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Genetic and Physiological Architecture of Aluminum Tolerance in Maize: From QTL Hotspots and Candidate Loci to Causal Genes and Breeding Prospects

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Abstract: Aluminum toxicity limits root growth in acid soils, and maize has several mechanisms for dealing with it. This critical evidence-mapping review examines what segregation studies, quantitative trait locus mapping, gene-function work, and root physiology tell us about tolerance, with a particular focus on the distinction between biochemical activity, causality for natural allelic variation, and field value. The evidence is drawn from the existing literature and targeted checks of the primary papers published to October 2026. Historical screening data were not reconstructed, so no claims are made to systematic or Preferred Reporting Items for Systematic Reviews and Meta-Analyses Extension for Scoping Reviews coverage. Among the candidate genes, ZmMATE1 is supported by the most compelling evidence. Findings from aluminum-induced citrate exudation, variation in gene copy number and expression, and analyses of near-isogenic maize lines all suggest that the quantitative trait locus qALT6 contributes to aluminum tolerance in particular genetic backgrounds. Nevertheless, variation independent of qALT6 has been observed in some maize populations, indicating that the roles of other candidate genes are still not fully resolved. Work on native maize has implicated another gene, ZmPGP1, in auxin-mediated root growth responses and ZmAT6 in protection against aluminum-associated cellular injury. Heterologous work supports ZmMATE6, ZmXTH, ZmNRAMP4 and ZmALDH as candidate genes, but there is no evidence for natural allelic causality or for any field yield benefit in relation to any of these genes. We conclude that maize tolerance is the outcome of three somewhat overlapping processes: limiting Al activity outside the root, protecting root apex growth, and managing aluminum-associated cellular injury. A useful breeding target will be one that improves performance under a well-defined Al challenge, that effect is retained in independent genetic backgrounds and that results in an improvement in grain yield on characterized acid soils. This framework defines the experiments needed to distinguish a promising mechanism from a deployable allele.)

Keywords: *Zea Mays; Aluminum Toxicity; Acid Soils; Zmmate1; Citrate Exudation; Quantitative Trait Loci; Maize Genetics; Root-Apex Physiology; Gene Validation; Phosphorus Acquisition.*

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I. INTRODUCTION

Aluminum (Al) is naturally present in soil minerals. As the soil pH falls below 5.0, the amount of Al that enters solution increases, and this can be injurious to plants. In maize, the response of the growing root apex is very rapid: there is a reduction in cell elongation, a decline in root exploration, and a consequent difficulty in taking up water and nutrients. Phosphorus deficiency and irregular soil moisture will often exacerbate the damage. pH is of course only part of the picture. The amount of free, phytotoxic Al present is also a function of the organic ligands, ionic strength, and other cations [1,2,3].

Maize genotypes vary enormously in their capacity to maintain root growth in the presence of Al. Early segregation studies in some crosses suggested control by one or a few major genes. Subsequent quantitative trait locus (QTL) and association studies have shown a much broader genetic basis, with genes involved in organic acid transport, root development, cell walls and oxidative stress responses [4,5,6,7]. The findings of the genetic investigations are fully consistent with this interpretation. A major allele can be the sole determinant of segregation in one family, whilst smaller effects will be present across a wider range of germplasm.

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ZmMATE1 is the most compelling molecular example of maize Al tolerance. The gene works by promoting the release of citrate from the root, which in turn reduces the activity of the toxic Al in the immediate vicinity of the root apex. A Kenyan mapping population, however, failed to identify any significant chromosome 6 QTL and showed dramatically lower levels of parental ZmMATE1 expression than in the tolerant Cateto-derived line [8,9,10,11]. The two observations are fully compatible and serve to underline the point that the question is not simply which genes are responsive to Al, but which alleles are important in a given genetic background and whether their effects are actually seen under the soil conditions that farmers encounter.

Three questions underpin this review. How do the inheritance patterns and QTLs vary with the population and assay? Which of the putative genes have been tested directly in maize and which are based on heterologous work (i.e. mapping, expression, orthology etc)? And what would be required to actually translate these genetic and physiological findings into robust acid soil adaptation, without making any assumptions about unproven effects on P use or grain yield?

II. REVIEW APPROACH, EVIDENCE BOUNDARIES, AND TRANSPARENCY

We employed a critical narrative evidence-mapping approach. The source bibliography was assessed in conjunction with targeted searches and checks on selected primary papers with a focus on inheritance experiments, maize MATE transporters, root wall and redox work, the Kenyan QTL work, and more recent tropical maize phenotyping and association analyses. Our search concepts were maize, Zea mays, aluminium/aluminum toxicity and tolerance, acid soil, relative root growth, QTL, GWAS, MATE, ALMT, NRAMP, cell wall, ROS, STOP1 and phosphorus. Where possible, we checked references against journal, bibliographic, and institutional records. Given that full database exports and inclusion/exclusion decisions are not available, this is a purposive rather than an exhaustive approach. The conclusions are about the strength and breadth of the evidence as we have assessed it rather than the frequency of results in a complete systematic corpus [12,13].

For the maize allele statements we put the primary maize data first. The data from other species can be used to provide a plausible biochemical explanation, but they do not in any way prove that the same pathway is responsible for tolerance in maize. The reviews are purely for context. We also ensured that the effects reported are linked to the original populations, the original treatments, and the original measurements. Root growth percentages from different assays should not be combined as if they were measuring the same thing. Appendix A contains the information required for the subsequent formal scoping review.

For each gene, we specify what was actually tested. A is native maize functional perturbation with an Al-related phenotype, B is a functional experiment in a heterologous system, C is genetic mapping or association study, and D is candidate evidence from expression, location, or orthology. Fig. 1 also shows E, which is mechanistic hypotheses derived from models or other species. These classes define the scope of inference rather than representing a universal scale of study quality. A gene can have very good transport biochemistry and still have no evidence that its native maize orthologs are the ones that confer Al tolerance.

III. EVIDENCE FROM INHERITANCE STUDIES AND GENETIC MAPPING

Understanding the genetic basis of aluminum (Al) tolerance in maize requires integrating evidence from several complementary lines of investigation, including inheritance studies, QTL mapping, association analyses, and functional characterization of candidate genes. Each approach contributes different types of information, ranging from the identification of genomic regions associated with tolerance to the validation of underlying molecular mechanisms. However, the strength of inference that can be drawn from these studies varies according to the genetic materials, experimental designs, and analytical methods employed. The following sections critically examine the scope, strengths, and limitations of the available evidence and consider what each category of study can reliably establish regarding the genetic and physiological basis of Al tolerance.

➢ *Scope and Limitations of the Evidence*

The body of evidence that underpins the inheritance of Al tolerance is drawn from a range of different studies, which vary in both the genetic material that they have looked at and the questions that they have sought to answer (Table 1). The earliest segregation work looked at a very limited number of crosses. QTL work subsequently utilized F2 families, F2:3 derivatives and RILs. Association studies have looked at a greater number of alleles, of course, but have also brought with them the well-known issues of population structure and linkage disequilibrium. Transport assays in oocytes, yeast or other model systems have established the biochemical activity in the trait. Native maize studies have been relatively rare. There is still a paucity of direct links to grain yield on well-characterized acid soils (7,8,14).

Experimental conditions add another domain of variation. The hydroponic studies differ in nutrient formulation, nominal Al concentration, exposure length, and root growth endpoint. Simply stating the AlCl3 concentration says nothing about the free Al^{3+} activity without the solution chemistry. Seedling elongation is of some use but cannot be used as a proxy for yield when the soils also differ in available phosphorus (P), exchangeable acidity, water, and biological activity. Two recent studies on 250 tropical inbreds have gone further with respect to root phenotyping and Genome-Wide Association Study (GWAS). They use the same germplasm and very similar methods so they are not really independent replications [15,16].

➢ *What Each Type of Evidence can Establish*

The evidence answers different questions. Treating them as the same creates a false sense of certainty. A QTL is only a chromosomal region that is associated with a trait of interest. A transport function assay shows that a protein can perform

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the function under the assay conditions. An overexpression phenotype shows that extra gene activity will change the response (sometimes in a different species). A native maize knockout or complemented mutant can go some way to establishing necessity. That said, the link to a naturally segregating favorable allele still has to be established. For a breeding claim, the ultimate test is an allele contrast that shows a difference in performance in the relevant germplasm rather than just a correlation with an unrelated linked variant [7,8,9,10].

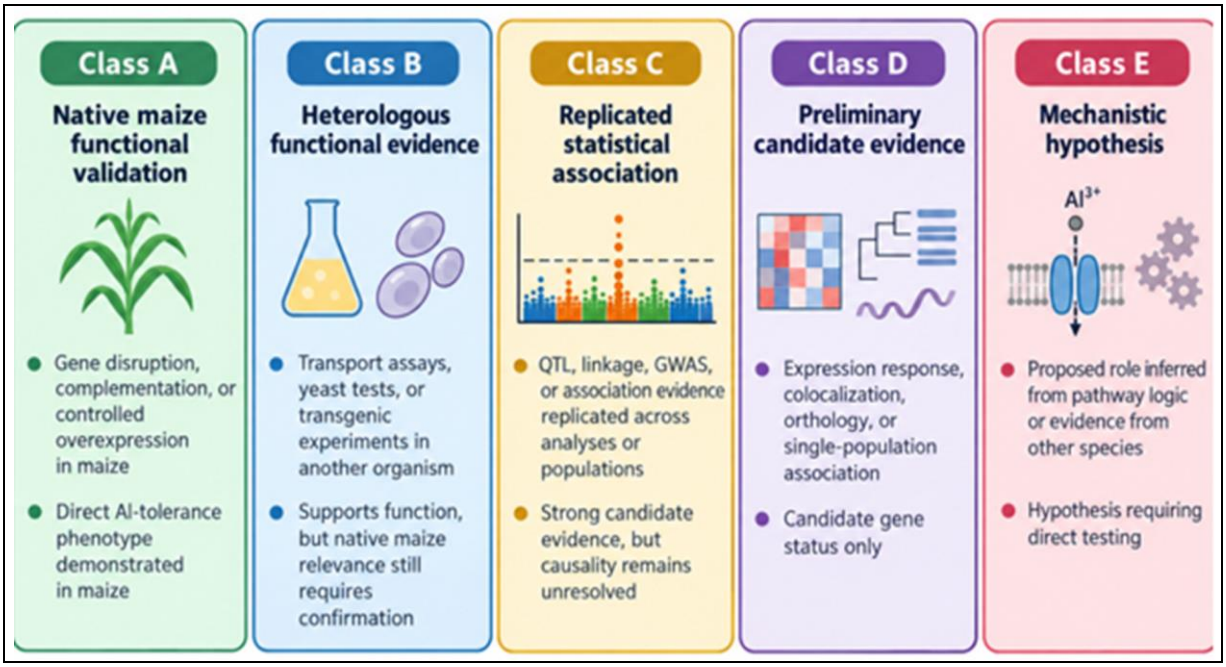

Fig 1 Operational Evidence Classes for Genes Associated with Aluminum Tolerance in Maize. These Operation Classes Refer to the Claim Tested and are Supported by Evidence, Not the Intrinsic Quality of a Study

Table 1 Key Original Studies Underpinning the Evidence Base for the Inheritance of Aluminum Tolerance

Study	Genetic material used	Principal approach	Key findings	Interpretive limit
[4]	Segregating maize crosses	Root response in nutrient solution	Major-locus segregation within studied crosses	Not population-wide genetic architecture
[17]	Cat-100-6 × S1587-17	RFLP/BSA, Al response	Two linked regions on chromosomes 6 and 10	Gene labels require direct map verification
[6]	Al237 × L53	168 $F_{3:4}$ families; root growth	Five QTL on chromosomes 2, 6 and 8	Population and assay dependent
[7]	282 inbreds; three linkage sets	Candidate association plus linkage	ZmASL, ALMT2, SAHH and ME candidates	Not equivalent to gene cloning
[8,9]	Al237-derived and diverse germplasm	Transport, transgene and CNV	ZmMATE1 citrate transport; three-copy allele	Allele distribution and mechanism differ
[10]	Cateto-derived recombinant lines/NILs	High-density SNPs; NILs	qALT6 validated; ZmMATE2 unlikely qALT5 cause	ZmNrat1 distant from qALT5 in this map
[11]	Kenyan maize cross	QTL and expression	Five QTL, four epistatic interactions	Candidate genes need functional tests
[14,22,23]	Maize and heterologous systems	Native/heterologous transgenes	ZmAT6, ZmMATE6, ZmXTH, ZmALDH	Evidence levels are heterogeneous
[15,16]	250 tropical inbreds	Root imaging and GBS GWAS	Trait variation; 44 significant SNPs	Paired investigations, not independent replication

The distinction is why a candidate gene list can grow faster than biological understanding. As mapping resolution increases with more markers, several correlated Single Nucleotide Polymorphisms (SNPs) can tag a single causal haplotype. Conversely, a structural variant such as the ZmMATE1 triplication may not be well represented by standard SNP genotyping. The most compelling studies are those that use complementary approaches to rule out alternative explanations rather than simply amassing a number of measurements of the same association.

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IV. FROM MAJOR-GENE SEGREGATION TO QUANTITATIVE INHERITANCE

Early inheritance studies proposed one or a few major Al-tolerance loci. Reference [4], found a major dominant component in certain maize crosses and Prioli et al. (2002) reported a segregation pattern that was compatible with relatively simple control in their material. These are the alleles segregating between those parents, not all the genes that would be involved across the species. The thresholds used to define tolerance, dominance, environmental variation, and parental divergence can all have an effect on the segregation ratio that is observed [19].

Reference [17] used bulked segregant analysis and Restriction Fragment Length Polymorphism.(RFLP) markers to map two regions on chromosomes 6 and 10 and reported a much stronger effect on chromosome 10 in the cross they studied. The historical Alm1 and Alm2 labels they proposed for identifying the regions have not been used consistently in the subsequent literature. We therefore just stick with the chromosome locations and do not attach any uncertain gene label to either locus. The significance of their work is primarily that they identified segregating regions of substantial effect, rather than any proof that two genes account for Al tolerance across the maize germplasm.

Evidence for quantitative inheritance became increasingly clear as more diverse families and continuous root growth traits were studied. Reference [5] provided evidence of genetic variation and additive effects. In 168 F3:4 families derived from the Al237 x L53 cross, [6] identified five QTL on chromosomes 2, 6 and 8. Together, these accounted for approximately 60% of the variation in that experiment. The reported proportion of phenotypic variation explained is specific to the evaluated germplasm and the particular genotyping and analytical approaches employed. Consequently, it does not provide a comprehensive explanation of the genetic basis of aluminium tolerance across the broader maize genome. Reference [19] also found that the pattern of inheritance was a function of the tropical material and the experimental design used.

Reference [10] fine-mapped Cateto-derived regions using dense SNP markers and near-isogenic lines, which provided further support for the QTL qALT6 on chromosome 6. A rather different picture came out of Kenya, where [11] identified five QTLs on chromosomes 1, 5, 8, 9 and 10, and four epistatic interactions. These explained about 51% of the phenotypic variance in their population. There was no significant QTL on chromosome 6, and the level of parental ZmMATE1 gene expression was considerably lower than in Cateto Al237. Again, this highlights genetic variation between the populations rather than some fundamental contradiction in the biology.

Even within a population, the QTL effects tend to be stress-dependent, based on intensity of stress, assay duration, and experimental precision. A gene which protects the root apex in nutrient solution may be of little consequence in a limed field. For breeding purposes, the practical consideration is to validate the haplotypes in the germplasm of interest, replicate the mapping, and compare the same physiological traits under the same conditions. Table 2 summarizes the main inheritance and mapping results.

The apparent conflict in findings between the major gene and the quantitative inheritance model disappears once the unit of inference is specified. A biparental cross only samples the differences between the two parents. If one of the parents has a large effect tolerance allele, then its segregation in the cross can give rise to classes that resemble a Mendelian trait. A broad panel, by contrast, has many alleles, many different haplotype backgrounds, and many allelic combinations that cannot be captured by a single cross. The root growth thresholds also compress continuous variation into discrete classes. The genetic model is therefore best viewed as a description of the population that was actually tested rather than a fixed property of the species [4,5,6,11].

This has a very practical implication. A marker that predicts tolerance in the Cateto material may well be a non-predictor in some breeding populations if the favorable allele is not present or if its effect is dependent on different background loci. The lack of a qALT6 signal in [11] not in any way undermine the functional evidence for ZmMATE1 elsewhere. These two results point to two very different genetic pathways, and it makes sense to first characterize the alleles actually present in each breeding population. Successful deployment of an allele will of course require that kind of local genetic diagnosis.

V. THE PHYSIOLOGICAL ARCHITECTURE OF ALUMINUM TOLERANCE

Aluminum injury at the root apex is the convergence several distinct processes (Fig. 2) that are often studied in isolation. Al can bind to the negatively charged cell wall components, disrupt membrane-associated activity, alter cytoskeletal organization, and inhibit elongation or division. The distal transition zone in maize is particularly sensitive [1]. Tolerance will depend on how much active Al gets to that zone and on the ability of the surrounding tissues to carry on growth. The mechanisms described below are all plausible in maize to varying degrees, but they do not all have equally strong genetic evidence [2,3].

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Table 2 Inheritance Models and Genetic Mapping Results for Aluminum Tolerance in Maize

Inheritance or mapping signal	Reference	Experimental basis	Supported inference	Qualification
Major-effect segregation	Rhue et al., 1978; Prioli et al., 2002	F_2 and backcross response	Can resemble Mendelian control in the tested cross	Dominance and threshold effects vary
Two-locus linkage	Sibov et al., 1999	F_2 BSA/RFLP	Chromosomes 6 and 10; chromosome 10 stronger	Historic gene-label assignment unresolved
Five mapped QTL	Ninamango-Cárdenas et al., 2003	Al237 × L53, $F_{3:4}$	Chr 2, 6, 8; ~60% variance in that population	Do not generalize R^2 to all germplasm
qALT6 validation	Maron et al., 2013; Guimarães et al., 2014	CNV, expression and NILs	Three-copy ZmMATE1, citrate-related tolerance	Single-copy high-expression alternatives exist
Non-qALT6 variation	Matonyei et al., 2020	Kenyan QTL and epistasis	Five QTL; four interactions; ~51% explained	Candidates not yet causal genes
Genome-wide associations	Channapur et al., 2026b	250 tropical inbreds, 60,227 SNPs	44 SNP associations; eight QTL clusters	Seedling root architecture, not field yield

- *Organic-Acid Exclusion at the Root–Rhizosphere Interface*

Citrate released from root apices binds Al^{3+} in the rhizosphere and can thus reduce the concentration of phytotoxic Al^{3+} ions within the vicinity of the sensitive tissues. Of the proposed exclusion mechanisms this is the one with the most genetic support in maize. However, it is not universal. Reference [20] found that citrate efflux did not consistently correlate with tolerance across six maize genotypes. That result prefigured the non-qALT6 variation later identified in the Kenyan material by [11].

Malate efflux represents a plausible mechanism for aluminum detoxification; however, the evidence supporting a role for the associated gene, ZmALMT2, remains less conclusive than that for some other candidate genes. The gene encodes an anion transporter, and its expression in a heterologous Arabidopsis system results in constitutive malate exudation. Its expression pattern in maize is not what would be expected for a major exclusion locus (i.e., root tip-localised, or Al-induced), and the original paper that characterized it in fact cast some doubt about its role in Al resistance [21]. Family membership and the measured substrate transport are therefore not enough to assign a natural tolerance allele

- *Cell-Wall Retention and Remodeling*

The apoplast contains pectins and hemicelluloses, which can bind Al^{3+}. How much Al^{3+} is left in the wall is a function of genotype, tissue, and treatment, as well as the method of measurement used. A single figure such as 85-90% cannot apply to all maize roots. Bound Al^{3+} has an effect on wall extensibility. The degree of pectin methylesterification in turn affects the amount of carboxyl groups available for binding. These are all plausible explanations for the differences in root growth, although scientists still lack direct causal evidence for particular maize alleles [2].

The maize gene ZmXTH encodes a xyloglucan endotransglucosylase or hydrolase. Reference [22] expressed this gene in Arabidopsis and found that the increased root growth and lower Al accumulation in both roots and walls of the transgenic plants. That finding is consistent with a protective effect in Arabidopsis, but it does not establish that naturally occurring ZmXTH alleles in maize provide protection, nor can it be extrapolated to all XTH genes. Another gene, SAHH, identified by [7] via association and linkage analysis is involved in S-adenosylhomocysteine turnover, but there is no demonstrated causal pathway from variation SAHH to pectin methylesterification and hence lower Al^{3+} binding in maize. These are all plausible explanations for the differences in root growth, but scientists still lack direct causal evidence for particular maize alleles [2].

- *Internal Aluminum Handling and Detoxification*

Once Al^{3+} has crossed the plasma membrane, the cell may reduce its toxicity by ligand binding, moving it to a less sensitive compartment, or sequestering it. Rice's OsNrat1 gene has demonstrated a well-established role in Al^{3+} uptake, which involves transporting Al^{3+} into root cells, where the aluminum can then be detoxified and ultimately sequestered in vacuoles. This process reduces the accumulation of toxic aluminum in the root cell wall, thereby protecting root growth under acidic soil condition. However, thismechanismt does not extend to the gene's maize homolog. In the [10] population, the ZmNrat1 homolog was located some 40Mb away from the qALT5 region, making the researchers conclude that it was probably not the causal gene underlying qALT5, even tough it showed Al-induced expression in the root tips.

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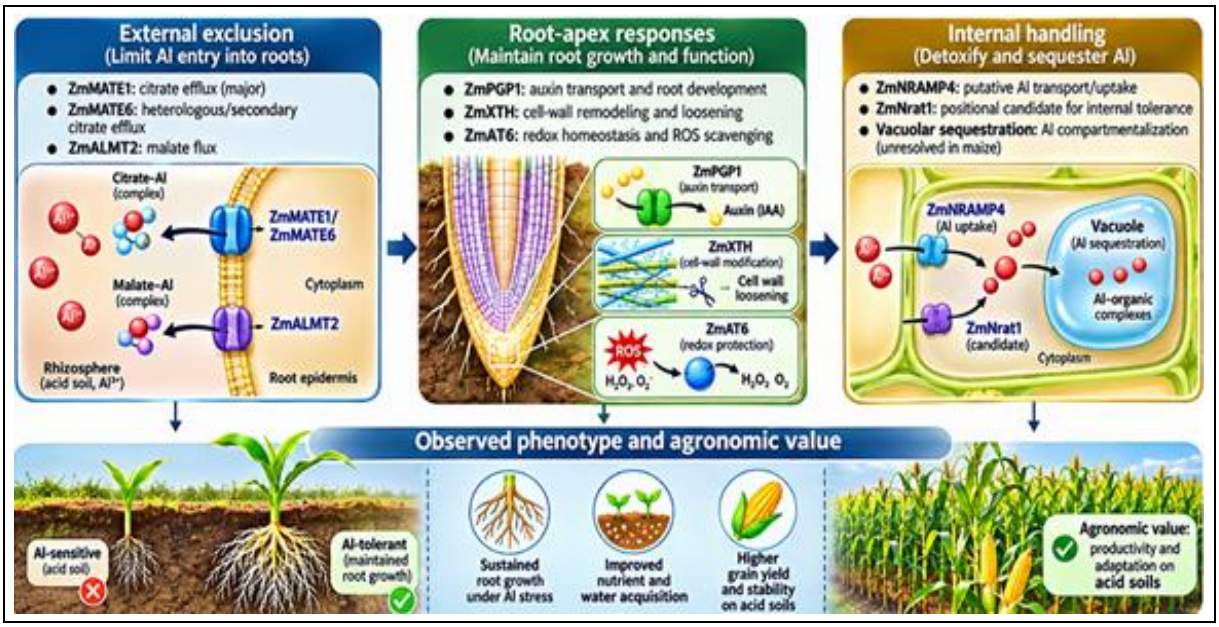

Fig 2 Conceptual Framework Illustrating the Integrated Physiological Pathways Potentially Underlying Aluminum Tolerance in Maize. Dashed Connectors Represent Putative Mechanistic Intersections and Proposed Links Between Physiological Responses and Field Performance that Remain to be Established Through Causal Testing

The finding highlights the importance of keeping QTL interpretation firmly rooted in the mapping population used. In yeast and transgenic Arabidopsis, the gene ZmNRAMP4 was associated with Al transport and improved Al tolerance, so it is considered a credible candidate for internal aluminum handling [23]. The cellular compartments in the maize root where this occurs are still to be defined. Increased cellular uptake would only be protective if it were followed by transport, complexation, and/or sequestration to prevent damage at sensitive sites. A direct test of this function would be to link the transported substrate to intracellular localization and a maize root phenotype. Without that full chain of evidence, "internal Al detoxification" remains just a working hypothesis rather than a verified Al-tolernace pathway.

➢ *Root Growth Regulation and the Auxin Response*

An ATP-Binding Cassette-type auxin efflux transporter gene, ZmPGP1, links Al injury to root development. In maize mutants, [24] found that aluminum treatment led to increased auxin efflux, depletion of auxin at the root apex and reduced elongation. Loss-of-function mutants responded quite differently to Al3+, with no reduction in root elongation. This is direct evidence of hormonal control of the root growth response in maize. However, it would be fallacious to interpret this as proof that ZmPGP1 is a gene that transports Al3+ out of the root.

How tolerance is measured is important. A genotype with fast-growing control roots may have longer roots under stress but the same proportion of root growth inhibition as a slower-growing genotype. Relative root growth and root regrowth thus capture different responses. Therefore, both stressed and unstressed measurements should be reported rather than be collapsed into a single index.

➢ *Redox Protection and Aldehyde Metabolism*

Aluminum exposure can lead to increased reactive oxygen species (ROS), lipid peroxidation, and membrane damage. Overexpression of the gene ZmAT6 in maize and Arabidopsis improved root growth and reduced Al accumulation, probably due to reduced ROS and malondialdehyde and increased antioxidant enzyme activity [14]). While this finding further supports a protective role for ZmAT6 in maize, it does not show that the gene facilitates ROS scavenging. Its effect could be via upstream control of the oxidative stress response.

The maize aldehyde dehydrogenase gene ZmALDH is another candidate in the redox protection pathway. Arabidopsis plants overexpressing the gene were more tolerant of Al and showed altered markers of oxidative injury [25]. Detoxification of reactive aldehydes fits this protein family. Howver, the contribution of natural ZmALDH alleles to Al tolerance and yield in maize remain uncharacterized. Enzymatic function, altered gene expression, and the subsequent downstream antioxidant responses are all separate alternative propositions.

➢ *A Physiological Interpretation of Root Protection*

Aluminum exclusion, root cell wall properties, hormone distribution, and redox responses all operate at different stages of Al^{3+} toxicity. Citrate efflux can reduce the amount of biologically active aluminum in the vicinity of the root surface. Alterations in cell wall chemistry may affect the binding of Al^{3+} and hence its effect on extensibility of the root. Auxin transport determines whether or not the root will continue to elongate following Al^{3+} exposure, whilst antioxidant responses influence the degree of secondary cellular damage. These are not mutually exclusive processes. A given genotype could have multiple of these processes operating simultaneously, and the relative contribution of each

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may vary with the Al^{3+} dose, plant developmental stage and/or nutrient status [1,2,14,24].

A useful distinction is resistance to Al^{3+} entry as opposed to maintenance of growth in the face of exposure. Root aluminum content, measured in a non-spatial way, cannot distinguish between those two possibilities. Two roots could have the same total aluminum content yet very different amounts in the sensitive transition zone, in the wall, or in the viable cells. Likewise, a reduction in ROS following treatment could be the result of a protective antioxidant mechanism, or it could simply be a consequence of less Al^{3+} exposure. Therefore, demonstrating the prevalent mechanism requires simultaneous measurements of Al^{3+} distribution, Al^{3+} transport, redox injury, and root elongation rather than just a single endpoint assessment at harvest.

VI. EVIDENCE FOR GENE-SPECIFIC CAUSALITY

Association studies have identified several aluminum-responsive candidate genes within genomic regions linked to tolerance (Table 2). However, finding a gene near an associated genomic region does not necessarily mean that the gene is responsible for the trait. Fig. 3 provides a schematic of the QTL regions reported and the genes that have been identified. The intervals and chromosome lengths shown are for illustrative purposes only and are not validated genome assembly coordinates. The candidate gene labels are not to be taken as fine-mapped causal positions. Evidence of causality for any individual gene (Table 3) is best assessed in the context of the experimental system, the actual phenotype, the alleles that have been tested, and whether the result is replicated in an independent maize background.

Cloning a gene and finding out what it does are not the same as proving that it causes the observed variation in a maize population. Nor does a statistically significant QTL automatically identify a causal gene. A useful gene-by-gene comparison asks four practical questions: Which organism was tested? What changed? Does the result explain segregating maize variation? What experiment would settle the remaining uncertainty?

ZmMATE1 is the gene supported by the most compelling body of evidence. Reference [8] demonstrated citrate transport in oocytes and improved Al tolerance in transgenic Arabidopsis. Reference [9] further found that a tandem triplication of the locus in maize was associated with increased expression. Reference [10] then showed very clear effects in maize near-isogenic lines but identified a highly expressing single-copy genotype, implying that copy number does not account for the total variation observed in the trait.

Although ZmMATE2 was not identified as the causal gene underlying qALT5 in a specific genetic background [10], the gene should not be dismissed as a candidate for aluminum tolerance in maize. The gene mediated anion efflux in oocytes, but not the citrate transport shown for ZmMATE1 [8] In the near-isogenic lines examined by [10], the relevant qALT5 segment did not confer tolerance. That argues against a causal role for ZmMATE2 at this QTL in those lines, though not against every possible biological function of the gene. ZmMATE6, by contrast, is supported citrate release and better Al tolerance in transgenic Arabidopsis [26]. Although the gene has been shown to function as an Al-inducible citrate transporter in heterologous expression studies, evidence that natural allelic variation at ZmMATE6 contributes to aluminum-tolerance variation in maize populations remains limited.

Reference [7] identified four candidate genes, ZmASL, ZmALMT2, SAHH, and malic enzyme (ME), that were supported by both candidate-gene association and linkage analyses. Although this provides strong evidence for prioritizing these genes, it does not establish a direct role in aluminum tolerance. In Kenyan maize, [11] reported four additional QTL-associated candidates, ZmNrat1, ZmMATE3, ZmWRKY, and ZmART1. While their predicted functions are compatible with known aluminum-tolerance mechanisms, neither QTL co-localization nor stress-induced expression demonstrates causality. The critical next step is to show that variation in a specific allele affects gene function and produces a measurable difference in aluminum tolerance in the same maize breeding material.

VII. PRIORITIZING GENES BY THE MISSING CAUSAL STUDY

The strongest candidate gene is not always the one with the most literature behind it, but the one with the most robust supporting genetic, molecular, and functional data. For ZmMATE1 the main questions that remain to be answered regard the extent of allelic variation, the pattern of expression, and the consistency of its effect across different germplasm and soil types. With respect to ZmMATE6, citrate transport activity has been demonstrated in heterologous systems, but its contribution to natural variation in Al tolerance in maize is still unknown. For ZmNRAMP4 and ZmNrat1, direct evidence is still required to confirm substrate specificity and to define their intracellular transport pathways in maize. The same applies to ZmXTH and SAHH where the link between the cell wall changes and the resultant effect on root growth under Al stress needs to be established. For the regulatory genes ZmART1 and ZmWRKY, the key issue is to define their downstream targets and their regulatory function in maize itself rather than simply extrapolating from the orthologs which have been characterized in rice and Arabidopsis [7,11,22,23,26]

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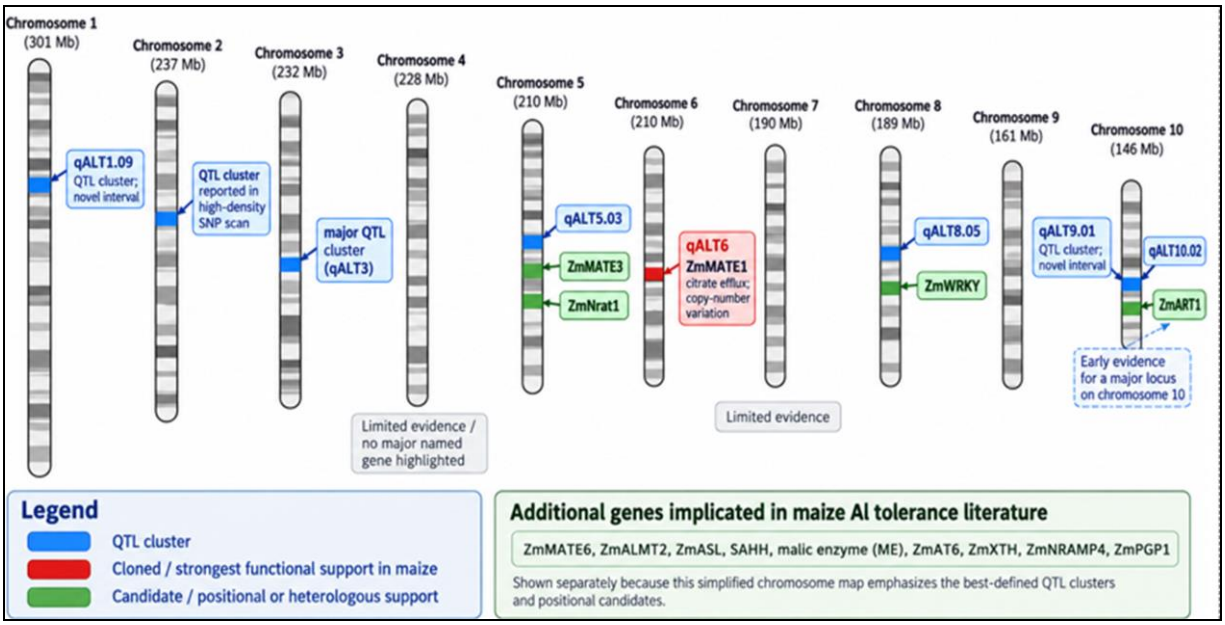

Fig 3 Chromosomal distribution of quantitative trait loci (QTL) clusters and cloned or candidate genes associated with aluminum (Al) tolerance in maize (Zea mays L.). The ten maize chromosomes are shown approximately in proportion to their physical lengths. Colored markers indicate reported QTL regions and genes implicated in Al tolerance: blue markers denote major QTL clusters, red markers identify loci with strong functional evidence in maize, and green markers represent candidate genes supported by positional mapping, association studies, or functional evidence from other species.

These are different experimental bottlenecks. Repeating expression measurements will not resolve a transport question, and another GWAS alone will not identify a transcription factor's direct targets. Research investment should follow the weakest necessary link in the causal chain: gene to molecule, molecule to root response, and root response to field performance. This approach offers a more productive basis for ranking candidates than a single undifferentiated list of "Al tolerance genes."

VIII. REGULATORY CONVERGENCE OF MAIZE RESPONSE ALUMINUM AND PHOSPHORUS

In acidic tropical soils, Al toxicity is rarely occurs in isolation. Phosphorus (P) deficiency and other nutrient imbalances are often constrain the same root system. A mechanism that improves root growth under high Al^{3+} levels may also affect P uptake. However, the fact that two stresses occur together does not prove that one allele is responsible for both traits. Citrate and other organic acids can reduce the activity of $Al^{\#+}$ at the rhizosphere and, under certain soil conditions, can have an effect on phosphate mobilization. The soil net effect depends on the mineralogy, the sorption and the buffering capacity [3,27,28]. However, evidence of pleiotropy of ZmMATE1 requires a defined allelic contrast that retains separate effects under Al-only and P-only treatments, followed by a test of their interaction when both stresses occur simultaneously [27,28,29]

The strongest evidence for pleiotropy comes from regulatory genes characterized in other plant species. In both Arabidopsis and rice, the transcription factors STOP1 and ART1, respectively, play central roles in regulating aluminum-tolerance responses. In Arabidopsis, STOP1 has also been implicated in root adaptation to P deficiency through mechanisms involving ALMT1 gene-mediated malate exudation.(Iuchi et al., 2007; Yamaji et al., 2009; Balzergue et al., 2017; Mora-Macías et al., 2017). These findings suggest that some regulatory networks may influence responses to multiple soil stresses. However, whether the corresponding maize orthologs exert similarly pleiotropic effects remains to be demonstrated experimentally. Geneticists have also examined SUMOylation and other post-translational modifications in Arabidopsis [34,35,36]. However, this is not evidence of a complete Al regulatory circuit in maize. For example, the ZmART1 gene identified by [11] as a candidate gene for aluminum tolerance based on its co-localization with an aluminum-tolerance QTL and its elevated expression in the favorable parent still requires native genetic validation and studies demonstrating its downstream regulatory targets and DNA-binding activity to establish its causal role.

Reference [29] mapped maize Phosphorus-Starvation Tolerance 1 (PSTOL1) gene-related regions associated with root traits and P accumulation under low P. In principle, breeders could stack those loci with Al tolerance alleles, but performance under low P does not necessarily equate to Al tolerance. ZmMATE1-mediated citrate release also does not necessarily confer a dual benefit in the field against both Al toxicity and P deficiency. That question is best answered by experiments that vary Al and P independently in matched genetic backgrounds and in chemically characterized soils.

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Table 3 Evidence Matrix for Cloned and Candidate Genes Linked to Aluminum Tolerance in Maize

Gene	Locus or context	Proposed role	Experimental support	Evidence type[a]	Required validation	Source
ZmMATE1	qALT6, Chr 6	Al-activated citrate efflux	Oocytes, Arabidopsis; maize CNV and NILs	A/B/C	Strong locus-function evidence; quantify yield	**[8–10]**
ZmMATE2	qALT5 region, Chr 5	Anion flux, citrate not demonstrated	Oocytes and maize NILs	B/C	Not causal for tested qALT5; other role possible	**[8,10]**
ZmMATE6	Position not validated as maize QTL	Al-associated citrate efflux	Transgenic Arabidopsis	B	Test maize alleles and knockouts	**[26]**
ZmALMT2	Candidate from association/linkage	Constitutive malate efflux	Transport assays, maize expression	B/C	Native tolerance causality unproven	**[7,21]**
ZmASL	Candidate from association/linkage	AltSB-like putative transport role	Candidate association and linkage	C	Biochemical and maize mutant tests needed	**[7]**
ME	Candidate from association/linkage	Malate carbon metabolism	Candidate association and linkage	C	Confirm causal allele and metabolic effect	**[7]**
SAHH	Candidate from association/linkage	Methylation-associated metabolism	Candidate association and linkage	C	Wall methylation link untested	**[7]**
ZmPGP1	Chr 1 developmental locus	Auxin efflux; Al-dependent arrest	Maize loss-of-function mutants	A	Determine natural allelic and field effects	**[24]**
ZmAT6	Chr 8 candidate region	Redox-associated protection	Transgenic maize and Arabidopsis	A	Direct target and agronomic effects unresolved	**[14]**
ZmNRAMP4	Chr 5 reported	Al-associated uptake/handling	Yeast and transgenic Arabidopsis	B	Native transport localization/sequestration unresolved	**[23]**
ZmNrat1	Population-dependent QTL relationship	Putative internal Al transport	Colocalization; root expression	C/D	Native metal transport tests required	**[10,11]**
ZmXTH	Not established as natural QTL	Xyloglucan-wall remodeling	Transgenic Arabidopsis	B	Maize knockout and wall chemistry needed	**[22]**
ZmALDH	No validated natural QTL	Aldehyde and oxidative protection	Transgenic Arabidopsis	B	Native maize effects and alleles unknown	**[25]**
ZmMATE3	Kenyan QTL candidate	Putative organic-acid transport	QTL proximity; expression	C/D	Transport and mutant assays needed	**[11]**
ZmWRKY	Kenyan QTL candidate	Putative transcriptional regulation	QTL proximity; expression	C/D	Identify native targets and causal variants	**[11]**
ZmART1	Kenyan QTL candidate	Putative regulatory ortholog	QTL proximity; expression	C/D	Maize binding targets and mutant tests needed	**[11]**

[a] A = native maize functional phenotype; B = heterologous functional evidence; C = mapped genetic association; D = preliminary candidate evidence. Multiple classes may apply to different claims.

These Al- and P-stress tolerance pathways are however likely connected through their effects on cell wall structure, organic acid metabolism, root elongation, and carbon allocation. Correlations in gene expression may point to such connections, but they cannot demonstrate directionality or establish causality. Determining the actual interactions will require promoter binding assays, targeted gene knockouts, rescue experiments and direct measurements of transport activity and metabolic flux. As the integrated mechanistic networks are developed, it will be vital to distinguish relationships demonstrated experimentally in maize from those proposed by analogy with other species, which therefore remain hypothetical in the maize context [37,38].

The Arabidopsis STOP1–ALMT1 literature is a perfect example of why the difference between pleiotropy and mere genetic association is important. Low P can trigger a pathway in which malate-bound Fe accumulation leads to root growth

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restriction rather than mere enhancement of nutrient acquisition [32,33]. That mechanism is obviously useful in terms of framing maize hypotheses, but it would be a massive leap of judgement to assume that the direction of the effect would be the same when moving from one species to another or from agar to soil. A factorial design of a maize experiment that examines free Al activity, P availability, root exudates, and growth would be one practical way to determine whether a candidate pathway is beneficial, neutral, or costly under combined Al and P stress.

IX. ADVANCES IN MODERN TRAIT PHENOTYPING AND QUANTITATIVE-GENETIC ANALYSIS

High-throughput root imaging coupled with a dense set of molecular markers has allowed the search for maize Al tolerance alleles to progress more rapidly. The value of this approach is that it can measure a number of root traits at once and look for associations across a wide range of germplasm. The downside is just as obvious: association is not causation. An SNP near a candidate gene is not in itself evidence that altering that gene will confer tolerance, and an association at the seedling stage is a long way from proving it will be of any benefit to adult plants in the field.

Reference [15] optimized hydroponic root imaging and examined 250 tropical maize inbreds. A related GWAS on a very similar panel identified 44 significant SNP associations and 8 QTL clusters [16]. These are companion studies which together add to the set of root traits and genomic intervals that are available for analysis. However, they do not validate each other. Their value is essentially one of discovery in that they identify the variation and the candidate regions that now need to be replicated, fine-mapped, and functionally tested.

A root stress index is only useful when interpreted against baseline vigor. A stressed-to-control root length ratio can in fact favor plants which have small control roots, while the absolute length under stress may simply be rewarding roots with faster growth. It is safer to report both. The study should also report total Al, estimated free-Al activity, soil pH, Ca and Mg concentrations, P availability, ionic strength, maize developmental stage and exposure duration.

Different genome assemblies present another issue because a chromosome bin or coordinate from one study will not necessarily map directly to another. Candidate intervals should be mapped to a named assembly. Duplicated genes and structural variants also make ortholog assignment a challenge. For breeding markers, authors should report the chromosome interval, assembly, allelic state, and assay performance. This is particularly important in ZmMATE1, where a SNP in the immediate vicinity may not distinguish between the relevant copy number and expression haplotypes.

The new phenotyping imaging work also highlights a measurement issue. An absolute root length value will reveal growth under stress but will not reflect the proportional injury Al is causing. A stress:control ratio will tell you about relative tolerance, but could result in a small, inherently weak root being deemed better. Root diameter can in fact increase as elongation decreases. Rather than pick on any one index, it would be better to report both the stressed and unstressed trait values, the % inhibition, and the genotype-by-treatment interaction. Root length, surface area and tip number can then be considered in conjunction with baseline vigor [15,16].

Genetic analyses should also take into account the dependence between these various measures. Total root length and total root surface area are obviously very closely related measures of the same underlying architecture, so many of the significant associations seen between those traits are not really independent findings. A sensible analysis would ask whether the same genomic region drives variation in multiple correlated traits and whether that effect still exists when total root size is accounted for. The analysis would then assess the stability of that effect across different maize germplasm and Al regimes before considering a candidate marker for use in breeding.

X. INTEGRATED INTERPRETATION: WHAT IS KNOWN AND WHAT REMAINS UNRESOLVED

- *Three Interacting Paths to the Observed Tolerance Phenotype*

There is sufficient genetic and physiological evidence to support a provisional model of Al tolerance in maize that involves three potentially interacting mechanisms. The first of these acts to limit Al^{3+} exposure at the root apex itself, via ZmMATE1-mediated citrate efflux, which in turn reduces the activity of the toxic Al species in the rhizosphere. The second mechanism is the maintenance of root elongation under Al stress through a combination of unique cell wall properties and auxin distribution. The third concerns the mitigation of cellular injury that results from Al^{3+} exposure (e.g., oxidative stress, accumulation of reactive aldehydes, etc.). This provides a useful framework for interpreting the data that has been published to date, although the extent of interaction between the mechanisms and their relative importance is still not fully resolved. Each of the mechanisms makes a distinct experimental prediction: reduced Al^{3+} availability at the root apex, sustained root elongation under the same aluminum exposure, or reduced cellular injury at the same internal aluminum burden [8,9,14,24,25]

This provides a basis for assessing whether beneficial alleles act via complementary mechanisms or converge on the same physiological constraint. It would be possible to combine alleles that confer different tolerance responses and thus assess their individual and combined effects under controlled Al exposure. A double genotype that is more tolerant than would be predicted from the effects of its component alleles would be evidence of a beneficial genetic interaction, whereas the absence of any further benefit would be consistent with the alleles acting via the same physiological constraint or being limited by some other process. Such an interpretation would require an explicit genetic model and measurement of the relevant physiological parameters. The responses would also need to be examined across a range of Al concentrations and nutrient regimes since

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the benefit of a given allele may vary with the soil environment. Such experiments would help establish whether the proposed mechanisms act independently, whether they interact genetically, or whether they are the limiting factor at different levels of Al stress, and would therefore provide a much more robust basis for predicting the performance of combined tolerance alleles.

➢ *Why Field Yield Remains a Separate Hypothesis*

There is sufficient The translation of Al tolerance into grain yield depends on a series of physiological and agronomic responses that vary across environments. A beneficial allele must first improve root performance at Al concentrations representative of the soil in question. That advantage must then be maintained till later stages of crop development and must ultimately result in improved water and/or nutrient uptake, sustained crop growth, and increased grain yield. Even when such responses occur, the magnitude of the effect may depend on soil mineralogy, rainfall, fertilizer management, and genetic background. Benefits seen under controlled conditions may therefore be lost in the field where other constraints limit crop growth or where the physiological advantage is associated with a cost. Thus, improved seedling tolerance does not necessarily lead to higher grain yield under field conditions [3,11].

Field evaluation should therefore look at the physiological basis of tolerance as well as the agronomic implications. A comparison between acidic and limed plots can assess whether the favorable alleles behave differently under these contrasting soil acidity conditions (although liming will also change Al availability, nutrient chemistry, and a whole host of other soil properties). A more informative set of experiments would be to have defined allelic contrasts and to measure exchangeable Al, free Al activity, P availability and repeated measures of root and shoot development. The P treatments would separate responses to Al toxicity from those to nutrient limitation. The aim is to determine whether improved root tolerance leads to sustained benefits in P uptake, crop growth, and grain yield. It is equally important to report effect sizes, confidence intervals, and the non-significant responses. The primary breeding objective is to identify alleles that maintain productivity in Al-toxic soils rather than those that simply improve the tolerance index in the laboratory.

XI. IMPLICATIONS FOR MAIZE IMPROVEMENT

Breeding starts with a phenotype, not with an identified gene of interest. In the target acidic soils, the trials should assess root performance and grain yield, as well as Al saturation and P availability. Comparable amended soil controls would also help separate out Al injury from general weakness and the wider degree of adaptation to poor soils.

Where favorable ZmMATE1 alleles are present, copy-number-aware assays and locally validated haplotypes could transfer them into adapted breeding material. A single SNP marker may not be sufficient to distinguish a tandem triplication from other high-expression alleles. Near-isogenic trials should therefore assess both root responses and crop performance (including genetic background, linkage drag, and stability across sites) [9,10].

Other germplasm require a different starting point. The Kenyan QTL and expression data point to mechanisms other than ZmMATE1, but no specific candidate genes should be assembled into breeding pyramids at this stage, as if their effects have been established. Fine mapping, near-isogenic comparisons and direct perturbation will be required to identify the underlying variants [11].

Genomic selection could capture many small effects and G×E if the training data represent the target breeding material (i.e., diverse tropical germplasm, consistent seedling assays, and field trials in acid soil environments). The approach should be tested for predictive ability in unfamiliar genetic backgrounds and locations. Accuracy measured only within the training population will give a misleading impression of transferability.

As Al toxicity and P deficiency are both soil-borne but may be mediated by different genes, breeding programs should test them separately and in combination. The benefit of citrate efflux, for instance, may be very different depending on the level of P available, the presence of organic ligands, and the moisture content. Yield under field stress is still the ultimate breeding endpoint, but the component traits are needed to explain why a line has performed well [28,29]

A genetic marker should only be introduced into routine selection after it passes three tests. It must first of all identify the actual functional variant (particularly where structural variation makes a SNP in the vicinity ambiguous). The allele must then show a ability to predict a relevant Al tolerance phenotype in populations other than those used to discover the marker. Thirdly, the marker must confer that advantage in the environments where the test genotype will be grown. A marker that satisfies the first two criteria but not the third may still be useful for physiological studies; however, it cannot yet be considered an agronomically-relevant selection marker. For ZmMATE1 copy number-aware markers are of particular importance, and the non-qALT6 loci identified in Kenya will require independent locus-level validation [9,10,11].

XII. RESEARCH PRIORITIES AND TESTABLE HYPOTHESES

Several maize genes have been associated with Al tolerance, though functional validation across a range of genetic backgrounds and field environments is still very limited. The genes of interest are ZmMATE6, ZmNRAMP4, ZmXTH, ZmALDH, and those identified within Al tolerance QTL in Kenyan maize. Establishing the causal role of these genes will require gene disruption or allele replacement (ideally using independent mutant events), with genetic complementation and testing in a range of maize backgrounds. The experiments would need to incorporate measurements of root elongation, membrane transport activity, Al accumulation, and cellular injury to separate out the primary molecular gene function from the downstream

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physiological response (Table 4). This is particularly important for the transporter candidates where altered Al tolerance does not necessarily equate to a direct role in transport or exclusion of the cation.

Several mechanisms have been proposed that would allow for more specific experimental testing. If ZmXTH influences Al retention by modifying cell wall structure, then biochemical fractionation of wall components and spatial analysis of Al distribution should show the expected differences. The proposed role of SAHH in pectin modification would be testable by measuring pectin methylesterification, alongside appropriate biochemical controls. The same would be true of any proposed regulatory role for ZmART1 in Al tolerance, with the requirement being evidence of target gene regulation, promoter occupancy, and altered stress responses resulting from the genetic perturbation. Functional similarity to genes already characterized in rice or Arabidopsis provides useful mechanistic hypotheses, but sequence and expression conservation alone is not sufficient to prove the same function in maize.

Field validation of these molecular studies should be carried out in contrasting acid soils using replicated trials that compare untreated and appropriately limed soils. Liming can estimate yield losses associated with soil acidity, but its effects on nutrient availability, soil chemistry, and the microbiota make it difficult to disentangle Al toxicity from other liming effects. Factorial experiments with varying levels of Al exposure and P supply, supported by soil chemistry and plant tissue analyses, would be the way to establish the individual and interactive effects. The trials would need to establish whether the beneficial alleles can maintain root growth, nutrient uptake, and grain yield under the actual combinations of soil constraints encountered in the field.

Interpretation of the experimental results would need to include the positive, non-significant, and negative results. A non-significant knockout phenotype does not, by itself, rule out gene function (consider statistical power, functional redundancy, and experimental conditions). An altered expression profile under Al stress shows the gene is responsive, but that does not prove it contributes to tolerance. It is important to report effect sizes, confidence intervals, the experimental organism, the genetic background, the Al exposure conditions, and the precisely defined phenotype. This is especially important when distinguishing genes that have been experimentally shown to function from those that are merely candidates based on association, expression, or heterologous evidence.

XIII. REVIEW LIMITATIONS AND IMPLICATIONS FOR FUTURE SYNTHESIS

This article is a critical narrative assessment of the genetic and physiological evidence for Al tolerance in maize rather than a systematic review or meta-analysis. The primary literature lacks reproducible screening records, and studies covered differ widely in Al exposure, root phenotyping, and how they estimated genetic effects. The evidence classifications we have used are to distinguish between experimentally demonstrated functions and associations and put forward proposed mechanisms; they are not to be interpreted as a formal assessment of risk of bias. The methodological constraints of the available evidence mean that any claims regarding the prevalence, magnitude, and reproducibility of individual tolerance mechanisms across maize germplasm are necessarily limited. That said, a critical comparison is still possible, provided interpretation is contextualized to the experiment and the strength of evidence for each gene.

A future systematic review would be strengthened by reproducible database searches, archived search exports and clearly defined inclusion criteria. Data extraction would need to record the experimental species, maize genotype, genetic intervention and phenotyping method used in each study (distinguishing between free Al activity and nominal Al salt concentration). Effect estimates and their associated uncertainty would need to be recorded in a consistent fashion, and native maize validation would need to be separated from heterologous functional assays. Field yield responses would also need to be treated as a separate outcome. This would allow for a more reliable assessment of agreement and variation between studies and would enable assessment of the extent to which the proposed mechanisms have been independently replicated across maize populations. Quantitative synthesis would still be conditional on sufficient methodological comparability rather than be a direct outcome of the systematic evidence collection.

XIV. CONCLUSIONS

Al tolerance in maize is inherited in a manner that is highly dependent on genetic background and environment, with the trait being governed by both major gene effects and more complex quantitative variation. In certain crosses, large phenotypic differences are attributable to a major allele, but across wider germplasm, a mixture of major and minor effects, with multiple QTL, epistasis, pleiotropy, and stress-dependent gene effects, all play a part. Of the mechanisms identified, ZmMATE1-mediated citrate efflux associated with qALT6 is the clearest example of Al exclusion in maize. However, this locus's contribution depends on the population studied; QTL work in Kenyan maize has identified other sources of tolerance in addition to this locus. The genetic basis of Al tolerance is thus best viewed as population- and environment-dependent, with the alleles present in a population and the intensity and nature of the Al stress to which they are exposed as the key determinants. Interpreting this architecture requires a very clear distinction to be made between molecular function, genetic association, and agronomic performance. Transport assays reveal whether a molecule can carry out a function; differential gene expression shows which genes are expressed in response to Al toxicity, but does not necessarily prove they are responsible for tolerance. QTL mapping indicates where in the genome an association with a trait is localized, but that does not in itself indicate which gene or polymorphism is responsible. Native gene disruption, complementation, and allele substitution can provide much stronger evidence of causality if the right

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genetic controls are in place. Even then, it does not indicate an allele's breeding value without evidence of a consistent advantage in the field. Therefore, molecular, genetic, and physiological evidence must be integrated to determine which mechanisms drive naturally occurring variation and which are suitable for use in crop improvement. Future work should focus on functional validation of candidate genes under physiologically relevant Al exposure, with evaluation of their effects across a range of maize backgrounds and in acid soils. The work should include characterization of Al speciation and activity, assessment of gene-specific effects via targeted gene perturbation, and measurement of resulting transport, root growth, or cellular injury responses. Validated alleles should then be tested in independent germplasm and in replicated field trials to determine whether improved root tolerance leads to improved nutrient uptake, sustained crop growth and ultimately grain yield. The studies would also be identify the conditions under which the beneficial effect of a given allele is negated due to the presence of other soil constraints or interactions with the environment. The breeding value of Al-tolerance genes will ultimately be determined by their ability to confer consistent agronomic benefits, and this will only be possible if there is a clear link between the causal molecular mechanism and performance in the target production environment.

Table 4 Priority Experiments to Distinguish Plausible Pathways for Aluminum Tolerance from Validated Maize Breeding Targets.

Priority	Working hypothesis	Discriminating test	Decision criterion
Candidate causality	ZmMATE6 alleles contribute to maize Al tolerance	Native maize editing/complementation; citrate efflux	Concordant molecular and root phenotype
Internal handling	ZmNRAMP4 alters Al compartmentation	Subcellular Al flux and localization in maize	Transport route and growth effect both demonstrated
Cell wall	ZmXTH or SAHH modifies wall Al binding	Wall chemistry, Al fractionation, allele contrasts	Specific biochemical intermediate established
Regulatory network	ZmART1 regulates native maize transport genes	Binding assays, loss-of-function and rescue	Direct target and tolerance effect
Al × P pleiotropy	Same allele benefits both stresses	Factorial Al–P soil and hydroponic studies	Independent effects and interaction quantified
Agronomic relevance	Validated alleles increase yield on acid soils	Multi-environment trials with amended controls	Repeatable yield or stability advantage

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