency antagonizes P uptake, reduces N assimilation; impacts node development and fiber maturity via redox effects on S.	[22,29,32]
Mn	N, P, K, S	Vegetation, Pods, Pod Abortion, Fiber Quality	Mn synergizes with K for vegetation growth; excess antagonizes Fe and P, increasing pod abortion and reducing fiber quality via S oxidation imbalance.	[1,34–36,46,78]

Zn	N, P, K, S	Flowering, Nodes, Pods, Pod Abortion, Fiber Quality	Zn positively interacts with N and K for flowering and node formation; antagonizes P, reducing pod abortion; enhances fiber quality by improving S metabolism.	[3,11,23,37,58,86]

6. Genomic and Phenotyping Approaches for Breeding Nutrient-Efficient Cotton Varieties

Rising cotton yields have been accompanied by greater inputs of micronutrients such as boron B, Fe, Mn, and Zn, creating challenges including soil imbalances, potential toxicity, and low nutrient use efficiency (NUE), which increases production costs. Natural variation in micronutrient efficiency among cotton genotypes provides an opportunity to breed varieties with both high yield potential and improved NUE. High-throughput phenotyping, particularly using unmanned aerial vehicles (UAVs) equipped with multispectral and hyperspectral sensors, now enables precise assessment of chlorophyll content, biomass, and micronutrient status [93]. These technologies allow detection of nutrient deficiency symptoms, such as B-induced boll shedding or Fe-related chlorosis and support the identification of nutrient-efficient genotypes [93]. However, cotton's complex canopy and inconsistent correlations between biomass and yield under variable micronutrient conditions remain challenges [94]. Advances in UAV imaging and precision cameras are expected to overcome these limitations, enabling more accurate trait measurement [95,96].

Integrating UAV-based phenotyping with genome-wide association studies (GWAS) facilitates the discovery of genes controlling B, Fe, Mn, and Zn efficiency [93]. Field trials with gradient micronutrient treatments can further improve screening accuracy while conserving resources. Genes such as *GhbHLH121* (Fe regulation) and *GhZIP3* (Zn uptake) enhance NUE and yield stability under deficiency, while *BOR1* and *NRAMP1* regulate B and Mn transport, influencing boll development and nutrient allocation [20,66].

Breeding strategies focus on incorporating such genes into elite cotton lines. For example, overexpression of *BOR1* reduces B deficiency–related pod abortion, while enhanced *IRT1* expression improves Fe and Zn uptake under calcareous soils [42,53,74,97]. While marker-assisted selection (MAS), CRISPR-based editing, and transgenic approaches targeting genes such as *GhMTP11* (Mn homeostasis) are producing varieties with balanced micronutrient uptake and improved fiber quality [81]. Lessons from model plants like *Arabidopsis* and rice continue to inform these efforts (Table 6).

Table 6. Key Genes Regulating Boron, Iron, Manganese, and Zinc Use Efficiency and Their Roles in Cotton Growth and Yield.

Nutrient	Gene Example	Influence on Nutrient Use Efficiency	Reference
B	BOR1 transporters and miR408 genes	Enhances boron uptake under B deficient soils, reduces pod abortion, and improves cell wall integrity under deficiency; critical for reproductive growth.	[88,97]
B	NIP5;1	Facilitates B transport under low B conditions, improving root and boll development; enhances BUE.	[98]
Nutrients	PIP1;1, PIP2;1, PIP2;2	Enhances nutrient uptake and stress tolerance traits of cotton	[87]
Fe	IRT1	Improves Fe uptake in calcareous soils, reduces chlorosis, and enhances N and P assimilation for better vegetation and fiber quality.	[99]

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Fe	GhbHLH121	Regulates Fe deficiency responses, enhances Fe and Zn homeostasis, improving boll development and yield stability.	[100]
Mn	NRAMP1	Controls Mn uptake and homeostasis, prevents toxicity, and supports K and S metabolism for pod retention.	[101]
Mn	GhMTP11	Enhances Mn sequestration, reduces oxidative stress, and improves pod and fiber quality under Mn excess.	[101]
Zn	GhZIP3	Boosts Zn uptake and signaling, improves N and K assimilation, reduces pod abortion, and enhances fiber length.	[102]
Zn	HMA4	Facilitates Zn transport, enhances stress tolerance, and supports S metabolism for improved flowering and node formation.	[79]

7. Future Research Directions for B, Fe, Mn, and Zn Efficiency in Cotton

Although the physiology of B, Fe, Mn, and Zn uptake, assimilation signaling, and use efficiency in cotton is increasingly getting attention, their genetic, hormonal pathways and technological advances remain underexplored. Insights from other plants highlight that these could improve cotton's nutrient use efficiency (NUE) and yield stability. The future research should focus on high-throughput phenotyping using UAV-based imaging, combined with genome-wide association studies (GWAS), among other molecular techniques in identifying nutrient-efficient genotypes. Breeding strategies incorporating marker-assisted selection and CRISPR editing, for instance, overexpressing *BOR1* or *IRT1*, or editing *GhMTP11*, can enhance micronutrient uptake, fiber quality, and stress tolerance. Research focusing on nutrient interactions with macronutrients (N, P, K, S), adaptation to environmental stresses, and sustainable nutrient management is highly recommended. Integrating genetic improvements with precision fertilization, biofortification, and sensor-guided application can optimize nutrient efficiency, reduce environmental impact, and support high-yield, resilient cotton production

8. Conclusions

In conclusion, this review synthesizes the pivotal roles of boron, iron, manganese, and zinc in cotton physiology, signaling integration, and nutrient use efficiency, addressing overlooked yield gaps in macronutrient-focused programs. Key findings demonstrate that these micronutrients regulate photosynthesis, hormone signaling, stress responses, and reproductive success, with deficiencies exacerbated by soil pH and interactions causing hidden hunger; genes such as BOR1, IRT1, and GhZIP3 provide targets for optimization. Future directions include leveraging microRNAs, CRISPR gene editing, UAV phenotyping, and precision nutrition to enhance efficiency, mitigate environmental challenges, and sustain high-yield cotton production.

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Abbreviations

NUE	**Nutrient Use Efficiency**
BUE	Boron Use Efficiency
IUE	Iron Use Efficiency
MUE	Manganese Use Efficiency
ZUE	Zinc Use Efficiency
N, P, K, S	Nitrogen, Phosphorus, Potassium, Sulfur
	Molecular/Genetic Acronyms
IRT1	Iron-Regulated Transporter 1
BOR1	Boron Transporter 1
NIP5;1	Nodulin 26-like Intrinsic Protein 5;1
ZIP	Zinc/Iron-Regulated Transporter-like Protein family
NRAMP1	Natural Resistance-Associated Macrophage Protein 1
MTP11	Metal Tolerance Protein 11
HMA2/HMA4	Heavy Metal ATPase 2/4
GhbHLH121	Basic Helix-Loop-Helix transcription factor in cotton
GhZIP3	Zinc Transporter gene in cotton
GhMTP11	Cotton Metal Tolerance Protein gene
miRNAs	MicroRNAs
miR169, miR398, miR408	Specific microRNAs involved in nutrient regulation
NFYA	Nuclear Factor Y subunit A
ROS	Reactive Oxygen Species
RG-II	Rhamnogalacturonan II
SOD	Superoxide Dismutase
POD	Peroxidase
CAT	Catalase
GhbHLH121	Basic Helix-Loop-Helix transcription factor in cotton
GhZIP3	Zinc Transporter gene in cotton
GhMTP11	Cotton Metal Tolerance Protein gene
	Hormone and Signaling Acronyms
ABA	Abscisic Acid
CTK / CTKs	Cytokinins
ETH	Ethylene

GA / GAs	Gibberellins
BR / BRs	Brassinosteroids
JA / JAs	Jasmonic Acid
SA	Salicylic Acid
SL	Strigolactones
IAA	Indole-3-Acetic Acid (Auxin)
	Technology Acronyms
UAVs	Unmanned Aerial Vehicles
scRNA-seq	Single-Cell RNA Sequencing
GWAS	Genome-Wide Association Studies
MAS	Marker-Assisted Selection
CRISPR	Clustered Regularly Interspaced Short Palindromic Repeats

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