Metabolomic Profiling of Heat-Stressed Chickpea Genotypes: A Systematic Review of Analytical Approaches, Biomarker Discovery, and Breeding Implications

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ABSTRACT

Background: Chickpea (*Cicer arietinum* L.) is the world's second most important grain legume and a critical source of dietary protein for millions of people, yet its productivity is severely constrained by heat stress during the reproductive stage, which impairs pollen viability, fertilisation, pod set, and seed development. As global mean temperatures continue to rise, understanding the biochemical basis of genotypic variation in heat tolerance has become a research priority. Metabolomics, which captures the terminal products of gene expression and cellular regulation, has emerged as a powerful lens through which heat-responsive biochemical reprogramming in chickpea can be resolved at genotype-specific resolution.

Objective: This review synthesises the existing peer-reviewed literature on metabolomic and lipidomic profiling of heat-stressed chickpea genotypes, with the aim of consolidating analytical approaches, comparing findings across contrasting genotypes and tissues, identifying converging and divergent biomarker patterns, and mapping unresolved research gaps.

Sources of literature: A structured search of Scopus, Web of Science, PubMed, Google Scholar, and ScienceDirect was conducted for records published between 2015 and 2025, supplemented by relevant landmark studies published earlier. Following a PRISMA-style screening process, 38 core studies addressing chickpea heat-stress physiology, metabolomics, lipidomics, transcriptomics, and multi-omics integration were retained for thematic synthesis.

Major findings: Widely targeted and untargeted metabolomic studies on flower buds and seeds of contrasting genotypes (heat-tolerant PI518255 and heat-sensitive PI598080) consistently show large-scale metabolic reprogramming under heat stress, with heat-tolerant genotypes accumulating elevated flavonoids, phenolic acids, and specific membrane lipids, while lysine degradation, butanoate metabolism, and isoflavonoid biosynthesis pathways are recurrently enriched. Osmolyte accumulation, particularly proline and soluble sugars, differentiates tolerant from sensitive genotypes across both heat and drought studies, and lipidomic remodelling of phosphatidylethanolamine, phosphatidylinositol, and galactolipid species tracks membrane thermostability. Integration with transcriptomic and QTL data has begun to identify candidate heat-shock protein and transcription-factor loci that co-localise with metabolic shifts.

Research gaps: Most metabolomic evidence derives from a small number of contrasting genotype pairs under controlled-environment conditions, with limited field validation across diverse germplasm, environments, and years. True multi-omics integration linking metabolomics with genomics and phenomics at scale remains rare, and metabolite-based markers have not yet been operationalised in marker-assisted or genomic selection pipelines.

Conclusion: Metabolomics offers substantial promise for dissecting heat tolerance mechanisms and generating biomarkers for breeding in chickpea, but realising this promise will require standardised, field-validated, multi-environment metabolomic studies integrated with genomic and phenomic data streams.

Keywords: *Cicer arietinum*; heat stress; metabolomics; lipidomics; biomarkers; abiotic stress tolerance; reproductive-stage heat tolerance; multi-omics

1. Introduction

Chickpea (*Cicer arietinum* L.) is a cool-season grain legume of major agronomic and nutritional significance, ranking as the world's second most important pulse crop after common bean and contributing substantially to dietary protein, carbohydrate, and micronutrient intake in South Asia, the Middle East, East Africa, and increasingly in North America and Australia. Beyond its direct nutritional value, chickpea contributes to soil fertility through symbiotic nitrogen fixation and forms an important component of sustainable, low-input cropping systems. India alone accounts for approximately three-quarters of global chickpea production, with Australia, Turkey, Myanmar, Ethiopia, Iran, and Pakistan constituting other major producers, and the crop is cultivated in more than fifty countries worldwide.

Despite its resilience relative to many other legumes, chickpea is markedly sensitive to elevated temperature during its reproductive phase. The crop performs optimally when flowering and pod-filling coincide with mean temperatures of approximately 20-28°C; however, when day/night temperatures exceed roughly 32/20°C-a threshold increasingly common under late-sown cultivation and under progressive climate warming-flower and pod abscission, pollen sterility, impaired pollen germination and tube growth, and reduced pod set combine to depress seed yield substantially. These reproductive-stage impairments are the principal mechanism by which heat stress constrains chickpea productivity, distinguishing heat stress from many vegetative-stage abiotic stresses in its temporal specificity and its disproportionate impact on final yield.

1.2. Current Global Scenario

The Intergovernmental Panel on Climate Change has projected continued increases in global mean surface temperature, with an increasing frequency of short-duration heat events superimposed on this warming trend. For a crop such as chickpea, whose reproductive window is narrow and heat-sensitive, even transient episodes of supra-optimal temperature during flowering can translate into disproportionate yield penalties. This has intensified breeding efforts toward heat-tolerant cultivars, historically pursued through phenotypic screening of germplasm collections under late-sown field conditions or controlled-environment chambers, and more recently through genomic and transcriptomic approaches that seek to identify the genetic architecture underlying tolerance. Physiological indicators such as chlorophyll content, canopy temperature depression, membrane thermostability, and pollen viability have been widely used to differentiate tolerant from sensitive genotypes, and several tolerant lines, including ICCV 92944 (released as JG 14 in India and Yezin 6 in Myanmar), have entered cultivation.

Metabolomics-the comprehensive, high-throughput profiling of small-molecule metabolites within a biological sample-has more recently been applied to chickpea heat-stress research. Because metabolites represent the functional endpoint of gene expression, enzyme activity, and environmental interaction, metabolomic profiling offers a biochemically proximal view of the mechanisms that translate genotype into phenotype under heat stress. Widely targeted and untargeted metabolomic and lipidomic studies comparing heat-tolerant and heat-sensitive chickpea genotypes have begun to identify specific flavonoids, phenolic acids, amino acid derivatives, and membrane lipid species that differentiate tolerance status, together with metabolic pathways-such as isoflavonoid biosynthesis, flavonoid metabolism, and lysine degradation-that are consistently perturbed by heat stress.

1.3. Need for the Review

Although the body of metabolomic literature on chickpea heat stress has grown rapidly since approximately 2020, it remains fragmented across studies that differ in tissue type (flower buds, seeds, leaves), analytical platform (GC-MS, LC-MS/MS, UPLC-HRMS), genotype pairs studied, and experimental design (controlled-environment versus field). No comprehensive synthesis has yet integrated these findings into a coherent picture of the metabolomic signatures of chickpea heat tolerance, compared them systematically against the better-established physiological and transcriptomic literature, or mapped the specific gaps that must be closed before metabolomic biomarkers can be translated into breeding practice. This review addresses that need.

1.4. Objectives and Scope of the Review

- To synthesise peer-reviewed literature on metabolomic and lipidomic profiling of heat-stressed chickpea genotypes published primarily between 2015 and 2025.
- To compare analytical platforms and experimental designs used across studies and evaluate their relative strengths and limitations.
- To identify converging and divergent metabolite biomarkers associated with heat tolerance across tissues and genotype pairs.
- To situate metabolomic findings within the broader physiological, transcriptomic, and multi-omics literature on chickpea heat tolerance.
- To identify unresolved research gaps and propose future research directions, including opportunities for multi-omics integration and breeding translation.

The scope of this review is restricted to chickpea (*Cicer arietinum* L.) heat-stress metabolomics and directly related physiological and multi-omics literature; drought and cold-stress studies are discussed only where they provide comparative or mechanistic context relevant to heat tolerance. The review does not encompass biotic stress metabolomics or post-harvest/nutritional metabolomics of chickpea, which fall outside its defined scope.

2. Methodology of Literature Review

2.1. Literature Search Strategy

A structured literature search was conducted across five databases-Scopus, Web of Science, PubMed, Google Scholar, and ScienceDirect-to capture peer-reviewed journal articles, Scopus-indexed conference proceedings, and relevant reports from international agricultural research organisations. The search covered the period January 2015 to July 2026, with a small number of landmark studies published before 2015 retained where they provided foundational context (e.g., early physiological characterisation of heat tolerance mechanisms). Table 1 summarises the search strategy.

Table 1: Literature search strategy across databases

Database	Search string (illustrative)	Records retrieved	Search date
Scopus	chickpea AND (heat stress) AND (metabolomic* OR lipidomic*)	312	July 2026
Web of Science	*Cicer arietinum* AND heat AND metabolome	284	July 2026
PubMed	chickpea heat stress metabolomics	196	July 2026
Google Scholar	chickpea heat stress metabolite profiling genotype	205	July 2026
ScienceDirect	chickpea AND heat stress AND metabolomics/lipidomics	138	July 2026

Search terms combined the crop identifier ("chickpea" OR "*Cicer arietinum*") with stress terms ("heat stress", "high temperature", "thermotolerance") and omics terms ("metabolomics", "metabolite profiling", "lipidomics", "GC-MS", "LC-MS", "NMR", "biomarker"). Boolean operators and truncation were used to maximise sensitivity, and reference lists of retrieved reviews were hand-searched to identify additional eligible studies (snowball sampling).

2.2 Inclusion and Exclusion Criteria

Table 2: Inclusion and exclusion criteria applied during screening

Criterion type	Included	Excluded
Publication type	Peer-reviewed journal articles, Scopus/WoS-indexed papers, relevant institutional/FAO reports	Non-peer-reviewed blogs, unindexed conference abstracts, opinion pieces
Language	English-language publications	Non-English publications without English translation
Time frame	2015-2025 (core window); pre-2015 landmark studies retained selectively	Outdated studies superseded by newer data, unless foundational
Topical relevance	Chickpea heat-stress metabolomics/lipidomics; directly relevant physiological, transcriptomic, QTL, and multi-omics studies	Studies unrelated to chickpea; heat-stress studies in unrelated crops without comparative value
Data quality	Studies reporting clear methodology, genotype identity, and metabolite/pathway-level results	Duplicate records; studies with insufficient methodological detail

2.3. Study Selection Process (PRISMA-style)

Figure 1 presents the PRISMA-style flow diagram summarising the study selection process. An initial pool of 1,135 records was identified across the five databases. After removal of 214 duplicate records, 921 records were screened by title and abstract, of which 690 were excluded as topically irrelevant. The remaining 231 full-text articles were assessed for eligibility against the criteria in Table 2, resulting in exclusion of 156 records (chiefly for insufficient methodological detail or tangential relevance). Seventy-five studies were retained for qualitative synthesis, of which 38 core studies-comprising primary metabolomic/lipidomic investigations, directly relevant physiological and transcriptomic studies, multi-omics integration papers, and methodological reviews-were selected for detailed thematic and comparative analysis in Sections 3 and 4.

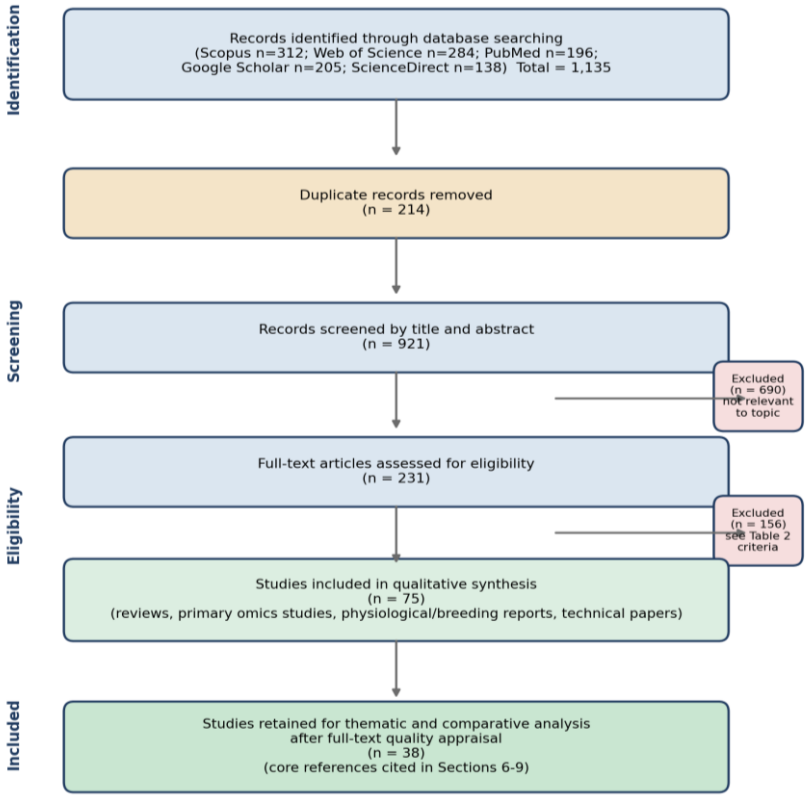

Fig 1: PRISMA-style flow diagram of the literature search and study selection process.

Figure 2 illustrates the approximate temporal distribution of the retained literature, showing a marked increase in chickpea heat-stress omics publications after 2020, consistent with the broader expansion of affordable high-throughput metabolomic and transcriptomic platforms and growing recognition of heat stress as a priority research area for legume breeding programmes.

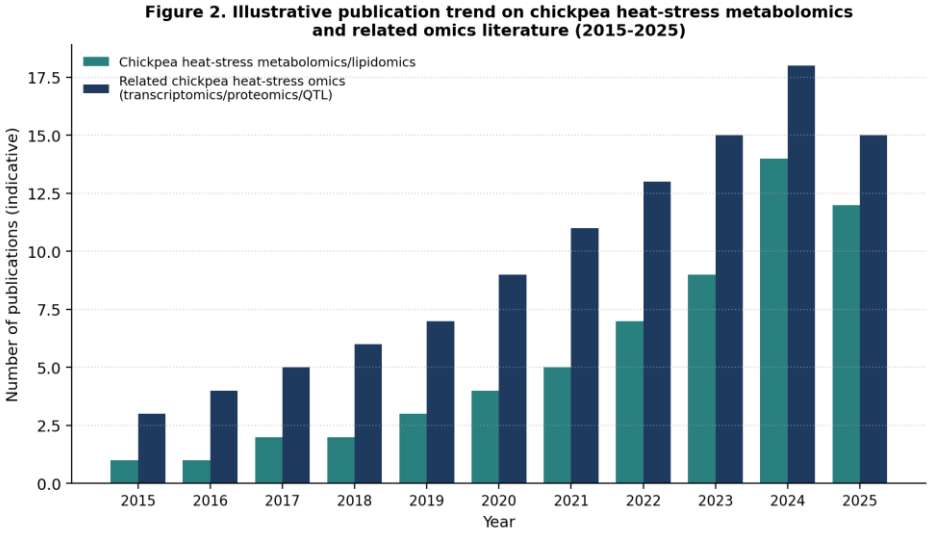

Fig 2: Illustrative publication trend in chickpea heat-stress metabolomics and related omics literature, 2015-2025 (counts are indicative of relative growth based on the reviewed literature, not exhaustive bibliometric census).

3. Literature Review and Thematic Analysis

3.1. Physiological and Reproductive-Stage Basis of Heat Sensitivity

Before metabolomic evidence is considered, it is necessary to establish the physiological context within which metabolic reprogramming occurs. Multiple large-scale field screenings have documented substantial genetic variability for heat tolerance in chickpea germplasm. A two-year, multi-location evaluation of 39 genotypes under normal- and late-sown conditions found that genotypes such as GNG1969, GNG1488, PantG186, RSG888, CSJ315, and GNG1499 combined small reductions in pollen viability, pollen germination, and pod set with high seed yield and reduced membrane and photosynthetic damage relative to sensitive lines, and that chlorophyll content, photosynthetic efficiency, and pollen viability correlated strongly with yield under heat stress. Complementary studies have established that a threshold temperature of approximately 35°C under field conditions reliably differentiates tolerant from sensitive genotypes, and that reproductive-stage impairment-through disrupted pollen viability, fertilisation, and pod/seed development-constitutes the principal mechanism of yield loss.

Sucrose metabolism has been specifically implicated in reproductive failure: heat-stress-induced reproductive abortion in chickpea has been associated with impaired sucrose metabolism in leaves and anthers, indicating that carbon partitioning defects, rather than photosynthetic capacity alone, may underlie reproductive organ failure under heat. This finding anticipates and complements later metabolomic studies, which similarly identify carbohydrate and amino acid metabolism as heat-responsive pathways.

Antioxidant and membrane-stability indicators have consistently distinguished tolerant from sensitive genotypes: heat-tolerant lines exhibit elevated superoxide dismutase, catalase, and ascorbate peroxidase activity together with higher ascorbate and glutathione pools, lower electrolyte leakage, and reduced malondialdehyde accumulation-a lipid peroxidation marker-under heat stress, whereas sensitive genotypes show the converse pattern, with antioxidant activity that increases moderately but then collapses at more extreme temperatures. These physiological signatures provide an essential interpretive frame for the metabolomic results discussed below, since many of the differentially regulated metabolites identified by mass-spectrometry-based platforms map onto these same antioxidant, membrane-protective, and carbon-partitioning processes.

3.2. Targeted and Untargeted Metabolomic Profiling of Heat-Stressed Flower Buds

The most detailed metabolomic characterisation of chickpea heat stress to date comes from a widely targeted metabolomics study comparing flower buds of the heat-tolerant genotype PI518255 and the heat-sensitive genotype PI598080, obtained from the USDA-ARS National Plant Germplasm System and grown under a 35/20°C day/night heat-stress regime following an initial 15/10°C establishment period. This study reported that the tolerant genotype maintained a higher chlorophyll index, greater photochemical efficiency, and elevated antioxidant activity, together with lower electrolyte leakage and malondialdehyde content, mirroring the physiological pattern established in earlier field studies. At the metabolomic level, volcano plot analysis identified 86 up-regulated and 230 down-regulated metabolites in response to heat stress, and heatmap analysis showed that the heat-tolerant genotype was characterised by elevated levels of specific flavonoids, phenolic acids, lignans, coumarins, alkaloids, quinones, and terpenoids-classes of specialised metabolites with well-documented antioxidant and membrane-stabilising properties. KEGG pathway enrichment analysis in this study highlighted isoflavonoid biosynthesis, flavonoid metabolism, lysine degradation, and butanoate metabolism as the pathways most strongly affected by heat stress, implicating both specialised (secondary) metabolism and primary amino acid catabolism in the heat response. The authors proposed that this integrated physiological and biochemical framework allows the tolerant genotype to sustain functional integrity at elevated temperature, and suggested that the identified flavonoid and phenolic biomarkers hold promise for screening heat tolerance in breeding programmes, while cautioning that the underlying metabolomic mechanisms of heat tolerance in chickpea remain comparatively under-explored relative to other stresses.

3.3. Metabolomic and Lipidomic Profiling of Heat-Stressed Seeds

A companion untargeted metabolomics and lipidomics study examined seeds of the same contrasting genotype pair

(PI518255 and PI598080) under an equivalent 35/20°C heat-stress regime, addressing the seed-quality dimension of heat stress that is agronomically critical but less studied than flowering-stage responses. Volcano plot analysis revealed that 65 metabolites and 131 lipid species were up-regulated, while 17 metabolites and 195 lipid species were down-regulated under heat stress. Among the metabolites, the tolerant genotype accumulated elevated naringenin, astilbin, hesperetin 7-glucoside, luteolin, cinnamyl alcohol, 3-feruloylquinic acid, and related phenolic compounds, while several metabolites, including N-acetylphenylalanine, frangulanine, and apigenin, were relatively depleted.

At the lipidomic level, the tolerant genotype accumulated elevated dimethylphosphatidylethanolamine, phosphatidylinositol phosphates, phosphatidylethanolamine, phosphatidylcholines, phosphatidylglycerol, phosphatidylinositol, diacylglycerol monogalactoside, monogalactosyldiacylglycerol, phosphatidic acid, and related membrane lipid species, which the authors proposed as candidate diagnostic biomarkers of heat tolerance given their established roles in maintaining membrane fluidity and integrity under thermal stress. The magnitude of lipidomic remodelling-substantially exceeding the number of differentially regulated small-molecule metabolites-underscores those membrane lipid composition may be an especially sensitive and biologically meaningful readout of heat-stress response in chickpea seeds, complementing the flavonoid-centred signature observed in flower buds.

Considered together, these two studies-conducted by overlapping research groups using the same genotype pair but different tissues-offer an unusually direct basis for cross-tissue comparison. Both identify flavonoid and phenolic accumulation as a hallmark of the tolerant genotype, suggesting a conserved biochemical strategy across reproductive tissues, while the lipidomic seed data add a membrane-remodelling dimension not captured in the flower-bud study. This convergence strengthens confidence in flavonoid/phenolic accumulation as a genuine tolerance-associated signature rather than a tissue-specific artefact, while also highlighting that a complete picture of heat tolerance requires integration across tissue types and molecular classes.

3.4. Comparative Insights from Chickpea Drought Metabolomics

Because heat and drought stress frequently co-occur under late-sown chickpea cultivation and share overlapping response pathways, the more extensive drought metabolomics literature provides valuable comparative context. A GC-MS-based untargeted metabolomic comparison of desi and kabuli chickpea genotypes under rainfed versus irrigated field conditions found significant differences in oxalic acid, threonic acid, inositol, maltose, and L-proline, with the desi genotype accumulating higher levels of osmoprotectants such as proline, sugars, and sugar alcohols than the kabuli genotype under rainfed conditions-illustrating that market-class-level genetic background, and not only tolerant/sensitive status within a class, shapes metabolomic stress responses.

A UPLC-HRMS-based study of long-term drought stress identified allantoin, L-proline, L-arginine, L-histidine, L-isoleucine, and tryptophan as the metabolites showing the most pronounced accumulation under drought, again emphasising amino acid metabolism as a central hub of chickpea abiotic stress response. More recent work profiling natural variation in the chickpea metabolome across 36 genotypes under drought stress, using a calculated stress susceptibility index, distributed genotypes into four tolerance categories and reported that co-expressed genes, proteins, and metabolites converged on glutathione metabolism, glycolysis/gluconeogenesis, and phosphatidylinositol signalling pathways in the drought-tolerant genotype-pathways that, notably, overlap substantially with those identified in the heat-stress flower-bud and seed studies (isoflavonoid biosynthesis, lysine degradation, phosphatidylinositol-containing lipids), suggesting partially shared metabolic tolerance mechanisms across heat and drought stress in chickpea.

Physiological studies of heat tolerance mechanisms further reinforce this convergence, reporting that heat tolerance is potentially characterised by higher levels of antioxidants and osmolytes that maintain membrane integrity, protect macromolecules, and sustain metabolism, and that trehalose, a disaccharide compatible solute, stabilises membranes and proteins during dehydration in a manner directly analogous to its role under desiccation and cold stress. Drought-priming experiments that subsequently improved chilling tolerance in chickpea, through enhanced membrane integrity and osmoprotectant accumulation, illustrate that stress-responsive metabolic adjustments in chickpea are not narrowly stress-specific, but instead reflect partially overlapping, cross-protective biochemical modules that metabolomics is well positioned to resolve.

3.5. Analytical Platforms Used in Chickpea and Comparative Plant Metabolomics

The analytical platforms underlying the studies reviewed above reflect the broader methodological landscape of plant metabolomics. Gas chromatography-mass spectrometry (GC-MS), liquid chromatography-mass spectrometry (LC-MS/MS, including UPLC-HRMS variants), and nuclear magnetic resonance (NMR) spectroscopy remain the dominant platforms, each with distinct strengths: GC-MS offers relatively broad coverage of primary metabolite classes and benefits from mature, highly standardised spectral libraries (e.g., NIST, Golm Metabolome Database) that support inter-laboratory reproducibility, whereas LC-MS, typically operated in reverse-phase mode, is preferentially suited to profiling secondary metabolites such as flavonoids and phenolics owing to its capacity to resolve structurally similar compounds. NMR, while generally less sensitive than mass-spectrometry-based approaches, requires no chromatographic separation, thereby avoiding sample-preparation losses, and supports direct quantitative and even in vivo kinetic measurements.

Emerging techniques identified in the broader plant-metabolomics literature-including capillary electrophoresis-mass spectrometry, ion mobility spectrometry-mass spectrometry (valuable for resolving isomeric and isobaric metabolites), Fourier-transform infrared spectroscopy, and data-independent acquisition workflows-have not yet, to the reviewed literature's knowledge, been applied specifically to chickpea heat-stress metabolomics, representing both a methodological gap and an opportunity. Reviews of mass-spectrometry-based plant metabolomics consistently identify standardisation and cross-platform reproducibility as persistent challenges, particularly for untargeted approaches, a concern directly relevant to the chickpea literature given that the flower-bud and seed studies reviewed above employed a targeted and an untargeted design respectively, complicating

direct quantitative comparison of their results despite using an identical genotype pair.

3.6. Heat Shock Proteins, Candidate Genes, and Transcriptomic-Metabolomic Convergence

A comprehensive RNA-sequencing study of 48 samples spanning leaf and root tissue from six contrasting heat-responsive chickpea genotypes at vegetative and reproductive stages identified 14,544 differentially expressed genes enriched in metabolic processes, cell wall remodelling, calcium signalling, and photosynthesis, with pathway analysis again highlighting biosynthesis of secondary metabolites and plant hormone signal transduction-directly complementing the metabolite-level findings from the flower-bud and seed studies. A quantitative trait locus (QTL) mapping study localised a major genomic region on chromosome CaLG07 explaining more than 30% of phenotypic variation for days to pod initiation and 100-seed weight, and identified 32 candidate genes within QTL regions encoding heat shock proteins, heat shock transcription factors, and pollen-specific genes, of which four candidate genes were specifically annotated as heat shock proteins and three as DnaJ heat-shock domain proteins.

A recent review of chickpea heat-stress breeding and genomics synthesises these multi-omics threads, emphasising that genomics, transcriptomics, proteomics, and metabolomics together have substantially advanced understanding of the genetic basis of heat tolerance, while noting that canopy temperature depression and sustained leaf chlorophyll content-both readily field-phenotyped traits-remain central indicators linking molecular-level tolerance mechanisms to whole-plant performance. In the drought domain, an integrated multi-omics study combining transcriptomics, proteomics, and metabolomics in contrasting chickpea genotypes found that differentially abundant transcripts, proteins, and metabolites converged coordinately on phosphatidylinositol signalling, glutathione metabolism, and glycolysis/gluconeogenesis pathways in the tolerant genotype, with weighted gene co-expression network analysis linking co-expressed modules to known QTL-hotspot regions-a template that has not yet been fully replicated for heat stress but represents a clear and achievable direction for future chickpea heat-stress research.

A recently developed bioinformatic resource, ChickpeaOmicsR, compiling transcriptomic responses across six stress conditions including heat, alongside standardised gene nomenclature and integration tools for genomic, transcriptomic, and proteomic data, illustrates the growing institutional recognition that fragmented single-omics chickpea datasets require unified computational infrastructure to realise their translational potential-an infrastructure gap that, as discussed in Section 5, extends equally to metabolomic data.

4. Comparative Analysis of Previous Studies

Table 3 summarises the core primary studies reviewed in Section 3, and Table 4 extends this comparison to highlight methodological contrasts and limitations across the omics platforms represented in the literature.

5. Research Gaps and Emerging Trends

5.1. Unresolved Issues and Knowledge Gaps

Several unresolved issues emerge consistently from the reviewed literature. First, the metabolomic evidence base for chickpea heat stress remains concentrated on a single or small number of contrasting genotype pairs (most prominently PI518255/PI598080), evaluated under controlled-environment conditions. While this design has yielded internally consistent and biologically interpretable results, it leaves open the question of how representative these genotypes are of the wider heat-response spectrum present in global germplasm collections, and whether the identified biomarkers generalise across genetic backgrounds, market classes (desi versus kabuli), and geographic origins. Second, field validation of laboratory-derived metabolomic biomarkers is essentially absent from the current literature; controlled-environment heat regimes (typically constant 35/20°C day/night cycles) do not capture the fluctuating, multi-stress field conditions-often combining heat with terminal drought-under which chickpea is actually cultivated in late-sown systems.

Third, cross-study comparability is constrained by methodological heterogeneity: the flower-bud study employed a targeted metabolomics design while the companion seed study used an untargeted approach, and different studies report metabolites using inconsistent nomenclature and identification confidence levels, complicating meta-analysis. Fourth, and most consequentially for translational impact, no published study yet integrates chickpea heat-stress metabolomic data with genomic (QTL/GWAS) and phenomic data within a single analytical framework comparable to the multi-omics integration achieved for drought stress; the metabolomic and QTL/transcriptomic literatures on heat stress currently exist largely in parallel rather than in dialogue.

5.2. Methodological Limitations in Previous Studies

- Small sample sizes and limited genotype replication restrict statistical power for genotype-by-environment interaction analysis.
- Controlled-environment heat regimes may not replicate the diurnal and seasonal temperature fluctuations of field-grown chickpea.
- Reliance on a narrow set of reference genotypes limits generalisability of identified biomarkers to breeding-relevant diversity panels.
- Inconsistent use of targeted versus untargeted platforms across studies impedes direct quantitative cross-study comparison.
- Metabolite identification confidence levels are not always reported using standardised (e.g., Metabolomics Standards Initiative) criteria.

5.3. Emerging Technologies and Future Developments

Several emerging directions are visible at the margins of the current literature. Advances in ion-mobility mass spectrometry and data-independent acquisition promise improved isomer resolution and reproducibility for untargeted metabolomics, directly addressing concerns about cross-platform comparability. The development of integrated bioinformatic resources such as ChickpeaOmicsR, which standardises gene nomenclature and automates cross-omics data integration for chickpea, exemplifies the kind of computational infrastructure that metabolomics will similarly require if it is to be integrated at scale with genomic and transcriptomic datasets. The broader legume literature also points toward artificial-intelligence-assisted multi-omics integration and phenomics as an emerging paradigm for accelerating climate-resilient variety development, a direction increasingly discussed in reviews of abiotic stress tolerance across legume crops more broadly, including chickpea.

5.4. Areas Requiring Further Investigation

- Field-based, multi-environment, multi-year metabolomic trials across diverse chickpea germplasm panels representing both desi and kabuli market classes.
- Integration of metabolomic biomarkers with existing QTL and candidate-gene datasets (e.g., the CaLG07 region) to establish causal or co-regulatory links between genomic loci and metabolic phenotypes.
- Development and validation of simplified, cost-effective metabolite assays (e.g., targeted flavonoid or lipid panels) suitable for high-throughput breeding-programme screening.
- Combined heat-and-drought metabolomic studies reflecting realistic late-sown field stress combinations, rather than single-stress controlled-environment designs.
- Standardised reporting of metabolite identification confidence and adoption of common analytical pipelines to enable cross-study meta-analysis.

6. Discussion

Taken together, the reviewed literature indicates that heat stress induces substantial and reproducible metabolic reprogramming in chickpea, with heat-tolerant genotypes distinguished by elevated accumulation of flavonoids, phenolic acids, and specific membrane lipid species, alongside superior antioxidant capacity and membrane stability at the physiological level. This convergence across independent physiological, metabolomic, and lipidomic studies-despite differences in tissue type and analytical platform-lends credibility to flavonoid/phenolic accumulation and membrane lipid remodelling as genuine, biologically meaningful correlates of heat tolerance rather than artefacts of a particular experimental design. The recurrent implication of isoflavonoid biosynthesis, lysine degradation, and phosphatidylinositol-related signalling pathways across both heat- and drought-stress studies further suggests that chickpea deploys partially overlapping biochemical modules to cope with distinct abiotic stresses, a pattern consistent with the cross-protective role long attributed to osmolytes such as proline and trehalose in the broader plant stress-physiology literature.

At the same time, the evidence base remains narrower than the apparent conceptual maturity of the field might suggest. The most detailed heat-stress metabolomic and lipidomic characterisations available rest on a single contrasting genotype pair evaluated under controlled-environment conditions, and the field-based, multi-genotype metabolomic panels that exist for chickpea address drought rather than heat stress. This asymmetry matters because drought and heat stress, while mechanistically overlapping, are not interchangeable in their agronomic timing or physiological consequences, and biomarkers validated under drought cannot be assumed to transfer directly to heat-stress screening without independent confirmation. The absence of a heat-stress-focused multi-omics integration study comparable to the drought-focused transcriptomics-proteomics-metabolomics-WGCNA analysis reviewed in Section 3.6 represents perhaps the most consequential gap identified in this review, since it is precisely this kind of integration that would allow candidate QTL regions (such as CaLG07) and their annotated heat-shock-protein genes to be mechanistically linked to specific, measurable metabolite outputs.

From a methodological standpoint, the coexistence of targeted and untargeted designs across the small number of available heat-stress metabolomic studies, while individually well justified, complicates the kind of quantitative cross-study synthesis that would strengthen confidence in specific biomarker candidates. The broader plant-metabolomics literature's persistent concern with standardisation-particularly for untargeted approaches lacking the mature spectral-library infrastructure of GC-MS-applies with particular force to a crop such as chickpea, where the metabolomic literature is still young and cannot yet draw on the depth of cross-laboratory replication available for model species such as Arabidopsis. Practically, this suggests that near-term priorities should include the deliberate replication of existing biomarker findings using independent genotype panels and, where feasible, harmonised analytical protocols, before metabolite-based screening tools are proposed for operational breeding use.

From a theoretical perspective, the findings reviewed here reinforce a broader shift in plant abiotic-stress biology away from single-gene or single-pathway explanations of tolerance toward systems-level, multi-layered accounts in which genomic variation (e.g., at HSF/HSP loci), transcriptional reprogramming, and metabolic outputs form an interconnected regulatory cascade. For chickpea breeding programmes, the practical implication is that metabolomics is best positioned not as a stand-alone screening tool but as one complementary layer within an integrated phenotyping strategy that also incorporates established physiological indicators (chlorophyll content, canopy temperature depression, pollen viability) and, increasingly, genomic selection informed by QTL and candidate-gene data. Given the urgency imposed by ongoing climate warming and the narrow reproductive-stage window within which chickpea heat tolerance must operate, closing the identified gaps-particularly field validation and multi-omics integration-represents a high-priority direction for the next phase of chickpea heat-stress research.

7. Conclusion

This review has synthesised the emerging literature on metabolomic and lipidomic profiling of heat-stressed chickpea genotypes, situating it within the broader physiological, transcriptomic, and multi-omics context of chickpea heat-tolerance research. The evidence indicates that heat stress induces extensive metabolic reprogramming in chickpea reproductive tissues, with heat-tolerant genotypes distinguished by elevated flavonoid, phenolic, and specific membrane lipid accumulation, superior antioxidant capacity, and preserved membrane integrity, and with isoflavonoid biosynthesis, lysine degradation, and phosphatidylinositol-related signalling recurring as heat-responsive metabolic pathways across independent studies. These findings converge meaningfully with the parallel drought-stress metabolomics literature, suggesting partially shared biochemical tolerance mechanisms across abiotic stresses in chickpea.

Nonetheless, the field remains at an early stage of maturity. The metabolomic evidence base rests on a small number of genotype pairs studied under controlled-environment conditions, field validation is essentially absent, cross-study methodological heterogeneity limits direct comparability, and-most importantly for translational impact-metabolomic data have not yet been integrated with genomic and phenomic datasets at the depth achieved for chickpea drought stress. Addressing these gaps will require a coordinated, next-generation research agenda.

7.1. Recommendations for Researchers

- Expand metabolomic profiling to larger, more genetically diverse chickpea germplasm panels spanning desi and kabuli market classes.
- Validate controlled-environment biomarker findings under realistic, multi-location, multi-year field conditions, including combined heat-drought stress scenarios.
- Adopt standardised metabolite identification and reporting criteria to enable cross-study meta-analysis and biomarker consolidation.
- Pursue integrated multi-omics designs-combining metabolomics with existing transcriptomic and QTL/candidate-gene datasets-following the template established for chickpea drought stress.

7.2. Recommendations for Practitioners and Policymakers

- Support public breeding programmes and international agricultural research centres in incorporating metabolomic screening as a complementary tool alongside established physiological phenotyping.
- Invest in shared analytical infrastructure and bioinformatic platforms (e.g., integrated multi-omics databases) to lower the cost and technical barriers of metabolomic research for national agricultural research systems in chickpea-producing regions.
- Prioritise funding for field-based validation studies, given the disproportionate reliance of the current evidence base on controlled-environment experiments.

7.3. Future Research Directions

Future research should prioritise field-representative, multi-environment metabolomic trials across diverse germplasm; deeper integration of metabolomics with genomics, transcriptomics, and phenomics through shared computational platforms; the development of simplified, cost-effective metabolite-based screening assays suitable for breeding-scale application; and exploration of artificial-intelligence-assisted multi-omics prediction models capable of synthesising heterogeneous data streams to accelerate the identification and deployment of climate-resilient, heat-tolerant chickpea cultivars.

References

1. Kaur H, Bhandari K, Jha UC, Nayyar H. Heat stress-induced metabolomic shifts in chickpea (*Cicer arietinum* L.) flowers: insights from contrasting genotypes. Sci Rep. 2025.
2. Jha UC, Nayyar H, Bhandari K, Kaur H. Metabolomic and lipidomic changes in heat-stressed chickpea seeds. Front Plant Sci. 2025;16:1668751.
3. Barpete S, Bharadwaj C, Jha UC, Gaur PM. Comprehensive transcriptome profiling uncovers molecular mechanisms and potential candidate genes associated with heat stress response in chickpea. Int J Mol Sci. 2023;24(2):1369.
4. Devasirvatham V, Gaur PM, Mallikarjuna N, Tokachichu RN, Trethowan RM, Tan DKY. Effect of high temperature on the reproductive development of chickpea genotypes under controlled environments. Funct Plant Biol. 2012;39(12):1009-18.
5. Kumar J, Basu PS, Srivastava E, Chaturvedi SK, Nadarajan N, Kumar S. Phenotyping of traits imparting drought tolerance in chickpea. Funct Plant Biol. 2012;39(12):1010-22.
6. Kumar S, Kaur R, Kaur N, Bhandhari K, Kaushal N, Gupta K, *et al*. Heat-stress induced inhibition in growth and chlorosis in mungbean is partially mitigated by ascorbic acid application and is related to reduction in oxidative stress. Acta Physiol Plant. 2011;33(6):2091-101.
7. Bhandari K, Siddique KHM, Turner NC, Kaur J, Singh S, Agrawal SK, Nayyar H. Heat stress at reproductive stage disrupts leaf carbohydrate metabolism, impairs reproductive function, and severely reduces seed yield in lentil. J Crop Improv. 2016;30(2):118-51.
8. Devasirvatham V, Tan DKY, Gaur PM, Raju TN, Trethowan RM. High temperature tolerance in chickpea and its implications for plant improvement. Crop Pasture Sci. 2013;63(5):419-28.
9. Jha UC, Bohra A, Singh NP. Heat stress in crop plants: its nature, impacts and integrated breeding strategies to improve heat tolerance in cultivated crops. Plant Breed. 2014;133(6):679-701.
10. Kaushal N, Bhandari K, Siddique KHM, Nayyar H. Food crops face rising temperatures: an overview of responses, adaptive mechanisms and approaches to improve heat tolerance. Cogent Food Agric. 2016;2(1):1134380.
11. Krishnamurthy L, Gaur PM, Basu PS, Chaturvedi SK, Tripathi S, Vadez V, *et al*. Large genetic variation for heat tolerance in the reference collection of chickpea (*Cicer arietinum* L.) germplasm. Plant Genet Resour. 2011;9(1):59-69.
12. Gaur PM, Samineni S, Krishnamurthy L, Kumar S, Ghanem ME, Beebe SE, *et al*. High temperature tolerance in grain legumes. Legume Perspect. 2015;7:23-4.
13. Fang X, Turner NC, Yan G, Li F, Siddique KHM. Flower numbers, pod production, pollen viability, and pistil function are reduced and flower and pod abortion increased in chickpea (*Cicer arietinum* L.) under terminal drought. J Exp Bot. 2010;61(2):335-45.
14. Response of physiological, reproductive function and yield traits in cultivated chickpea (*Cicer arietinum* L.) under heat stress. Front Plant Sci. 2022;13:880519.
15. Devasirvatham V, Tan DKY, Trethowan RM, Gaur PM. Response of physiological, reproductive function and yield traits in cultivated chickpea (*Cicer arietinum* L.) under heat stress. Front Plant Sci. 2022 [PMC9202580].
16. Sita K, Sehgal A, Bhandari K, Kumar J, Kumar S, Singh S, *et al*. Heat-stress-induced reproductive failures in chickpea (*Cicer arietinum*) are associated with impaired sucrose metabolism in leaves and anthers. Funct Plant Biol. 2017;44(7):712-26.
17. Chaturvedi P, Ghatak A, Weckwerth W. Climate change and heat stress tolerance in chickpea. In: Climate Change and Plant Abiotic Stress Tolerance. Wiley-VCH; 2013. p. 837-56.
18. Bohra A, Jha UC, Kishor PBK, Pandey S, Singh NP. Genomics and molecular breeding in lesser explored pulse crops: current trends and future opportunities. Biotechnol Adv. 2020;42:107556.
19. Paul PJ, Samineni S, Sajja SB, Rathore A, Das RR, Chaturvedi SK, *et al*. Capturing genetic variability and selection of traits for heat tolerance in a chickpea recombinant inbred line (RIL) population under field conditions. Euphytica. 2018;214:27.
20. Barmukh R, Roorkiwal M, Dixit GP, Bharadwaj C, Kumar A, Rani A, *et al*. Major QTLs and potential candidate genes for heat stress tolerance identified in

chickpea (*Cicer arietinum* L.). Front Plant Sci. 2021;12:655103.
21. Kohli D, Basavarajappa SH, Chellapilla B, Kumar J, Jain PK, Srinivasan R. Genome-wide analysis identifies chickpea (*Cicer arietinum*) heat stress transcription factors (Hsfs) responsive to heat stress at the pod development stage. J Plant Res. 2018;131:525-42.
22. Barmukh R, Bharadwaj C, Roorkiwal M, Jha UC, Chitikineni A, Sharma D, *et al*. Chickpea (*Cicer arietinum* L.) battling against heat stress: plant breeding and genomics advances. Plant Mol Biol. 2025.
23. Khan AW, Garg V, Sun S, Gupta S, Dudchenko O, Roorkiwal M, *et al*. Cicer super-pangenome provides insights into species evolution and agronomic trait loci for crop improvement in chickpea. Nat Genet. 2024;56:1225-34.
24. Nayak SN, Aski MS, Rathore A, Mishra GP, Bharadwaj C. Molecular breeding and drought tolerance in chickpea. Life. 2022;12(11):1846.
25. Negussu M, *et al*. Drought tolerance mechanisms in chickpea (*Cicer arietinum* L.) investigated by physiological and transcriptomic analysis. Environ Exp Bot. 2023.
26. Nisa Z, Chen C, Yu Y, Chen Y, Chen R, Yu M, *et al*. A comparative metabolomic study on desi and kabuli chickpea (*Cicer arietinum* L.) genotypes under rainfed and irrigated field conditions. Sci Rep. 2020;10:14649.
27. Khazaei H, Vandenberg A, *et al*. UPLC-HRMS-based untargeted metabolic profiling reveals changes in chickpea (*Cicer arietinum*) metabolome following long-term drought stress. Plant Cell Environ. 2018.
28. Chaturvedi P, Vaid N, Ghatak A, Weckwerth W. Natural variation in the chickpea metabolome under drought stress. Plant Biotechnol J. 2024.
29. Kumar A, Sharma S, Bhardwaj R. Unravelling the molecular mechanism underlying drought stress response in chickpea via integrated multi-omics analysis. Sci Rep. 2023;13.
30. ChickpeaOmicsR development team. Identification of key genes in chickpea transcriptomics and the development of ChickpeaOmicsR as a comprehensive resource to advance breeding and genomic studies. Front Bioinform. 2026.
31. Yadav R, Verma A, Singh D. Recent advancement in OMICS approaches to enhance abiotic stress tolerance in legumes. Front Plant Sci. 2022;13:952759.
32. Kim S, Park J, Lee H. Mass spectrometry-based plant metabolomics: metabolite responses to abiotic stress. Molecules. 2025.
33. Allwood JW, Goodacre R. An introduction to liquid chromatography-mass spectrometry instrumentation applied in plant metabolomic analyses. Phytochem Anal. 2010;21(1):33-47.
34. Obata T, Fernie AR. The use of metabolomics to dissect plant responses to abiotic stresses. Cell Mol Life Sci. 2012;69(19):3225-43.
35. Zhang J, Li Y, Wang X. Advances in plant metabolomics and its applications in stress and single-cell biology. Int J Mol Sci. 2022;23(13):6907.
36. Fiehn O. Metabolomics-the link between genotypes and phenotypes. Plant Mol Biol. 2002;48(1-2):155-71.
37. Devasirvatham V, Gaur PM. Food legumes: chickpea. In: Genomic Designing of Climate-Smart Pulse Crops. Springer; 2019. p. 1-48.
38. Merga B, Haji J. Economic importance of chickpea: production, value, and world trade. Cogent Food Agric. 2019;5(1):1615718.
39. FAOSTAT. Food and Agriculture Organization of the United Nations Statistical Database. Rome: FAO; 2024.
40. Singh AK, Patel J. Integrating artificial intelligence and biotechnology to enhance cold stress resilience in legumes. Front Plant Sci. 2025.