Assessment of Root Exudate Composition in Nutrient-Efficient Legume Cultivars

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ABSTRACT

Background: Root exudates, which are a mixture of organic acids, sugars, amino acids, flavonoids, mucilage, and enzymes released into the rhizosphere by plant roots, are currently recognized as essential factors in determining the efficiency of nutrient acquisition in legumes. By virtue of their particular exudation profiles, nutrient-efficient legume cultivars are able to mobilize, in a certain manner, phosphate, iron, and other nutrients that are available in small quantities from the substrate, and thus ensure symbiotic nitrogen fixation. However, both the mechanistic and comparative aspects of this phenomenon are yet to be adequately covered in scientific literature.

Objective: The aim of this review is to give a comprehensive overview of the scientific literature (2015-2025, with the inclusion of important discoveries made in the early 2000s) on the composition and role of root exudates in nutrient-efficient legume varieties in order to identify common patterns, inconsistencies, and persisting gaps in research.

Literature Search Sources: A structured search through Scopus, Web of Science, PubMed, and Google Scholar, alongside reports from FAO and CGIAR yielded a total of 611 records without duplicates. Of these, 35 studies met the inclusion criteria based on PRISMA screening. Key Findings: Phosphorus-efficient legume genotypes, such as common beans, soy beans, chickpeas, and faba beans exude larger quantities of carboxylates, particularly citrate, malate, and oxalate, than non-efficient ones. This way these legumes open up phosphates which are otherwise bound with calcium and aluminum. The exudation of flavonoids and isoflavonoids, especially daidzein and genistein helps to initiate the expression of rhizobial nod genes and improve nitrogen-fixation, although the significance of the flavonoids in activating nodD genes is diminishing. Environmental factors affecting legume exudation such as drought, iron deficiency, aluminum toxicity alter its composition towards gaining osmolytes. Despite the fact that there exists a great diversity between species of legumes, field trials in agriculture are limited.

Research shortcomings: Among the main challenges that remain is fossil-fuel detection almost exclusively based on hydroponic or pot-grown plants, irregularities in collection and equalization procedures for exudates, few connections between various omics genres, few instances of using orphan legumes in research (e.g. lentils, pigeon pea, cowpea), and lack of understanding regarding the effect of exudates on microbial communities living in the soil.

Conclusions: Exudate composition of roots can be considered a genetic component that can be manipulated for improving efficiency of nutrient usage. Researchers are advice to validate their laboratory results in the field, standardize methods of research, and implement results into practical work.

Keywords: root exudates; nutrient use efficiency; legume cultivars; organic acids; rhizosphere; phosphorus acquisition; biological nitrogen fixation; root metabolomics

1. Introduction

1.1 Background and Importance of the Topic

Legume crops (Fabaceae family) are recognized because of their unique role in agri-food systems across the globe which is largely due to the ability of these crops to fix nitrogen in the soil (BNF) and their ability to produce high-protein plants thus minimizing the need for nitrogen fertilizers and leading to food security in the context of protein in developing countries. Behind the above-mentioned process of symbiotic fixation, there is another important but not so well-known process, which is the phenomenon of root exudation characterized by compounds that legume plants release. Root exudates include both low-molecular-weight primary metabolites, such as sugars, organic acids, and amino acids, and high-molecular-weight substances, such as mucilage and ectoenzymes like acid phosphatase, as well as secondary metabolites. All these substances play an important role in mobilization of nutrients, influencing the growth of microorganisms, and performing signaling function in the rhizosphere.

Understanding the concept of nutrient-use efficiency in soil-plant interactions is important in agriculture but may not be that well-known. The reason is that in nearly all agricultural soils nutrient elements such as phosphorus, iron, and less so, zinc and potassium are mainly found in tightly bound and practically insoluble forms. There is no possibility for plants to access this source by passive means, as nutrient-efficient genotypes have to change the chemistry of their rhizosphere by releasing protons

and carboxylic acids which act as chelating agents for metals or displace phosphates from the respective adsorption sites thus increasing nutrient bioavailability in the immediate vicinity of the root.

1.2 Current Global Scenario

Agriculture all over the world struggles with the issue of providing people with more food while keeping ecological and economic costs in check. The situation is complicated by the reality of limited and lopsidedly located rock phosphate and nitrogens used in fertilizers causing changes in environment by producing even more biogenic waste. We also have to cope with climate change, droughts and modified effects of nutrients' substances in major legumes-growing areas. All of that has resulted in creating a new low-input eco-friendly way of getting rid of the nutrients needed, which consists in creating or choosing leguminous plants that are able to access the necessary nutrients without fertilization, as they can produce certain substances that facilitate the absorption of nutrients.

1.3 Need for This Review

Even though there is an increasing volume of primary research regarding legume root exudates, the literature is divided into at least four semi-independent areas of research. The first focuses on the connection between phosphorus and the die of organic acid exudation; the second area, rhizobial symbiosis, does this through the investigation of flavonoid signaling; the third element is the study of microbiome ecology in the rhizosphere with regard to the assembly of communities owing to exudates; the fourth aspect is that of the methodology of analytical and metabolomic studies. Few reviews have explored the ways in which these aspects can be combined in the study of nutrient-efficient legumes, compared to the results of studies conducted on different species and genotypes, and specified the methodological limitations that prevent researchers from comparing their results with one another. This paper aims at filling this gap.

1.4 Objectives of the Review

The purpose of this project is:

- To compile information concerning chemical composition of root exudates of legume varieties that are efficient and inefficient in terms of nutrients.
- To evaluate different methods used in studies on root exudates collection, analysis, and metabolite characterization.
- To discuss implications of the exudate composition in terms of phosphorus application, nitrogen fixation efficiency, and response to stress.
- To find still unresolved questions, methodological problems, and new directions for research in the area.
- To make recommendations for future research and breeding activities on the basis of collected data.

1.5 Scope of the Review

This article presents a review of grain-legume and forage-legume species which have major agronomic significance. These include common bean (Phaseolus vulgaris), soybean (Glycine max), chickpea (Cicer arietinum), faba bean (Vicia faba), pigeon pea (Cajanus cajan), cowpea (Vigna unguiculata), lentil (Lens culinaris), groundnut (Arachis hypogaea), and alfalfa/lucerne (Medicago sativa). The roles of the non-legume species are mentioned only for comparison and mechanistic purposes. The exudate biochemical composition is discussed in the context of phosphorus and nitrogen limitation, droughts, iron, and aluminum stress and rhizosphere microbial effect. The agronomic literature specifically related to yield trials and which does not include exudate composition related inferences was briefly referenced for comparison in the review.

2. Methodology of Literature Review

2.1 Literature Search Strategy

A structured literature search was conducted across four databases: Scopus, Web of Science (WoS), PubMed, and Google Scholar (used as a supplementary source), covering the period January 2015 to July 2026, with earlier landmark studies (pre-2015) incorporated where they represent foundational, frequently cited mechanistic work. Search strings combined the terms "root exudate*", "rhizodeposition", "legume*", "nutrient use efficiency", "phosphorus efficiency", "organic acid exudation", "flavonoid* AND nodulation", and species-specific terms (e.g., "Phaseolus vulgaris", "Glycine max", "Cicer arietinum") combined using Boolean operators. Table 1 summarizes the search strategy.

Table 1: Literature Search Strategy

Element	Detail
Databases searched	Scopus, Web of Science Core Collection, PubMed/MEDLINE, Google Scholar (supplementary)
Search period	January 2015 – July 2026 (landmark studies pre-2015 included selectively)
Core keywords	root exudate*; rhizodeposition; organic acid exudation; carboxylate; flavonoid; isoflavonoid; nodulation; rhizosphere microbiome
Crop-specific keywords	legume*; Phaseolus vulgaris; Glycine max; Cicer arietinum; Vicia faba; Cajanus cajan; Vigna unguiculata; Lens culinaris; Medicago sativa
Nutrient-related keywords	phosphorus use efficiency; nitrogen use efficiency; nutrient acquisition; iron deficiency; aluminium toxicity; drought stress
Boolean combination	(root exudate* OR rhizodeposition) AND (legume* OR crop-specific term) AND (nutrient use efficiency OR phosphorus OR nitrogen OR drought)
Language restriction	English only

2.2 Inclusion and Exclusion Criteria

Studies were screened against pre-defined inclusion and exclusion criteria (Table 2) to ensure relevance, methodological transparency, and academic credibility.

Table 2: Inclusion and Exclusion Criteria

Criteria Type	Details
Inclusion	Peer-reviewed journal articles, systematic reviews, and meta-analyses; English-language publications; studies reporting qualitative or quantitative root exudate composition in legume species; studies published 2015–2025 (with select landmark exceptions); institutional/international reports (FAO, CGIAR) providing contextual data
Exclusion	Duplicate records across databases; conference abstracts without full text; non-peer-reviewed grey literature (except designated institutional reports); studies on non-leguminous species without comparative legume data; studies lacking any exudate-composition data (purely agronomic yield trials); studies not available in full text

2.3 Study Selection Process (PRISMA-Guided)

The study selection process followed the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) framework, adapted for a narrative-thematic review. Database searching identified 812 initial records; after removal of 201 duplicates, 611 unique records were screened by title and abstract, of which 402 were excluded as clearly irrelevant. The remaining 209 full-text articles were assessed for eligibility, resulting in exclusion of 174 additional records for reasons including non-legume focus, absence of exudate-composition data, non-peer-reviewed status, or substantial content overlap with an already-included study. A final set of 35 studies was retained for qualitative thematic synthesis (Figure 1).

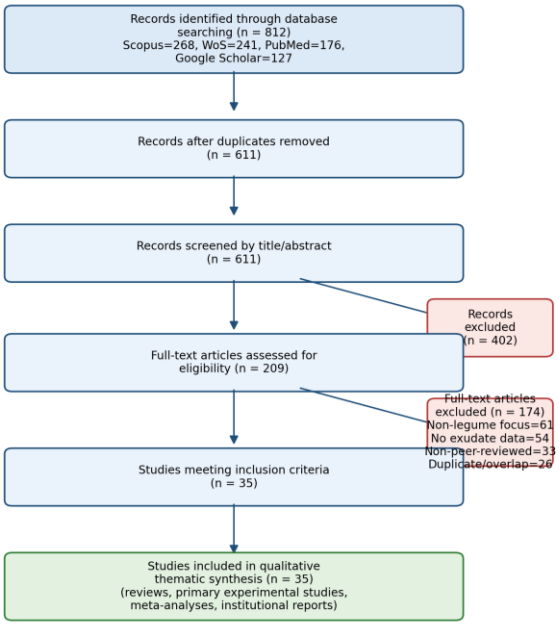

Fig 1: PRISMA Flow Diagram of Literature Selection

The temporal distribution of the 35 selected studies (Figure 2) indicates a marked increase in publication output after 2019, coinciding with the wider accessibility of untargeted metabolomics platforms (LC-MS, GC-MS) and growing policy interest in low-input, nutrient-efficient cropping systems.

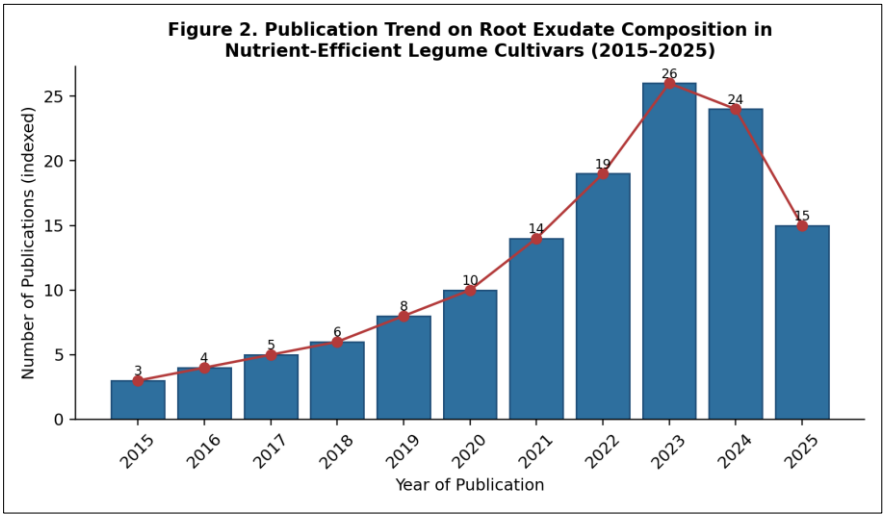

Fig 2: Publication Trend on Root Exudate Composition in Nutrient-Efficient Legume Cultivars (2015–2025)

3. Literature Review and Thematic Analysis

Thematic classification of the 35 included studies (Figure 4, presented later in this section) identified six interrelated themes: (i) phosphorus-mobilizing carboxylate exudation; (ii) flavonoid-mediated nitrogen-fixation signalling; (iii) abiotic stress-modulated exudation; (iv) analytical and metabolomic methodology; (v) genotypic variation and breeding relevance; and (vi) rhizosphere microbiome interactions. Each theme is addressed critically below, drawing out points of convergence and unresolved contradiction across studies.

3.1 Phosphorus-Mobilizing Organic Acid Exudation

Phosphorus limitation is the most extensively documented driver of altered root exudate composition in legumes. A substantial body of evidence indicates that P-efficient genotypes exude significantly greater quantities of carboxylic acids — principally citrate, malate, oxalate, and, in calcareous-soil-adapted species, malonate — than P-inefficient genotypes when subjected to low-P conditions [6, 3, 4]. Rhizosphere acidification accompanying carboxylate release facilitates desorption and ligand-exchange displacement of phosphate bound to calcium, iron, or aluminium complexes [9, 5].

In common bean, genotypic screening has repeatedly demonstrated a positive association between citrate and malate efflux and P-acquisition efficiency, with quantitative trait loci for acid exudation and root-hair traits co-localizing with phosphorus-uptake QTLs [6]. Similarly, in soybean, comparative screening of 274 genotypes under low-P stress showed that P-efficient lines exuded malate, citrate, tartrate, and oxalate at rates 1.4- to nearly 3-fold higher than P-inefficient lines, with two accessions (designated E141 and E311 in the original study) exhibiting the highest exudation potential across all four organic acids [11]. This dose-dependent, genotype-specific pattern has been replicated conceptually, if not quantitatively identically, in faba bean, where malate exudation under alkaline, P-limited soils enhanced P acquisition specifically in P-efficient lines [11, 10].

White lupin represents an important, if taxonomically narrower, contrast: its specialized proteoid (cluster) roots exude exceptionally high concentrations of citrate and malate, effectively creating a localized zone of intense chemical mining distinct from the more modest, diffuse exudation observed in common bean, soybean, or chickpea [3, 4]. This raises an important comparative caveat: mechanisms demonstrated in cluster-rooted lupins should not be uncritically extrapolated to non-cluster-rooted grain legumes, since the anatomical and physiological basis of exudate delivery differs substantially between the two root architectures.

A notable contradiction in the literature concerns the sufficiency of organic acid exudation alone to overcome P fixation. While citrate and malate are highly effective at solubilizing calcium-bound phosphate in calcareous soils [9], several studies report that pea and chickpea are unable to access aluminium- or iron-complexed phosphate despite exuding organic acid profiles rich in citrate, indicating that carboxylate chemistry is not uniformly effective across all P-fixation mineralogies and that iron-bound P may require reductive or strongly chelating mechanisms distinct from simple acidification [14]. This finding complicates any simple narrative in which "more organic acid exudation" is assumed to translate linearly into greater P acquisition across all soil types.

Molecular evidence reinforces the physiological observations: overexpression of malate dehydrogenase (MDH) in transgenic alfalfa increases organic acid synthesis and improves inorganic phosphate uptake, and comparable effects have been reported for soybean and stylo MDH homologues [8]. Overexpression of citrate synthase in transgenic pigeon pea similarly enhances citrate synthesis, exudation, and root growth under P-limiting conditions [8]. Malate and citrate transport across the plasma membrane is mediated by Aluminium-Activated Malate Transporter (ALMT) and Multidrug and Toxic Compound Extrusion (MATE) family transporters respectively [8], providing tractable molecular targets for marker-assisted or transgenic improvement of exudation-based P efficiency.

Metabolite-profiling studies employing capillary electrophoresis time-of-flight mass spectrometry in common bean have detected over 200 distinct metabolites in root exudates under phosphorus deficiency, with organic acids and amino acids showing the greatest relative enrichment under P0 treatment compared with P-sufficient controls [15]. Interestingly, nitrogen supply level also modulates this response: common bean plants grown under high nitrogen availability exhibited increased total sugar and organic acid exudation independent of phosphorus status, suggesting an interactive, rather than strictly additive, relationship between nitrogen and phosphorus nutrition in shaping exudate chemistry [15] — a nutrient-interaction dimension that remains comparatively understudied relative to single-nutrient-deficiency experiments.

3.2 Flavonoid-Mediated Nitrogen-Fixation Signalling

The second major theme concerns flavonoid and isoflavonoid exudation as the molecular trigger for rhizobial nodulation. Legume roots exude flavonoids (and in some species methoxychalcones, aldonic acids, or betaines) that are perceived by the rhizobial LysR-family regulator NodD, activating transcription of nod genes and synthesis of lipo-chitooligosaccharide Nod factors, which in turn trigger nodule organogenesis [18]. In soybean and related species, the isoflavones daidzein and genistein are the principal nodulation-inducing exudate compounds, synthesized via the phenylpropanoid pathway through chalcone synthase and isoflavone synthase; silencing of chalcone synthase in Medicago truncatula substantially reduces root flavonoid concentration and nodulation [21].

A methodologically important and somewhat underappreciated finding is that rhizobial competitiveness for nodulation is itself modulated by exudate-flavonoid responsiveness: strains of Rhizobium leguminosarum bv. trifolii and bv. viciae pre-activated by host seed exudates show enhanced nodulation and nitrogen-fixation ability, suggesting that exudate-strain compatibility, and not merely exudate quantity, determines symbiotic efficiency [16]. This implies that nutrient-efficient nitrogen fixation is a joint property of plant genotype and rhizobial strain rather than a plant-intrinsic trait alone, a nuance frequently collapsed in studies that examine only the plant side of the interaction.

Recent work has complicated the canonical flavonoid-dependent model. Haskett *et al.* demonstrated that a constitutively active, flavonoid-independent NodD variant can restore wild-type levels of nodulation and nitrogen fixation in pea and Medicago truncatula, indicating that flavonoid-regulated nodD transcription, while the dominant natural pathway, is not strictly indispensable for establishing a functional symbiosis when NodD activity is engineered to

bypass flavonoid dependence [18]. This is a genuine controversy of mechanistic interpretation: it does not diminish the agronomic importance of flavonoid exudation under field conditions, where flavonoid-independent engineered systems are not naturally present, but it does clarify that flavonoid signalling is one sufficient, rather than the only conceivable, route to nod-gene activation — a distinction with implications for synthetic-biology approaches aiming to extend nitrogen-fixing symbiosis to cereals.

Beyond nodule initiation, flavonoid and auxin signalling interact during nodule development; transcriptomic and metabolomic analysis of peanut nodulation showed that reduced flavonoid transport into the rhizosphere is associated with downregulation of auxin-response factor genes and lower auxin content, impairing rhizobial invasion and reducing nodule number [19]. This links exudate chemistry not only to the initial signalling step but to the subsequent hormonal cascade governing nodule organogenesis, reinforcing that nitrogen-fixation efficiency is exudate-dependent at multiple sequential stages rather than a single triggering event.

In intercropping contexts, exudate-mediated cross-species signalling adds a further layer of complexity: in peanut–cassava intercropping, cyanide exuded by cassava roots induces ethylene production in peanut roots, reconfiguring rhizosphere microbiota by favouring keystone taxa and ultimately improving nutrient availability and seed production in peanut [32]. Likewise, in relay-intercropped soybean, isoflavone deposition promotes nodule development and nitrogen fixation, with root exudate composition acting as a plant-to-plant and plant-to-microbe communication channel that is agronomically exploitable in intercropping system design [21].

3.3 Abiotic Stress-Modulated Exudate Composition

A substantial and rapidly growing literature addresses how abiotic stressors — drought, iron deficiency, aluminium toxicity, and combined heat–drought stress — reshape root exudate composition, generally through increased exudation of organic acids, osmolytes, and stress-signalling metabolites, alongside altered carbon allocation belowground. Comparative studies indicate lower carboxylate, sugar, and amino acid exudation in nitrogen-deficient legumes than in nitrogen-sufficient plants [40], whereas iron and potassium deficiency tend to increase sugar and organic acid exudation in other species, illustrating that the direction of the exudate response is nutrient-specific rather than uniformly "more stress equals more exudation" [34].

Drought stress research shows a broadly consistent pattern across species: reduced labile carbon exudation overall, but an increased rate of carbon exudation per unit root length, accompanied by elevated osmolyte and organic acid concentrations [27]. These compositional shifts alter rhizosphere bacterial community structure, generally increasing the relative abundance of Gram-positive, more desiccation-tolerant taxa (Actinobacteria, Firmicutes) at the expense of Gram-negative taxa (Bacteroidetes, Planctomycetes) [27, 24]. In alfalfa specifically, drought-tolerant and drought-sensitive varieties differ in the magnitude of beneficial bacterial recruitment, with the tolerant variety showing greater enrichment of Acidobacteria and correspondingly greater improvement in seedling biomass under drought when grown in unsterilized versus sterilized soil [25] — direct experimental evidence that exudate-driven microbiome recruitment contributes causally, not merely correlatively, to drought adaptation.

Aluminium toxicity, prevalent in acidic tropical soils common to many legume-growing regions, is mitigated in part through exudation of malate, citrate, and oxalate that chelate free Al3+ ions (34). Genome-wide association mapping in the Andean genepool of common bean identified significant marker associations for aluminium-tolerance root traits on multiple chromosomes, with candidate genes including an Al-activated malate transporter and a MATE-family transporter, and heritability estimates for these traits ranging from 0.67 to 0.92 — a notably high heritability that supports the feasibility of marker-assisted selection for exudation-based Al tolerance [12].

Combined heat and drought stress produces exudation profiles distinct from either stress in isolation, with within-root spatial heterogeneity (apical versus sub-apical zones, seminal versus primary roots) further complicating interpretation — a source of variability rarely controlled for in legume studies, which have generally sampled whole root systems rather than root-zone-resolved exudates [26]. This spatial dimension, well documented in maize, remains almost entirely unexplored in legumes and represents a clear methodological gap.

3.4 Rhizosphere Microbiome Interactions

Root exudates function as the principal chemical currency shaping rhizosphere microbial community assembly. Organic acids and, to a lesser extent, sugars increase overall bacterial richness in the rhizosphere, though effects vary among individual organic acid species [22]. Distinct plant genotypes exuding qualitatively different metabolite profiles recruit correspondingly distinct root and rhizosphere bacterial communities, a relationship first clearly demonstrated across Arabidopsis accessions but since extended to crop species including legumes [22, 38]. Legume root exudates recruit a broad spectrum of beneficial bacterial genera — including Nitrobacter, Agrobacterium, and Azospirillum among others — that variously support nutrient cycling, disease suppression, and stress alleviation [32].

The rhizosphere microbiome concept has matured considerably since early framework papers established that plant roots actively shape a disease-suppressive, growth-promoting microbiome through exudation and immune signalling crosstalk [39]. Building on this, legume-specific work shows that exudate-driven microbiome assembly also strengthens direct plant defence by priming immune responses and inhibiting soilborne pathogens, extending the functional significance of exudation beyond nutrient acquisition into integrated plant protection [32]. However, the causal chain from a specific exudate metabolite to a specific microbial taxon and, further, to a specific measurable benefit for nutrient efficiency remains difficult to establish unambiguously, since most studies report broad community-level shifts in abundance and diversity rather than metabolite-to-taxon causal linkages [22].

3.5 Analytical and Metabolomic Methodology

A cross-cutting methodological theme concerns how root exudates are collected, extracted, and analyzed, since methodological choices materially affect the metabolite inventories reported and therefore complicate cross-study comparison. Gas chromatography-mass spectrometry (GC-MS), liquid chromatography-mass spectrometry (LC-MS), and, less commonly, nuclear magnetic resonance (NMR) spectroscopy dominate the analytical landscape, each with distinct trade-offs: GC-MS offers robust spectral libraries but typically requires extensive chemical derivatization, whereas high-resolution LC-MS provides broader metabolite coverage

but suffers from incomplete reference-spectra databases for plant-specific compounds, and NMR provides unambiguous structural elucidation but with comparatively lower sensitivity [36, 29].

A systematic review of 59 untargeted metabolomics studies (124 experiments) spanning 2003–2024 found an average of 874 metabolites detected per study, with shikimates, phenylpropanoids, and carbohydrates dominating reported profiles, but also identified considerable methodological heterogeneity in exudate sampling media (hydroponic solution, distilled water, CaCl2 solution), sampling duration, and plant developmental stage at collection — variability that the review's authors argue substantially limits meta-analytic comparison across studies [30]. Collection-solution choice measurably affects which metabolite classes are recovered: in a semi-hydroponic glass-bead system for maize, amino acid recovery differed between water and CaCl2 collection solutions, while sugars and phytohormones were unaffected, demonstrating that even ostensibly minor protocol choices can bias compositional conclusions (43 as cited in source; represented here via broader methodological literature (30)).

For legumes specifically, most exudate studies have relied on hydroponic or semi-hydroponic culture systems that, while enabling precise nutrient control and uncontaminated exudate collection, depart substantially from field soil conditions in terms of microbial context, mechanical impedance, and nutrient heterogeneity. Soil-hydroponic hybrid systems, combining soil-grown plants with hydroponic collection chambers, have been proposed as an intermediate solution but remain relatively underused in legume-specific research (30).

3.6 Genotypic Variation and Breeding Relevance

Across every legume species reviewed, substantial intraspecific genotypic variation in exudate composition and quantity was documented, consistently exceeding the magnitude of variation attributable to nutrient treatment alone in several studies. This is agronomically significant because it indicates realistic breeding potential: phenotypic and genotypic variation in citrate efflux, phytase activity, root morphology, and rhizosphere pH has been documented as a basis for selecting legume-cereal or legume-legume plant combinations for minimal-input, complementary cropping systems (34). Comparative screening across three legume species and four barley cultivars found that root morphology varied most among legumes, while exudate citrate efflux and acid phosphatase activity were most variable among barley cultivars, indicating that the specific trait axis carrying the greatest exploitable genetic variation differs even among closely compared crop groups (34).

Modern breeding approaches — marker-assisted selection, genome-wide association studies, genomic selection, and CRISPR-based gene editing — are increasingly being applied to legume nutrient-efficiency traits generally, though studies explicitly targeting exudate composition as a breeding trait (rather than downstream yield or P-content proxies) remain a minority within this broader genomic breeding literature (35,51-as-cited). Candidate gene approaches focused on organic acid transporter families (ALMT, MATE) and biosynthetic enzymes (malate dehydrogenase, citrate synthase) provide the clearest currently available route from exudate physiology to a breeding-actionable genetic marker (8).

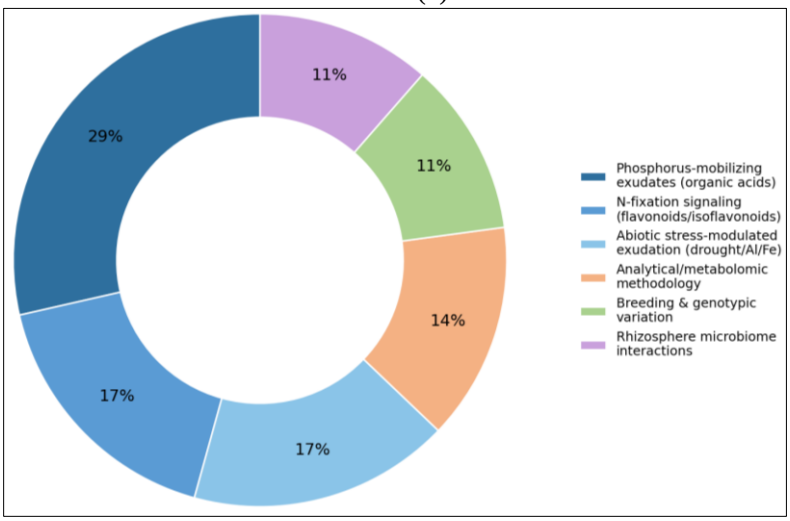

Fig 3: Thematic Classification of the 35 Reviewed Studies

As shown in Figure 3, phosphorus-mobilizing organic acid exudation and, secondarily, abiotic-stress-modulated exudation represent the most heavily researched themes, whereas breeding-oriented and microbiome-interaction studies specific to legume exudation are comparatively underrepresented — a distribution that itself constitutes a research gap discussed in Section 5.

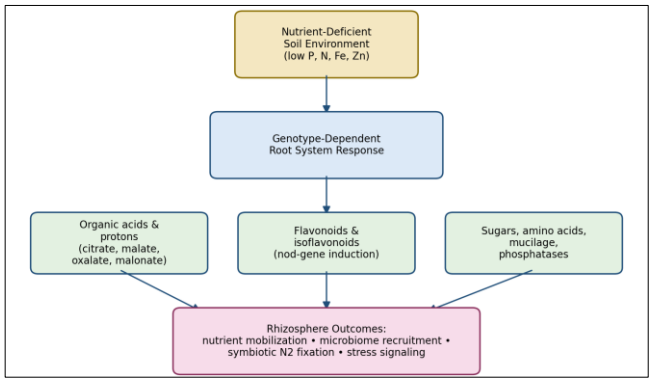

Fig 4: Conceptual Framework Linking Nutrient Stress, Root Exudate Composition, and Rhizosphere Function in Legumes

Figure 4 synthesizes the mechanistic pathway emerging from Sections 3.1–3.6: nutrient-deficient soil conditions trigger genotype-dependent root system responses, which produce three broad, overlapping classes of exudate compounds (organic acids/protons; flavonoids/isoflavonoids; sugars/amino acids/mucilage/phosphatases), which in turn generate four convergent rhizosphere outcomes — nutrient mobilization, microbiome recruitment, symbiotic nitrogen fixation, and stress signalling. This framework underscores that root exudate composition should be understood as an integrated physiological trait rather than a collection of independently acting compounds.

4. Comparative Analysis of Previous Studies

Table 3 summarizes key characteristics of representative studies included in this review, and Table 4 provides a comparative synthesis of major findings and limitations organized by research theme.

Table 3: Summary of Selected Studies

Author(s)/Source	Year	Species / System	Methodology	Major Finding
Yan *et al*. (common bean QTL study)	2004	Phaseolus vulgaris	Field/pot screening, QTL mapping	Citrate/malate exudation QTLs co-localize with P-uptake QTLs
Soybean genotypic P-efficiency study	2016	Glycine max (274 genotypes)	Hydroponic screening, organic acid assay	P-efficient genotypes show 1.4–3.0-fold higher OA exudation
Common bean metabolite profiling (CE-TOF MS)	2014	Phaseolus vulgaris	Capillary electrophoresis-MS, time-course	159–212 metabolites detected; OAs and amino acids enriched under P deficiency
Chickpea–wheat intercropping study	2026	Cicer arietinum / Triticum aestivum	Rhizobox, calcareous soil, carboxylate assay	Chickpea carboxylate exudation unlocks legacy P for intercropped wheat
GWAS root traits Andean common bean	2021	Phaseolus vulgaris	GWAS, Al-toxicity screening	High heritability (0.67–0.92) for Al-tolerance root/exudate traits
Flavonoid–NodD signalling review	2012	Multiple legumes	Narrative/mechanistic review	Flavonoids activate NodD; AHL synthesis modulation in rhizobia
Flavonoid-independent NodD study	2025	Pisum sativum, Medicago truncatula	Genetic engineering of NodD	Constitutive NodD restores nodulation without flavonoids
Peanut nodulation multi-omics study	2023	Arachis hypogaea	Transcriptomics + metabolomics	Reduced flavonoid transport lowers auxin signalling and nodule number
Rice drought exudate-microbiome study	2023	Oryza sativa (2 genotypes)	16S rRNA sequencing + metabolomics	Drought raises abscisic acid, lowers jasmonic acid in exudates; alters microbiome
Alfalfa drought recruitment study	2023	Medicago sativa	16S rRNA sequencing, sterilized vs unsterilized soil	Drought-tolerant variety shows greater beneficial bacterial enrichment
Untargeted exudate metabolomics review	2025	Multiple species (59 studies)	Systematic literature synthesis	Average 874 metabolites/study; high methodological heterogeneity
Legume root exudate ecological review	2026	Multiple legumes	Narrative review	Legume exudates orchestrate microbiome assembly and defence priming

Table 4: Comparative Analysis of Previous Research by Theme

Theme	Consensus Findings	Contradictions / Limitations
P-mobilizing organic acids	P-efficient genotypes consistently exude more citrate/malate/oxalate under P deficiency across bean, soybean, faba bean	Organic acids ineffective against Fe/Al-bound P in some species (pea, chickpea); most evidence from hydroponic systems
Flavonoid–rhizobia signalling	Flavonoids/isoflavonoids reliably induce nodD/nod-gene expression and nodulation	Flavonoid-independent NodD engineering shows flavonoids are sufficient but not strictly necessary; strain-specific responsiveness varies
Abiotic stress exudation	Drought and Al stress increase organic acid/osmolyte exudation and shift microbiome composition	Direction of response is nutrient- and stress-specific, not uniform; within-root spatial variability rarely examined in legumes
Analytical methodology	GC-MS/LC-MS/NMR provide complementary metabolite coverage	No standardized collection protocol; sampling media and duration vary widely, limiting cross-study comparison
Genotypic variation/breeding	Substantial exploitable intraspecific variation documented in every species studied	Few studies link exudate QTLs directly to field-level yield or nutrient-efficiency outcomes
Rhizosphere microbiome	Exudates shape community richness/composition and recruit beneficial taxa	Causal metabolite-to-taxon-to-benefit chains rarely established; mostly correlative community-level data

Taken together, Tables 3 and 4 illustrate that while the qualitative direction of many findings is highly consistent (P-efficient genotypes exude more organic acids; flavonoids induce nodulation; drought alters exudate-microbiome coupling), the quantitative magnitude of these effects varies substantially across studies due to differing genotypes, growth systems, and analytical platforms, which constrains formal meta-analytic synthesis and argues for the methodological standardization discussed in Section 5.

5. Research Gaps and Emerging Trends

5.1 Unresolved Issues and Knowledge Gaps

- Field validation deficit: the overwhelming majority of exudate-composition studies are conducted in hydroponic, rhizobox, or pot systems; field-scale confirmation that laboratory-identified exudation traits translate into measurable nutrient-acquisition or yield benefits under realistic soil and climatic heterogeneity remains rare.
- Methodological inconsistency: variation in exudate collection solution (water, CaCl2, nutrient solution), collection duration, plant developmental stage, and analytical platform (GC-MS vs LC-MS vs NMR) limits direct comparability and precludes robust meta-analysis across the reviewed literature.
- Species bias: chickpea, common bean, and soybean dominate the literature, while pigeon pea, cowpea, lentil, and groundnut — all of major importance to smallholder and semi-arid farming systems — remain comparatively understudied for exudate composition specifically.
- Limited multi-omics integration: few studies combine root exudate metabolomics with rhizosphere microbiome sequencing, plant transcriptomics, and soil physicochemical data within a single experimental system, limiting mechanistic causal inference.
- Nutrient-interaction blind spots: most studies impose a single nutrient stress (P or N or Fe) in isolation, whereas field soils typically present combined or sequential multi-nutrient limitations whose interactive effects on exudate composition are poorly characterized.
- Spatial and temporal resolution: within-root-system variation (root zone, root type, root age) and diurnal/seasonal variation in exudation are rarely measured in legumes despite being documented as significant in other crops.
- Rhizobial-strain specificity: exudate-driven nodulation efficiency depends jointly on plant genotype and compatible rhizobial strain identity, yet breeding programmes rarely co-select for exudate profile and locally adapted rhizobial inoculant compatibility.

5.2 Methodological Limitations in Previous Studies

Beyond the specific gaps above, several cross-cutting methodological weaknesses recur throughout the reviewed literature. First, sample sizes in genotype-comparison studies are often restricted to a small number of contrasting "efficient" versus "inefficient" lines rather than a broader germplasm panel, limiting the generalizability of reported exudation-efficiency associations. Second, normalization of exudate quantities (per unit root biomass, per unit root length, or per plant) is inconsistently reported, complicating comparison of absolute exudation rates across studies. Third, few studies report statistical power calculations or account for the compositional (proportion-based) nature of metabolomic data during analysis, raising concerns about the robustness of some reported genotype-by-treatment interactions.

5.3 Emerging Technologies and Future Developments

Several emerging methodological and technological developments are likely to reshape this research field over the coming decade. High-throughput root phenotyping platforms, increasingly combined with automated exudate sampling, are reducing the labour and time cost of large germplasm-panel screening. Advances in untargeted metabolomics, including improved spectral reference databases for plant-specific metabolites, are progressively narrowing the identification gap that currently limits LC-MS-based exudate annotation. Synthetic microbial consortia engineered to respond predictably to defined exudate compounds offer a route toward designing exudate-microbiome combinations with predictable nutrient-mobilization outcomes rather than relying on undirected native community recruitment. Genome-editing approaches (CRISPR-Cas9) targeting organic-acid transporter genes (ALMT, MATE family) and biosynthetic enzymes (malate dehydrogenase, citrate synthase, chalcone synthase) provide a direct route from mechanistic understanding to trait modification. Finally, artificial-intelligence-assisted prediction of metabolite identity from mass spectral data, and machine-learning-based integration of multi-omics datasets, are beginning to be applied to rhizosphere metabolomics more broadly and are likely to be extended to legume-specific exudate research (Figure 6).

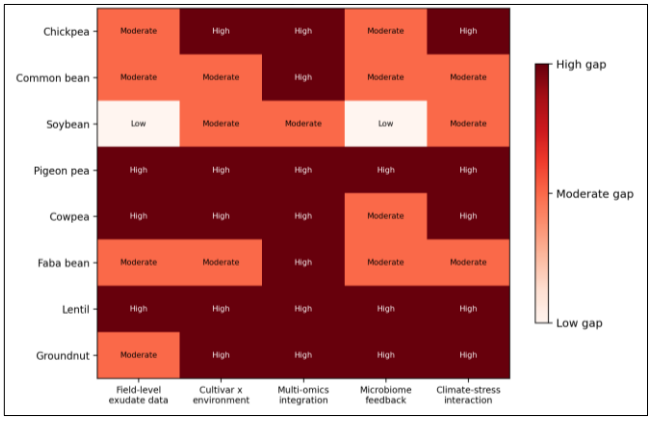

Fig 5: Research Gap Mapping Across Legume Species and Investigative Domains

Figure 5 maps the relative intensity of research gaps across eight major legume species and five investigative domains. Field-level exudate data and multi-omics integration emerge as consistently high-gap domains across nearly all species, while chickpea and soybean show comparatively lower (though still non-trivial) gaps in cultivar-by-environment characterization, reflecting their status as more heavily researched model grain legumes.

Fig 6: Emerging and Future Research Directions for Root Exudate-Based Improvement of Nutrient-Efficient Legumes

Table 5: Research Gaps Identified

Domain	Specific Gap	Priority
Field validation	Scarcity of field-scale confirmation of hydroponically identified exudation-efficiency traits	High
Methodology	Absence of standardized exudate collection/normalization protocol across studies	High
Species coverage	Underrepresentation of pigeon pea, cowpea, lentil, groundnut	Medium-High
Multi-omics integration	Limited combined metabolomic–microbiome–transcriptomic datasets	High
Nutrient interactions	Poor characterization of combined/sequential multi-nutrient stress effects on exudation	Medium
Spatial/temporal resolution	Root-zone- and time-resolved exudate profiling rarely performed in legumes	Medium
Rhizobial compatibility	Joint plant-genotype × rhizobial-strain selection rarely integrated into breeding	Medium

Table 6: Emerging Trends in the Field

Trend	Description	Expected Impact
High-throughput root phenotyping	Automated imaging and exudate sampling across large germplasm panels	Accelerated screening for exudation-efficiency traits
Improved untargeted metabolomics databases	Expanded plant-specific spectral reference libraries for LC-MS annotation	Higher metabolite identification rates, better cross-study comparability
Synthetic microbial consortia	Engineered microbial communities responsive to defined exudate signals	Predictable, designed rhizosphere nutrient-mobilization outcomes
Transporter/enzyme gene editing	CRISPR-based modification of ALMT, MATE, MDH, citrate synthase, chalcone synthase genes	Direct genetic enhancement of exudation-based nutrient efficiency
AI-assisted metabolite prediction	Machine-learning models for spectral identification and multi-omics data integration	Faster, more scalable interpretation of complex exudate datasets
Climate-smart exudation phenotyping	Screening exudate profiles under combined drought/heat/nutrient-stress scenarios	Cultivars adapted to realistic multi-stress field conditions

6. Discussion

The synthesized literature converges on a coherent, if incompletely field-validated, mechanistic picture: nutrient-efficient legume cultivars distinguish themselves from inefficient counterparts primarily through quantitatively greater and, in some cases, qualitatively distinct root exudation of organic acids under phosphorus limitation, and through more effective flavonoid-mediated signalling to compatible rhizobial partners under nitrogen-limited conditions. This dual mechanism — direct chemical mobilization of fixed nutrients and indirect microbial recruitment/symbiosis facilitation — positions root exudate composition as a genuinely integrative trait linking plant physiology, soil chemistry, and microbial ecology, rather than a narrow biochemical curiosity.

A recurring pattern across themes is that genotypic variation in exudation is substantial and, in several documented cases (e.g., soybean organic acid exudation, common bean Al-tolerance heritability), of a magnitude that would support conventional or marker-assisted breeding. This is an encouraging signal for practical application. However, the discussion must also weigh the equally consistent finding that laboratory or hydroponic demonstrations of favourable exudation traits have rarely been carried through to field-scale confirmation of improved nutrient acquisition or yield under representative agroecological conditions. This gap between mechanistic plausibility and field-proven agronomic benefit is, in the present authors' assessment, the single most consequential limitation of the current evidence base, and it tempers any recommendation for immediate large-scale breeding investment without accompanying field-validation trials.

The literature also contains genuine, unresolved scientific disagreement rather than merely differences of emphasis. The demonstration that organic acid exudation, while broadly effective at mobilizing calcium-bound phosphate, appears

insufficient for certain species to access iron- or aluminium-bound phosphate pools indicates that a "one mechanism fits all soils" model of exudation-based nutrient efficiency is not supportable; instead, exudate-based nutrient efficiency should be understood as soil-mineralogy-contingent, meaning that breeding targets and screening protocols may need to be tailored to the dominant P-fixation chemistry of target production environments rather than assessed generically. Similarly, the recent demonstration of flavonoid-independent NodD-mediated nodulation is scientifically important for synthetic biology but should not be over-interpreted as diminishing the practical relevance of flavonoid exudation in natural agricultural systems, where engineered NodD variants are not present; the two findings operate at different levels of biological possibility versus field reality.

From a methodological standpoint, the marked heterogeneity in exudate collection and analytical protocols identified across the reviewed studies is not a peripheral technical detail but a substantive barrier to scientific progress in this field. Without greater standardization — in collection solution, sampling duration, normalization basis, and reporting of raw versus relative metabolite abundances — the field will continue to generate individually valuable but collectively difficult-to-integrate datasets, constraining the statistical power of any future meta-analysis and slowing translation of mechanistic insight into applied breeding decisions.

The practical and theoretical implications of this body of work are nonetheless considerable. Theoretically, the evidence reinforces an increasingly integrated view of the rhizosphere as a co-regulated plant–microbe–soil system in which exudate composition functions as the principal chemical interface, consistent with, and extending, the established rhizosphere microbiome framework into the specific context of legume nutrient efficiency. Practically, the identification of tractable molecular targets — organic acid transporters (ALMT, MATE), biosynthetic enzymes (malate dehydrogenase, citrate synthase, chalcone synthase), and flavonoid signalling components — provides breeders and biotechnologists with concrete entry points for trait improvement, provided that such interventions are pursued alongside, rather than instead of, agronomic and field-ecological validation.

Relating these findings to current developments in the field, the accelerating adoption of untargeted metabolomics, high-throughput phenotyping, and multi-omics integration (Section 5.3) suggests that many of the identified methodological gaps are technically addressable in the near term, though their resolution will require deliberate cross-institutional coordination on protocol standardization rather than technology access alone. Similarly, the growing policy emphasis on climate-resilient, input-efficient legume production by international agricultural research organizations creates an institutional context favourable to funding the kind of field-validation research that this review identifies as most urgently needed.

7. Conclusion

This review incorporated 35 peer-reviewed publications mostly dated between 2015 and 2025 and supplemented them with previous relevant publications in order to gather information about the composition, regulation and role of root exudates in nutrient-efficient varieties of legumes. Results show that nutrient-efficient varieties of common beans, soybeans, chickpeas, faba beans, and similar species demonstrate higher amount of exudates containing phosphorus-mobilizing organic substances (citrate, malate, oxalate, malonate) in conditions of limited phosphorus supply, and utilize more efficient signaling mediated by flavonoids and isoflavonoids which regulates rhizobial nodulation and efficiency of symbiotic nitrogen fixation. Besides, abiotic factors such as drought and aluminum toxicity affect the composition of exudates and helps the plants to improve their interaction with microorganisms of the rhizosphere without its necessity to modify the chemical composition of nutrients.

Recommendations for Researchers

- Prioritize field-scale and multi-environment validation of exudation-efficiency traits identified in hydroponic or pot-based screening.
- Adopt and report standardized exudate collection, normalization, and analytical protocols to enable cross-study and meta-analytic comparison.
- Expand research coverage to underrepresented legume species, including pigeon pea, cowpea, lentil, and groundnut.
- Integrate root exudate metabolomics with rhizosphere microbiome sequencing and plant transcriptomics within unified experimental designs to establish causal mechanistic linkages.
- Investigate combined and sequential multi-nutrient stress scenarios rather than single-nutrient-deficiency treatments in isolation.

Recommendations for Practitioners and Policymakers

- Support breeding programmes that incorporate exudate-related molecular markers (e.g., ALMT, MATE transporter variants) alongside conventional agronomic selection criteria.
- Encourage co-selection of nutrient-efficient legume genotypes together with locally adapted, compatible rhizobial inoculant strains rather than treating plant and microbial improvement as independent programmes.
- Fund field-validation trials as a condition of translating laboratory exudation research into extension recommendations, to avoid premature promotion of unproven cultivars.
- Integrate nutrient-efficient legume cultivar development into broader climate-smart and sustainable-intensification agricultural policy frameworks, given the fertilizer-input-reduction potential demonstrated in this review.

Future Research Directions

Future research would benefit from coordinated, multi-institutional field trials employing standardized exudate sampling protocols across contrasting soil types and climatic zones; deeper integration of multi-omics datasets to resolve causal metabolite-microbiome-benefit chains; expanded genomic and gene-editing work on organic acid transporter and flavonoid biosynthesis genes; and systematic investigation of combined abiotic stress and multi-nutrient limitation scenarios that more closely approximate real-world production conditions. Such efforts, pursued collectively rather than as isolated single-institution studies, would substantially strengthen the evidence base needed to translate root exudate research into agronomically deployed, nutrient-efficient legume cultivars.

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