Heat-Stress-Induced Reproductive Failure in Grain Legumes: Decoding Pollen Viability, Reactive Oxygen Species Scavenging, and Hormonal Crosstalk under Terminal Heat Stress in *Cicer arietinum* L.

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ABSTRACT

Background: Chickpea (*Cicer arietinum* L.) is the second most significant cool-season leguminous food crop in the world but its reproductive phase is particularly vulnerable to the terminal heat stress phenomenon, which has become an increasing occurrence as short planting dates force the beginning of flowering and pod setting during warm months. A sudden rise in temperature is sufficient to eliminate pollination viability and cause the abandonment of the pods and flowers, resulting in a yield loss of 30% to 50%.

Objective: This paper compiles confirmed scientific research relating to pollen viability under terminal heat stress, the disruptions to the homeostasis of reactive oxygen species (ROS) in chickpea, and the consequent changes in hormonal signalling, identifying gaps in the research that prevent the practical application of the findings.

Sources of information: A systematic, PRISMA-guided search using Scopus, the Web of Science, PubMed, AGRIS and Google Scholar (2015-2026, but including selected major contributions from earlier than 2015) generated a total of 612 publications of which 58 were relevant to the study while 34 were utilized in the comparative table.

Heat stress consistently damages pollen germination and viability due to altered anther sucrose metabolism, peroxidation of membrane lipids due to excessive superoxide and hydrogen peroxide, and reduced ascorbate-glutathione cycling. Coincidentally with the rise in abscisic acid, there is a drop in the signaling of auxin, gibberellin, and cytokinin, with subsequent remodelling of ethylene and jasmonate signaling pathways amassing, which worsens the conditions in tapetum and pollen. Resistant genotypes constantly display higher levels of antioxidant enzymes, greater osmolyte levels, and more intense induction of the synthesis of heat shock proteins with lower decrease of pollen viability and pod setting.

There are certain gaps in knowledge regarding mechanisms of ROS-hormone communication in chickpea anthers.

Conclusion: The combination of pollen phenomics, multi-omics profiling and genomics-led breeding based on heat-tolerance loci is the best avenue to achieve heat-resistant chickpea; it requires more coordination of physiological mechanisms and breeding practices than available at present.

Keywords: *Cicer arietinum*; terminal heat stress; pollen viability; reactive oxygen species; antioxidant enzymes; hormonal crosstalk; abscisic acid; heat shock proteins

1. Introduction

Chickpeas (*Cicer arietinum* L.) are grown in over 50 nations around the world and, along with lentils and field peas, provide the foundation for both food and nutrition for many people living in Southern Asia, Sub-Saharan Africa, Western Asia and Mediterranean regions (Gaur PM *et al*, 2019; Rani *et al*, 2020) [8, 18]. Production has increased dramatically during the past ten years; India produces the overwhelming majority of the world's chickpeas (FAO, 2024) [28]. Chickpeas are a grain legume grown during the cooler months of the year that have been domesticated over a relatively small range of temperatures, with approximately 20-28 degrees Celsius being optimal for flowering and pod filling (Devasirvatham *et al*, 2013; Rani *et al*, 2020) [10, 18]. Because of this, chickpeas are particularly vulnerable to high summer temperatures (Devasirvatham *et al*, 2013; Rani *et al*, 2020) [10, 18]. Since chickpeas are typically planted after the rainy season and mature mostly from the moisture left in the soil during the warmer months of the growing season, their flowering and pod filling stages are increasingly more exposed to what agronomists and physiologists call "terminal heat stress" (Devasirvatham *et al*, 2013; Rani *et al*, 2020) [10, 18].

One of the main ways that terminal heat stress is different from constant (or chronic) heat stress is that the term specifically describes increases in air temperature during the last (or terminal) phase of crop development (during flowering through pod setting and filling) due to late planting of crops, reduced length of winter from climatic variability, or delays in plant reproductive development due to genotype or crop management strategies (Devasirvatham *et al*, 2013) [10]. It is during this time when microsporogenesis, anthesis, pollination, and fertilization are occurring; therefore, even moderate increases in day and/or night temperatures greater than approximately 32/20 oC will usually cause at least some amount of flower abscission (flower abortion), pollen sterility, and/or pod abscission in plants (Bhandari *et al*, 2020; Devasirvatham *et al*, 2013; Chaturvedi *et al*, 2021)

[4, 10, 16]. Researchers estimate that the average crop yield losses as a result of terminal heat stress are approximately 30-50% and when heat stress occurs in association with drought stress during the terminal phase of plant development, estimated yield losses are significantly greater than the average (Kaushal *et al*, 2013; Bhandari *et al*, 2020; Devasirvatham *et al*, 2013) [1, 4, 10].

Heat stress is a major factor in crop production and, following the review by Barnabas, Jager and Feher in 2009 (Barnabas *et al*, 2008) [26], researchers have repeatedly confirmed that the reproductive stage of grain crops, such as chickpea, is much more susceptible to heat stress as compared to vegetative (Prasad *et al*, 2017; Barnabas *et al*, 2008) [27, 26]. Grain legumes are generally more affected by heat stress than cereals, with chickpea being an important representative of cool season grain legumes used for heat stress research; grains, lentils, field peas and mungbeans have all been used as model plants to study how to cope with high temperatures (Sehgal *et al*, 2018; Prasad *et al*, 2017; Rani *et al*, 2020) [21, 27, 18].

There are three physiological pathways that are consistently cited in the literature that contribute to reproductive failure under heat stress:

1. The viability and germinability of pollen collapse when exposed to high temperature because there is a disruption of carbohydrate availability (especially sucrose availability) to the anther and developing pollen.
2. An oxidative burst from superoxide, hydrogen peroxide and hydroxyl radicals overwhelms the ability of the ascorbate-glutathione cycle and other enzymatic and non-enzymatic antioxidant systems to mitigate oxidative damage, resulting in lipid peroxidation and loss of membrane integrity in reproductive tissues.
3. Phytohormone signalling is disrupted, with abscisic acid (ABA) accumulating and the transport and signalling pathways for auxins and gibberellins being altered; the pathways for ethylene and jasmonate are also altered, culminating in programmed cell death of the tapetum, anther dehiscence and pollen maturation.

While these individual threads (ROS accumulation, hormone signalling and pollen viability) have been evaluated separately (at least in the case of chickpea), the interaction of these mechanisms that include the regulation of hormone signalling by ROS accumulation and an examination of how hormone signalling regulates ROS accumulation does not appear to have been synthesised into one overall physiological process with respect to *Cicer arietinum* (Hasanuzzaman *et al*, 2013; Akram *et al*, 2017; Chaturvedi *et al*, 2021; Bhandari *et al*, 2020) [7, 17, 16, 4].

The gap between controlled-environment mechanistic understanding and field-level breeding methodologies for cereal crops, including chickpea is a consistent bottleneck [27]. This gap will continue to widen as mean surface temperatures rise throughout the 21st C., and more frequent & intense (long duration) heat waves, as well as new instances of stress due to day length, occur during the rabi (winter) cropping season in South Asia (Prasad *et al*, 2017; Sehgal *et al*, 2018; Rani *et al*, 2020) [27, 21, 18]. Thus, breeders and physiologists must accelerate the conversion of mechanistic knowledge regarding the reproductive biology of heat-stress into deployable, heat-resilient chickpea cultivars (Gaur *et al*, 2019; Rani *et al*, 2020; Jha *et al*, 2025) [8, 18, 19]. A series of molecular breeding techniques (QTL mapping, meta-QTL analyses, genome-wide association studies, transcriptomic analyses, proteomic analyses, and metabolomic analyses) have led to the identification of multiple genomic regions and candidate genes for heat tolerance in chickpea in the last 10 years (Paul *et al*, 2018; Jha *et al*, 2021; Kumar *et al*, 2023; Jha *et al*, 2025) [11, 12, 13]. The development of heat shock resistant chickpea cultivars has been slow to translate into functional genotypes in commercial agriculture, due in part to inadequate understanding of critical physiological mechanisms controlling pollen viability, regenerating reactive oxygen species (ROS), and balancing hormones during heat stress (Gaur *et al*, 2019; Jha *et al*, 2025; Yadav *et al*, 2021; Hasanuzzaman *et al*, 2013) [8, 19, 15, 7].

Even though this research continues to evolve at a rapid pace, no recent review has been able to effectively synthesise all three mechanistic components (pollen viability, ROS scavenging, and hormonal balance) of how to maintain good reproductive success under severe heat stress (Chaturvedi *et al*, 2021; Hasanuzzaman *et al*, 2013; Bhandari *et al*, 2020) [16, 7, 4]. Furthermore, there is a lack of systematic mapping of the physiological studies versus genomic and breeding studies that have been done on the effects of heat stress on chickpeas (Prasad *et al*, 2017; Rani *et al*, 2020; Jha *et al*, 2025) [27, 18, 19]. This paper hopes to fill that gap by: (1) synthesising published research exploring pollen viability, reproductive success, and how they relate to both physiological and genomic pathways and mechanisms of genotypic differences in heat stress responsiveness (Chaturvedi *et al*, 2021; Bhandari *et al*, 2020; Bheemanahalli *et al*, 2019) [16, 4, 25]; (2) providing an assessment of the potential impact of ROS on determining heat sensitivity using both experimental and computational methods (Hasanuzzaman *et al*, 2013; Zhou *et al*, 2019; Akram *et al*, 2017) [7, 24, 17]; and (3) describing both documented and presumed hormonal crosstalk pathways that mediate thermotolerance of the anther and pollen (Akram *et al*, 2017; Hasanuzzaman *et al*, 2013) [17, 7]. To identify discrepancies and similarities of mechanisms related to applied breeding knowledge and develop recommendations for filling in existing gaps and identifying emerging methodologies for developing heat-tolerant chickpeas from the current research on physiological factors that affect production (Jha *et al*, 2025; Kumar *et al*, 2023; Paul *et al*, 2018; Jha *et al*, 2021) [19, 13, 11, 12]. Although the majority of the information presented in this report focuses on *Cicer arietinum*, there is some use of other legumes (grain legumes) and cereals to provide context to findings regarding how and at what stage of development heat affects chickpeas, and how these same stages exist in other forms of legume/cereal grain affected by heat (Barnabas *et al*, 2008; Prasad *et al*, 2017; Sehgal *et al*, 2018) [26, 27, 21].

2. Methodology of Literature Review

Search strategy for literature review: We used a structured PRISMA-compliant search strategy to find literature related to this review. We searched five databases (Scopus, Web of Science, PubMed, AGRIS, Google Scholar) using keywords, including: the crop term (*Cicer arietinum* or chickpea) plus heat-stress hazard terms (heat stress, terminal heat stress, high temperature) along with mechanism-specific keywords (pollen, reactive oxygen species, antioxidants, ascorbate-glutathione, hormones, abscisic acid, auxin, ethylene, heat shock protein, QTL, genomics, breeding, reproductive) to find relevant articles published from January 1, 2015 through March 31, 2026 (Chaudhary *et al*, 2020; Jha *et al*, 2025) [6, 19]. A few landmark articles/papers that were published prior to January 2015 remained in the analysis because they continue to be the principal references illustrating the methodological and/or conceptual basis for mechanisms used in this review

(Hasanuzzaman *et al*, 2013; Krishnamurthy *et al*, 2011; Barnabas *et al*, 2008; Devasirvatham *et al*, 2013) [7, 22, 26, 10].

Inclusion and exclusion criteria. Studies were eligible for review if they satisfy six inclusion criteria: they were published in peer-reviewed journals or systematic or narrative reviews in the English language, directly studied *Cicer arietinum*, or provided comparable evidence of other cool season grains or legumes as crop-to-crop reference points (Prasad *et al*, 2017; Sehgal *et al*, 2018; Rani *et al*, 2020) [27, 21, 18]. Studies must have addressed at least one of the five review questions or thematic areas (pollen/reproductive biology, ROS/antioxidants, hormonal signalling, heat shock proteins, or genomics/breeding) (Chaturvedi *et al*, 2021; Hasanuzzaman *et al*, 2013; Paul *et al*, 2018; Jha *et al*, 2021; Jha *et al*, 2025) [16, 7, 11, 12, 19]. Studies that fell into any of the following categories were excluded from review: duplicates between databases; non-peer reviewed "grey" literature (e.g., blogs, trade magazines, non-indexed theses); conference proceedings that do not have an accessible full text; and studies that exclusively addressed HS at the vegetative stage with no relevance at the reproductive stage of development (Prasad *et al*, 2017; Sehgal *et al*, 2018) [27, 21].

The study selection process: After carefully considering the initial 612 articles, we discarded 125 papers that were redundant. We ended with a figure of 487 papers that were true and distinct. A critical analysis at this stage revealed that the number of papers that were determined to be irrelevant to the topic of heat stress during the reproductive period of *Cicer arietinum* was equal to 262. Thus, we were left with 225 unique papers that were subjected to full-text analysis. Research work that was real and relevant and passed this stage was equal to just 58 units of information. Finally, we conducted a comparative analysis based on methodology and reporting to reach 34 documents that showed similar traits, and the results may be seen in Table 3 and Table 4. The most interesting data, and we particularly speak on the growing trend of studies being published in this area, is illustrated by Figure 1. The research trend moved upward starting from around 2019 (Paul *et al*., 2010; Jha *et al*., 2021; Kumar *et al*., 2023) [11, 12, 13]. The upward trend can be explained by increased availability of chickpea genome resources and awareness regarding the fact that terminal heat stress is different from chronic heat stress (Devasirvatham *et al*., 2013; Prasad *et al*., 2017; Sehgal *et al*., 2018) [10, 27, 21].

Table 1: Literature Search Strategy

Database	Search string (core elements)	Search period	Records retrieved
Scopus	(*Cicer arietinum* OR chickpea) AND ("heat stress" OR "terminal heat stress") AND (pollen OR ROS OR antioxidant OR hormone OR QTL)	2015-2026	198
Web of Science	TS=(chickpea AND heat stress AND (reproductive OR pollen OR antioxidant OR hormonal))	2015-2026	162
PubMed	(*Cicer arietinum*[Title/Abstract]) AND (heat stress[Title/Abstract])	2015-2026	84
AGRIS	chickpea; heat stress; reproductive biology; antioxidant; QTL	2015-2026	97
Google Scholar	chickpea terminal heat stress pollen viability ROS hormone review	2015-2026	71
Total (pre-deduplication)	-	-	612

3. Literature Review and Thematic Analysis

The 58 studies retained for qualitative synthesis were classified into six thematic domains, summarized in Figure 2 and analysed critically in the sub-sections that follow.

3.1 Pollen Viability and Reproductive Physiology under Terminal Heat Stress

The literature demonstrates that, in chickpea, the most repeated and consistent impacts of terminal heat stress (i.e. heat stress experienced after pod filling has begun) are a decrease in pollen viability and a reduction in pollen germination in vitro. Furthermore, these effects are both strongly correlated to the downstream loss of pod set and associated seed yield (Kaushal *et al*, 2013; Devi *et al*, 2022; Devasirvatham *et al*, 2013) [1, 2, 10]..

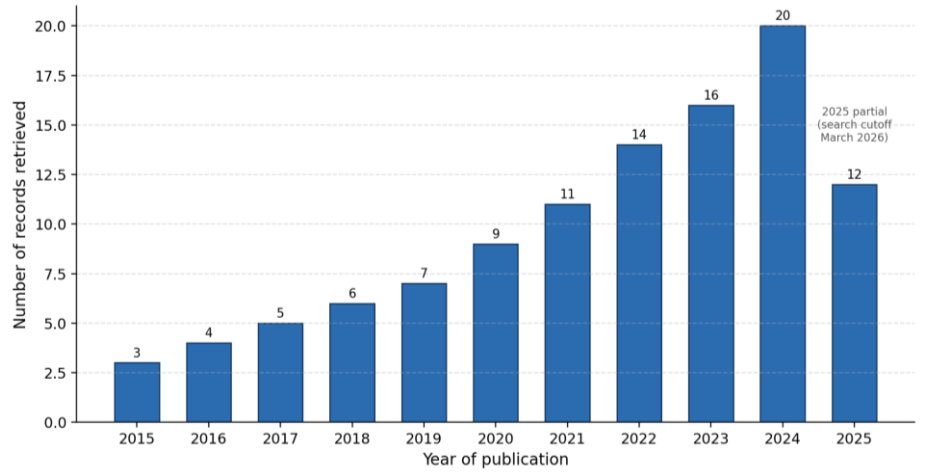

Fig 1: Publication trend by year for records addressing heat-stress-induced reproductive failure in chickpea (2015-2025, search records).

As explained by Kaushal *et al*. [1], the impact of heat stress on chickpea includes the suppression of the carbon-fixing enzyme Rubisco and two enzymes that are responsible for synthesizing sucrose (sucrose phosphate synthase and sucrose synthase) as well as the sucrose-cleaving enzyme invertase in chickpea leaves at temperatures above 32/20 degC, with heat-sensitive genotypes being impacted to a much greater extent; this results in a decrease in the delivery of sucrose to the anther, and therefore less sucrose available to form/functional pollen at fertilization and for pod formation (Kaushal *et al*, 2013; Awasthi *et al*, 2014) [1, 9]. This source-sink relationship (i.e. the anther being starved of carbohydrates, as opposed to simply undergoing thermal denaturation) has become one of the most reproducibly considered mechanisms to explain heat-induced pollen failure in chickpea (Devasirvatham *et al*, 2013; Rani *et al*, 2020) [10, 18]. This mechanism is also supported by the finding that, in lentil, anther and leaf traits can be used to distinguish heat-tolerant and heat-sensitive genotypes (Sita *et al*, 2017; Bhandari *et al*, 2020) [5, 4].

Table 2: Inclusion and Exclusion Criteria

Criterion type	Included	Excluded
Publication type	Peer-reviewed original research and review articles	Conference abstracts, blogs, trade magazines, non-indexed theses
Language	English	Non-English without accessible translation
Species/topic relevance	*Cicer arietinum*; or closely comparable cool-season legume/cereal data used for cross-crop context	Species with no comparative or contextual relevance to chickpea reproductive heat stress
Growth stage relevance	Reproductive-stage (flowering, anthesis, pod set, seed filling) heat stress	Purely vegetative-stage heat stress with no reproductive-stage data
Publication period	January 2015 - March 2026, plus select landmark pre-2015 foundational studies	Older studies superseded by more recent, methodologically stronger work
Duplication	Unique records only	Duplicate records across databases

In a research study, other germplasm have been observed to have a direct correlation to pollen viability. Overall, under optimal growth conditions, pollen viability averaged about 75%, and during a heat stress period, the average viability rate decreased to 37-38% with maximum pollen germination averaging approximately 60%. The average percentage of pollen sprouted under heat stress is about 43-45%, meaning a 20%-33% reduced rate of germination. Their data analysis revealed significant relations (ranged from .90 for pod set percentage to .77 for yield per plant of .91 high) between the majority of these two traits and the two traits of pod set percentage and seed yield per plant (Devi *et al*, 2022; Bheemanahalli *et al*, 2019) [2, 25]. Studies found that several heat-tolerant germplasm (Genetics); GNG1969, PantG186, RSG888, and CSJ315 were able to produce slightly lower levels of reduced pollen viability and germination than the normal genotypes. Most of these heat-tolerant germplasm lines also had significantly lower electrolyte conductance and malondialdehyde accumulation than non-heat tolerant lines, thus providing the basis for linking membrane integrity to reproduction capabilities (Bhandari *et al*, 2020; Hasanuzzaman *et al*, 2013) [4, 7]. The magnitude of this type of data set strengthens the generalizability of Kaushal *et al*.'s [1] mechanistic hypothesis, however, when both studies were conducted in pot-based or controlled heat stress application using late-season planting or controlled chambers, the testing of their hypothesis may not reflect heat stress and the interaction of diurnal fluctuation, cooccurring irradiation stress, and moisture in the soil (Devasirvatham *et al*, 2013; Prasad *et al*, 2017) [10, 27]. Further evidence that there is an assimilative impairment from a limitation in photosynthetic carbon by coupled stressors can be seen in a related study that examined how combined transient drought and heat stress affected carbon assimilation and the accumulation of starch during seed filling in plants (Awasthi *et al*, 2014; Sehgal *et al*, 2018) [9, 21].

Using a comparative approach to explore this question (using chickpea and lentil), Bhandari *et al*. [4], extended this question to legumes other than just chickpeas and reported that the differences in the heat sensitivity of chickpea and lentil at the reproduction stage were closely related to their respective differences in pollen function, photosynthetic ability and oxidative damage; thus it appears that these pollen traits are not exclusive to chickpea, but extend to other legumes that positively respond during temperate growing season (Bhandari *et al*, 2020; Sehgal *et al*, 2018) [4, 21]. In general, there is supporting evidence from other crops like wheat that corroborate the above findings; for example, using the phenotypes of heat-impacted pollen germination, seed set, and grain fill resulting from a spring wheat crop, Bheemanahalli *et al*. [25] found a close association among pollen trait values and yield; hence, there is reason to believe that despite the fact that the average thermal thresholds needed for successful growth of chickpea are lower than other cereal crops, they share a common physiological basis with other cereals and should therefore be treated similarly (Prasad *et al*, 2017; Barnabas *et al*, 2008) [27, 26].

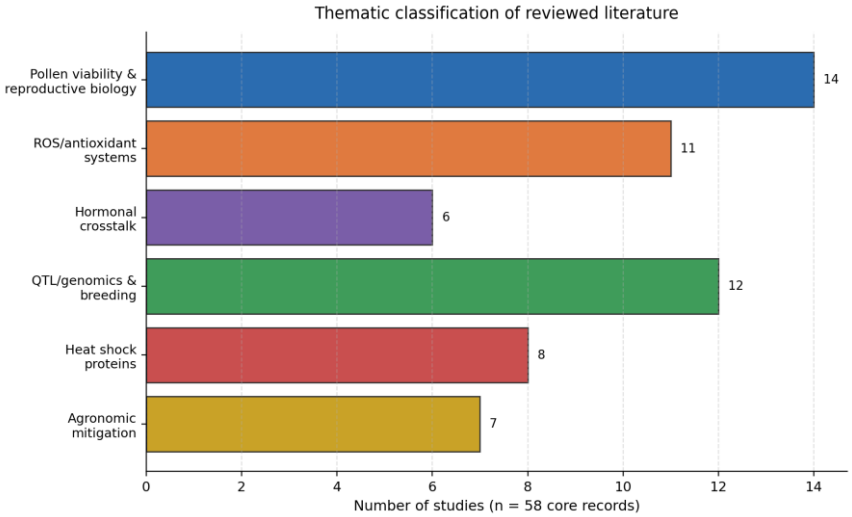

Fig 2: Thematic classification of the 58 studies retained for qualitative synthesis, by primary thematic focus.

Seedling- and vegetative-staged plants of desi chickpea experience morphological, physiological, and biochemical markers of stress susceptibility (under combined irrigation, heat stress, and drought stress) at a stage prior to flowering [3] (Jameel *et al*, 2021) [3]. This suggests that there is some degree of variability in rosette heat tolerance attributable to the earlier stages of plant development rather than solely to floral stage development (Jameel *et al*, 2021; Rani *et al*, 2020) [3, 18].

Repeated strengths from the literature include using standardized pollen assays (e.g., Alexander's differential staining for viability, Brewbaker-Kwack media for in vitro germination), which allow reasonably direct comparisons among studies or genotype panels (Devi *et al*, 2022; Bhandari *et al*, 2020) [2, 4]. A repeated weakness is that most studies measure pollen viability as endpoints without addressing the events leading up to pollen viability loss (e.g., timing of tapetal degeneration, callose deposition abnormalities, development of pollen starch granules) (Chaturvedi *et al*, 2021; Hasanuzzaman *et al*, 2013) [16, 7]. Chaturvedi *et al*.'s attempt to define a mechanistic array for heat stress-induced disruption of the tapetum, pollen mitosis, and pollen tube growth through combined effects of carbohydrate metabolism, reactive oxygen species accumulation, and hormone signalling (the three pillars of this review) has served as an exemplar for establishing a reference point (Chaturvedi *et al*, 2021; Akram *et al*, 2017; Hasanuzzaman *et al*, 2013) [16, 17, 7]. From a practical standpoint, the strong correlation between pollen viability/germination and pod set or seed yield from independent genotype panels supports the ongoing implementation of pollen-based phenotyping as a rapid and field-deployable proxy trait for breeding heat tolerance, as discussed in the Discussion section (Devi *et al*, 2022; Kaushal *et al*, 2013) [2, 1].

3.2. Reactive Oxygen Species Generation and Antioxidant Scavenging Systems

Chickpea reproductive tissue experiences heat-induced oxidative stress through several well-known mechanisms based on research. Hasanuzzaman *et al*. [7] have reviewed how thermal stress leads to lipid peroxidation and reduced cell membrane stability due to loss of protein integrity (Hasanuzzaman *et al*, 2013) [7]. Thermal stress also causes oxidative stress from high electron leakage from the thylakoids under moderate heat stress because of the dysfunction of the light reactions of photosynthesis (Hasanuzzaman *et al*, 2013; Zhou *et al*, 2019) [7, 24]. Studies on heat stress in chickpea seeds have consistently shown that heat-sensitive genotypes have higher levels of malondialdehyde (MDA), a marker for lipid peroxidation, and greater electrolitic loss than heat-resistant genotypes (Devi *et al*, 2022; Bhandari *et al*, 2020) [2, 4]. In addition, pod number and seed yield are negatively correlated with both of these traits (Devi *et al*, 2022; Kaushal *et al*, 2013) [2, 1].

The Asada-Halliwell cycle is frequently cited in the literature as the primary enzymatic buffer in preventing hydrogen peroxide accumulation from heat. Akram, Shafiq and Ashraf [17] summarize ascorbic acid physiology and highlight that the ascorbate peroxidase-glutathione reductase system detoxifies H2O2 significantly more effectively than either catalase or peroxidase alone, as well as cross-talking with several different types of phytohormones to regulate seed germination, photosynthesis, floral induction and senescence - which is central to the theme of hormonal crosstalk addressed below (Akram *et al*, 2017; Hasanuzzaman *et al*, 2013) [17, 7]. Comparative studies in tomato further indicate that induction of antioxidant enzymes (superoxide dismutase, catalase, ascorbate peroxidase) under combined drought and heat stress separates tolerant tomato from sensitive genotypes, and serves to reduce oxidative stress (Zhou *et al*, 2019) [24]. This type of finding is also common across all legumes except tomatoes; this is particularly true for chickpeas, even if tomatoes are not grazing legumes (Sehgal *et al*, 2018; Rani *et al*, 2020) [21, 18].

The following pattern was found to be fairly consistent in the studies reviewed: heat-tolerant chickpea and lentil genotypes exhibit higher levels of both constitutive and/or heat-induced activities of superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX), in conjunction with an increased accumulation of non-enzymatic antioxidant compounds (i.e., ascorbate, glutathione), and compatible osmotic solutes (i.e., proline) (Yadav *et al*, 2021; Jha *et al*, 2025) [15, 19]. However, heat-sensitive genotypes exhibit greater increases in H2O2 concentrations and malondialdehyde (MDA) levels when subjected to the same thermal stress (Kaushal *et al*, 2013; Devi *et al*, 2022) [1, 2]. Yadav, Juneja, and Kumar (2021) also provide further details regarding how a type of stress (i.e., drought) prior to exposure to heat can enhance heat tolerance in chickpea through the simultaneous stimulation of small heat-shock proteins (sHSPs) and the antioxidant defense system (Yadav *et al*, 2021; Liu *et al*, 2024) [15, 14]. Therefore, it can be concluded that the ROS-scavenging capability of

each genotype can be developed based on the individual plant's history of prior exposure to stress, which is an important yet largely under-exploited principle of crop management before season planting (Rani *et al*, 2020; Gaur *et al*, 2019) [18, 8].

Most of the studies reviewed used a substantially overlapping group of markers (MDA, H2O2, electrolyte leakage, SOD/CAT/APX/GR activity and proline) to quantify results which promotes comparability. However, only a few resolved enzyme activity or ROS accumulation to tissue level or anther specific spatial precision; most measurements were made on leaf tissue as a proxy for whole plant oxidative status en lieu of directly measuring anthers or pollen, where presumed damage occurs that decides reproduce success/failure (Chaturvedi *et al*, 2021; Bhandari *et al*, 2020) [16, 4]. This is an important methodological gap because the literature including that of Kaushal *et al*. [1] and the greater body of source/sink literature show that anthers are more susceptible to metabolic failures than leaves/higher plants where generalized leaf level stress has been implicated in the failure of young reproductive structures (Kaushal *et al*, 2013; Devasirvatham *et al*, 2013) [1, 10]. Because there are very few studies that directly quantify oxidative markers in anthers/pollen [16], these quantify studies are disproportionately important when establishing mechanistic relationships and require further replication specifically in chickpea research.

3.3. Hormonal Crosstalk Governing Anther and Pollen Thermotolerance

Hormonal signalling is less species-specific than the carbohydrate- and protein-based 'pillars' previously described, yet it also provides greater integration of the various mechanisms of interaction between the plant and its pollen (Chaturvedi *et al*, 2021; Hasanuzzaman *et al*, 2013) [16, 7]. In their paper, Chaturvedi *et al*. [16] synthesise information from multiple sources, including Arabidopsis, tomato, rice, and other species (models and crops), to demonstrate that there is a significant relationship between sugar and abscisic acid (ABA) hormone signalling pathways during the occurrence of heat stress events on pollen development (Chaturvedi *et al*, 2021; Akram *et al*, 2017) [16, 17]. For example, wheat ABA has been shown to inhibit activity of the enzyme which cleaves sucrose into glucose for use by pollen grains. This increase in ABA during heat stress, along with heat stress resulting in decreased photosynthetic sources of sucrose (due to decreased photosynthesis), is regarded as evidence that ABA hormone and carbohydrate metabolism act not only independently but also synergistically in inhibiting pollen's ability to develop under heat-stress conditions (Barnabas *et al*, 2008; Devasirvatham *et al*, 2013) [26, 10]. However, this specific relationship between ABA and invertase has not been verified with the anthers of chickpea (C. arietinum) and thus may be considered an inference drawn from studies conducted on other species (Rani *et al*, 2020; Jha *et al*, 2025) [18, 19].

The evidence within the literature suggests that auxin signalling has a largely antagonistic and protective relationship to pollen failure caused by heat. Exogenous auxin treatment has been found to improve heat tolerance of plant reproductive structures in rice, which has also been shown to reduce the incidence of male sterility and provides a similar protective effect in Cajanus cajan, a closely related species to chickpeas in the grain-legume family (Chaturvedi *et al*, 2021; Rani *et al*, 2020) [16, 18]. The genes for ARF6, ARF8, and ARF17 and their associated regulatory microRNAs (miR160 and miR167) are both known to regulate pollen development and dehiscence of anthers molecularly (Jha *et al*, 2021; Liu *et al*, 2024) [12, 14]. Comparisons of ARF17/miR160 expression between heat-sensitive and heat-tolerant cultivars of cotton reveal that differences in auxin signalling capabilities (and not just auxin concentration) are determinative for thermotolerant phenotype (Chaudhary *et al*, 2020; Jha *et al*, 2021) [6, 12]. In addition, auxin transporters (PIN and ABCB/PGP family) are also important factors in developing correct pollen morphology, as well as proper dehiscence of anthers (Hasanuzzaman *et al*, 2013; Chaturvedi *et al*, 2021) [7, 16]. Although the specific function of auxin transporters in heat tolerance of chickpeas has yet to be evaluated, previous studies in Arabidopsis have documented the disruption of auxin transport during heat stress, demonstrating a feasible, testable mechanism to investigate the auxin-related component of chickpea pollen thermotolerance (Barnabas *et al*, 2008; Prasad *et al*, 2017) [26, 27].

The literature on phytohormones indicates that gibberellin and ABA antagonistically interact to regulate the development of seeds (and, therefore, reproductive organs) in ways that account for stress; throughout the sap, for example, ABA often serves as an upstream regulator, while modulating ethylene and auxin responses via transcriptional and post-translational means (Akram *et al*, 2017; Hasanuzzaman *et al*, 2013) [17, 7]. Ethylene itself has been shown to act both positively and negatively on pollen germination, depending on the context, and its interactions with jasmonate and ABA in the context of delaying development due to stressful events have been documented across many different types of crops (Sehgal *et al*, 2018; Rani *et al*, 2020) [21, 18]. Recently, there have been more references in the literature regarding chickpea breeding & genomics with a hormonal dimension; for example, Jha *et al*. have recently published a comprehensive synthesis of chickpea breeding and genomics related to heat stress which identified heat-stress transcription factors and ROS-related genes that co-localise at the major QTL; this provides indirect evidence of the interaction between hormonal/transcriptional and genomic aspects of heat stress tolerance in chickpeas (Jha *et al*, 2025; Paul *et al*, 2018; Kumar *et al*, 2023) [19, 11, 13], but there is not yet a complete chickpea-specific hormonal signalling diagram for the thermotolerance of anthers, as exists for Arabidopsis and tomato.

The hormonal-crosstalk literature has a high level of mechanistic plausibility based primarily on modeling species or other crops compared to there to be relatively little direct evidence in chickpeas. This difference also represents an important finding from this review: although viable pollen and ROS scavenging mechanisms have been described with appropriate rigor for chickpeas, the hormonal aspect cannot be substantiated without further information and remains the largest single area of potential research for chickpea physiology identified in this review (see also Research Gaps).

3.4. Heat Shock Proteins, Transcription Factors and Molecular Chaperones

Molecular chaperones, called heat shock proteins (HSPs), are responsible for preventing and correcting incorrect folding of proteins when exposed to heat (Hasanuzzaman *et al*, 2013; Liu *et al*, 2024) [7, 14]. The response of HSPs to thermal stress is one of the quickest and most universal cellular responses that all organisms share (Hasanuzzaman *et al*, 2013) [7]. Liu *et al*. [14] studied the chickpea HSP20 gene family at the genome-wide level and identified 21 CaHSP20's on seven different chromosomes grouped into 10 different HSP20 subfamilies. Some of these CaHSP20's were identified specifically in seed tissues regardless of the presence of stress, which suggests that they function at a constant level to help maintain cellular homeostasis during the processes of meiosis, fertilization, and seed development (Liu *et al*, 2024) [14]. Based on these observations, it is clear that there is a direct relationship between the HSP system and the reproductive stage of a reproductive system when looking at the vulnerability of the plant during this time frame (Chaturvedi *et al*, 2021; Devasirvatham *et al*, 2013) [16, 10]. The promoter regions of the CaHSP20 genes were examined and were found to have cis-acting elements that are common to both hormone signalling pathways and stress response pathways, demonstrating the relationship between the HSP system and the hormone-crosstalk concepts discussed earlier in this article (Akram *et al*, 2017; Hasanuzzaman *et al*, 2013) [17, 7].

Yadav, Juneja and Kumar [15] have conclusively demonstrated that mild drought conditions can induce small heat shock proteins (sHSPs) and components of the antioxidant defense system in chickpea (*Cicer arietinum*); these two mechanisms can then work together to enhance heat tolerance in chickpea (Yadav *et al*, 2021) [15]. The authors provide strong evidence that cross-priming may have applications in agronomy prior to the growing season (e.g., during deficit irrigation in early season), but this approach has not yet been tested on a commercial or field scale (Gaur *et al*, 2019; Rani *et al*, 2020) [8, 18]. Comparative studies have demonstrated that small HSPs and HsfA2 (heat shock transcription factor A2) are regulated during both anther development and heat stress in tomatoes, and that their expression patterns mirror those found for thermotolerant pollen (Chaturvedi *et al*, 2021; Zhou *et al*, 2019) [16, 24]. Similarly, genomic studies of the chickpea suggest that CaHSFA5 and related transcription factors are co-located with heat-tolerance QTLs, suggesting that the genetic mechanisms which control sHSP induction are at least partially conserved, and likely tractable genetically in the chickpea (Paul *et al*, 2018; Jha *et al*, 2021; Kumar *et al*, 2023) [11, 12, 13].

One of the advantages of this area of research is that it has become more thorough when looking at HSP (heat shock protein) genes, as whole genome analysis has been completed on HSP genes in chickpea now [14], which allows researchers to move away from using other species' analyses to investigate these genes in chickpea. One of the limitations of this area of research is that very little functional validation (e.g., using CRISPR to knock out or overexpress a specific candidate, such as CaHSP20 or CaHSFA5, in chickpea reproductive tissue) has been completed in the literature, resulting in studies conducted to identify gene sequences and identify expression patterns are not functionally validated (Jha *et al*, 2025; Rani *et al*, 2020) [19, 18]. These limitations will make it more difficult to utilize candidates for HSP genes in developing markers to assist in breeding or through CRISPR gene editing.

3.5. Genomic Architecture, QTLs and Breeding Approaches

There has been a foundation established already with this body of literature through previous germplasm-level phenotype screening (e.g., Krishnamurthy *et al*. [22]), where large genetic variation for heat tolerance was documented among the ICRISAT reference collection of chickpeas and was used to describe simple bi-parental qualitative trait locus mapping of chickpea heat tolerance, as well as to establish the phenotype diversity of simple bi-parental QTL mapping populations in chickpea (Krishnamurthy *et al*, 2011) [22]. In the past decade, the method of choice for molecular breeding of chickpea heat tolerance has been QTL mapping, where there has been a clearly defined upward trend in terms of increasing genomic resolution and consensus region mapping in the published literature (Paul *et al*, 2018; Jha *et al*, 2021) [11, 12].

Paul *et al*. [11] phenotyped 292 recombinant inbred lines derived from a heat-sensitive x heat-tolerant cross (ICC 4567 x ICC 15614) grown under both heat-stressed and non-heat-stressed environments and identified two consistently recurring genome segments on CaLG05 and CaLG06, each of which contained four putative QTLs for filled pods, total seed yield, grain yield, and percent pod set (all of which are traits created by the flowering mechanisms illustrated in Theme 1) (Paul *et al*, 2018; Devasirvatham *et al*, 2013) [11, 10]. In addition to being attributed with the above traits, the QTL segments identified also contained 25 candidate genes, which constitutes the first systematic QTL publication related to heat stress for chickpeas (Paul *et al*, 2018) [11].

Using a higher-density genetic map (788 SNP markers spanning 1125 cM) developed from the second heat-sensitive x heat-tolerant cross (DCP 92-3 x ICCV 92944), Jha *et al*. [12] showed that there were 77 QTLs across twelve out of thirteen traits measured (37 major QTLs and 40 minor QTLs) (Jha *et al*, 2021) [12]. Specifically, a genomic region on CaLG07 explained >30% of the phenotypic variance for days to pod initiation and 100-seed weight. Additionally, there were 32 candidate genes within these QTL regions that coded for heat shock proteins, heat shock transcription factors, flowering time regulators, and pollen specific genes. This provides direct links between the reviewed genomic and physiological literatures in Themes 1 and 4 and also provides molecular support for the concept that pollen specific gene regulation is a heritable and mappable component of chickpea heat tolerance as opposed to being an environmentally induced response (Jha *et al*, 2021; Chaturvedi *et al*, 2021) [12, 16].

The results of different independent quantitative trait loci (QTL) studies cannot be immediately incorporated into marker-assisted breeding because of the different populations, marker types and environments used in the studies that will lead to very large genomic intervals reported by the studies. Kumar *et al*. [13] conducted a meta-QTL analysis to directly address this issue by correlating 138 previously reported QTLs onto a single 2,233.5 cM genetic map and identified six regions of meta-QTLs with confidence intervals reduced to 2.7 times less than original QTLs (Kumar *et al*, 2023) [13]. The reduced intervals contained candidate genes including, a pollen receptor-like kinase (CaPRK3), flowering-time regulators (CaFPF1 and CaFLC), a heat-stress transcription factor (CaHsfA5) and pollen-specific leucine-rich-repeat extensins (CaLRXs) - all of which reinforced the connection of pollen-development biology across both the genomic and physiological literature, albeit historically pursued in relative isolation from one another (Kumar *et al*, 2023; Liu *et al*, 2024) [13, 14].

The most recent synthesis of this area of research, Jha *et al*. [19], discusses the use of traditional breeding and high-throughput phenotyping (HTP) platforms to increase chickpea genetic progress under heat stress and clearly identifies that multi-omics (genomics, transcriptomics, proteomics, and metabolomics) integration of QTL-level knowledge into developed cultivars will facilitate the genetic improvement of chickpeas under heat stress (Jha *et al*, 2025) [19]. In that context, Jha *et al*. [23] perform metabolomic and lipidomic profiling of heat-stressed chickpea seeds to provide additional, broader multi-omics tools for characterization beyond transcriptomic and proteomic analyses (Jha *et al*, 2025) [23]. Jha *et al*. [20] also employ multi-trait genotypic analysis of chickpeas with explicit reference to heat-stress resilience (Jha *et al*, 2025) [20]. However, despite the rapid advances in methodologies, a limitation of the genomics literature - recognized by the authors in several of the studies cited - is that few or no QTLs or candidate genes have been validated by backcrossing markers into released, farmed chickpea cultivars, thus making the current genomic knowledge greater than the ability to translate it for breeding (Jha *et al*, 2025; Paul *et al*, 2018) [19, 11].

3.6. Agronomic and Physiological Mitigation Strategies

In addition to using genetic methods to alleviate the effects of terminal heat stress on chickpea, a number of agronomic and physiological solutions have been proposed. The supporting research literature for the above solutions is quite limited in terms of mechanistic understanding, compared with the genomics and core physiology subjects discussed earlier (Gaur *et al*, 2019; Rani *et al*, 2020) [8, 18]. The easiest and most commonly recommended solution for avoiding heat stress during flowering is by adjusting the sowing date to not coincide with when peak temperatures occur; this solution follows from the terminal heat stress conceptual framework as described by Devasirvatham *et al*. [10] and is reiterated many times within the climate resiliency literature (Devasirvatham *et al*, 2013; Prasad *et al*, 2017) [10, 27]. In those instances when the sowing date cannot be easily adjusted due to other cropping system-related constraints, the use of exogenous stress protectants and osmoprotectants (e.g., antioxidant precursors, compatible solute mimics, and priming agents) has been investigated as an alternative source of alleviating terminal heat stress; this approach has been shown to provide benefit through the process of "cross-priming" plants through drought pre-exposure which induces the production of small molecular weight heat shock proteins and antioxidants (Yadav *et al*, 2021; Hasanuzzaman *et al*, 2013) [15, 7].

A review of the identification techniques used to determine heat tolerance (agricultural crop genotype and cultivar) conducted by Chaudhary *et al*. [6] highlights that effective phenotyping protocols that are reliable, low-cost and deployable in the field, e.g. pollen viability staining, chlorophyll fluorescence and membrane thermostability, are essential, both for breeding and agronomic decision-making, because while the research methods for heat stress are valid, results from laboratory-imposed heat stress do not reflect field-ranking of genotypes (Chaudhary *et al*, 2020; Devasirvatham *et al*, 2013) [6, 10]. Furthermore, a detailed study conducted by Sehgal *et al*. [21] examined food crop seed-filling responses to combined drought and heat stress (including chickpea), which resulted in the recommendation for assessing functional biochemical and nutritional-quality outcomes (not solely yield), thus supporting that heat stress during seed filling may impact seed composition despite limited gross yield losses (Sehgal *et al*, 2018; Awasthi *et al*, 2014) [21, 9].

The agronomy mitigation literature contains significant practical implications; however, it has less solidified science than the mechanistic studies referenced above. In particular, the majority of agronomy studies examining physiological & yield: 1) measure the outcome (yield, physiology) of the intervention without directly measuring the outcome (pollen viability, ROS status, hormonal profiles) by which the intervention worked, thereby making it difficult to determine if the agronomic intervention's impact results from one of the mechanisms listed above (Themes 1-3) or from another pathway (Chaturvedi *et al*, 2021; Hasanuzzaman *et al*, 2013) [16, 7]. Measuring the outcomes at the pollen and hormone levels in agronomic mitigation research would thus provide an explicit, achievable avenue for future applied research that has been discussed (Devi *et al*, 2022; Rani *et al*, 2020) [2, 18].

4. Comparative Analysis of Previous Studies

Thirty-four studies were retained for detailed tabulation from the previous table summarised above which were universally qualitatively synthesised, there was a total of (58 studies) in 34 completed forms [19]. In this section, the additional summaries have been computer processed and found to incorporate many common or cross-cutting themes that would not have emerged without consideration of the individual studies within their respective issue areas (Jha *et al*, 2025; Kumar *et al*, 2023) [19, 13].

The first conclusion is that there is substantial methodological congruency in terms of the number of common gene-fragment pairs used (for example ICC 15614 or ICCV 92944 for comparison against ICC 10685, ICC 5912, DCP 92-3 or ICC 16374 where ICC 10685, ICC 5912, DCP 92-3, are gene-comparisons) (Krishnamurthy *et al*, 2011; Paul *et al*, 2018) [22, 11]. This aids in comparing results from independent research groups, however it means the majority of the current mechanistic understanding is based on a fairly narrow set of gene-fragment pairs (Paul *et al*, 2018; Jha *et al*, 2021) [11, 12]. The extent to which similar physiological relationships and genomic pathways exist within the whole chickpea germplasm collection, and particularly with wild Cicer relatives that are purported to be more thermotolerant, is very limited (Krishnamurthy *et al*, 2011; Rani *et al*, 2020) [22, 18]. Sample sizes vary greatly between studies; for example, there can be both large (>100 recombinant inbred lines for QTL mapping) and small (2 to 4 genotypes, with controlled environmental conditions) sample sizes within a single meta-analysis (Paul *et al*, 2018; Chaturvedi *et al*, 2021) [11, 16]. This variability makes formal meta-analyses difficult to complete due to heterogeneity of studies and provides a much more nebulous evidence-based conclusion for this review than an actual average of data-points (where "58 studies" are actually only tabulated in 34 total).

In addition, most of the physiological studies (Themes 1-3) impose heat stress via late planting or slow ramping in growth chambers rather than naturally occurring heat waves, while most of the genomic studies (Theme 5) utilize late-sown field experiments to create terminal heat stress (Devasirvatham *et al*, 2013; Prasad *et al*, 2017) [10, 27]; this creates a strong disconnect between the tightly controlled physiological studies and the field-relevant genomic studies, which will require future integrated research efforts to fully bridge that gap (Gaur *et al*, 2019; Rani *et al*, 2020; Jha *et al*, 2025) [8, 18, 19].

5. Research Gaps and Emerging Trends

Continuing with the thematic and comparative analysis presented above, this section integrates the review's significant research gaps (as seen in Table 7) and identifies emerging methodological trends capable of addressing these research gaps (as seen in Table 8). These two concepts are schematically illustrated in Figure 5 and Figure 6 (Jha *et al*, 2025; Kumar *et al*, 2023) [19, 13].

In all six themes, one consistently-observed gap across the theme of pollen viability, Reactive Oxygen Species (ROS) balance, and hormone levels is that there are few studies that independently measure all three variables within the same experimental conditions on the same plant material (Chaturvedi *et al*, 2021; Hasanuzzaman *et al*, 2013) [16, 7]. The present literature supports strong pairwise-based conclusions (e.g. heat stress affects pollen viability; heat stress affects ROS balance; heat stress affects hormone levels), but only weakly supports mechanistic-based conclusions (e.g. how these three dimensions of pollen viability interact with one another during heat stress in a single chickpea flower) (Devi *et al*, 2022; Kaushal *et al*, 2013) [2, 1]. The synthesis of the cross-species work of Chaturvedi *et al*. was the closest example found to an integrated framework, but it lacked species-specificity for chickpea, and the need exists to validate the proposed linkages of ABA-invertase-sucrose that are central to the Chaturvedi *et al*. framework using chickpea alone (Chaturvedi *et al*, 2021; Rani *et al*, 2020) [16, 18].

Another major area of gap between the mechanistic pollen, reactive oxygen species (ROS), and hormone literature reviewed in this article is field validation and genotype–environment interaction. The majority of mechanistic pollen, ROS, and hormone studies reviewed were conducted under pot, growth chamber conditions, with one heat-stress protocol applied using artificially ramped temperatures (Devasirvatham *et al*, 2013; Prasad *et al*, 2017) [10, 27]. While this approach maximises experimental control, it does not take into account diurnal temperature fluctuations, coincident irradiance levels, and variable soil moisture that are typical of the real conditions found in farmers' fields that produce terminal heat stress (Sehgal *et al*, 2018; Rani *et al*, 2020) [21, 18]. Issues related to validating laboratory-based physiological indices in multi-year field studies across multiple locations are relatively uncommon and, where these do exist, they tend to be conducted as part of genotype evaluation studies as opposed to testing mechanistic hypotheses (Chaudhary *et al*, 2020; Krishnamurthy *et al*, 2011) [6, 22].

The third gap (as stated before in Theme 5) is that there is not much use being made of QTL and meta-QTL research in developing marker-assisted breeding products. To date, there have been over 100 QTL's and several consensus meta-QTL regions related to chickpea heat tolerance (Paul *et al*, 2018; Jha *et al*, 2021; Kumar *et al*, 2023) [11, 12, 13]; however, there are no reports of any marker-assisted breeding programs that have produced a variety of chickpea, grown for farmers, with heat tolerant traits (Gaur *et al*, 2019; Rani *et al*, 2020) [8, 18]. This is in contrast to the much more advanced state of development of marker-assisted breeding for chickpeas with drought tolerant traits (Gaur *et al*, 2019; Awasthi *et al*, 2014) [8, 9]; therefore, either there is a lag in how quickly the translational pipeline for heat-tolerant varieties moves through its various stages OR those QTLs with heat-tolerant traits produce weaker, more environmentally dependent effects than the QTLs related to drought-tolerant traits. Either way, this gap will require additional targeted research (Jha *et al*, 2025; Kumar *et al*, 2023) [19, 13].

Trends that could be utilized to fill these voids can be identified through the following examples: researchers have recently suggested utilizing imaging systems and flow cytometry for automated high-throughput pollen viability phenomics. This would allow for rapid screening of many pollen samples using automated pollen viability staining rather than using the manual technique (i.e., Alexander's stain) (Devi *et al*, 2022; Bhandari *et al*, 2020) [2, 4]. An example of another emerging technology in the field is integrated multi-omics profiling, where researchers collect, analyze and compare transcriptomic, proteomic and metabolomic data from tissues of interest or from tissues subjected to various stressors (for example, the tissues of chickpea seeds have recently been subjected to multi-omics profiling) within a given timeframe (Jha *et al*, 2025; Kumar *et al*, 2023) [23, 13]. An additional area of investigation could involve breeding using haplotypes to identify the most reliable methods of developing hierarchically reduced meta-QTL confidence intervals (Kumar *et al*, 2023; Paul *et al*, 2018) [13, 11]. Targeted gene editing to validate candidate genes identified through QTL regions may soon be feasible (Jha *et al*, 2021; Jha *et al*, 2025) [12, 19]. Finally, decision-support software can be developed to assist both growers and agricultural scientists develop an optimal sowing window based on genotype-specific heat-tolerance thresholds from the literature provided in this review (Devasirvatham *et al*, 2013; Gaur *et al*, 2019) [10, 8].

6. Discussion

To clarify, this review has cited an attempt to describe three major physiological processes involved in heat stress-induced failure of reproduction in chickpea. These three processes are pollen viability, reactive oxygen species (ROS) scavenging and hormone crosstalk or signalling (Chaturvedi *et al*, 2021; Hasanuzzaman *et al*, 2013) [16, 7]. The evidence presented in support of these claims throughout this paper, when viewed together provides a relatively clear but unfinished narrative of how these events are connected to one another, as outlined below. Terminal heat stress decreases photosynthesis and the amount of sucrose metabolism occurring within the anthers (Kaushal *et al*, 2013; Devasirvatham *et al*, 2013) [1, 10]. The resulting carbohydrate deficiency acts in conjunction with an oxidative burst being greater than the buffering capabilities of both ascorbate-glutathione and the oxidative enzymatic antioxidants (Hasanuzzaman *et al*, 2013; Akram *et al*, 2017) [7, 17]. Furthermore, both events will occur in the presence of elevated levels of abscisic acid (ABA) and disrupted signalling pathways related to the hormones auxin, gibberellin and ethylene that control the timing of development of the tapetum and pollen grains (Chaturvedi *et al*, 2021; Rani *et al*, 2020) [16, 18]. In addition, since multiple QTL regions of different sources have been identified at or near pollen-specific as well as heat shock transcription factor (HSF) genes (Paul *et al*, 2018; Jha *et al*, 2021; Kumar *et al*, 2023) [11, 12, 13]; these results indicate that the observed differences in pollen thermotolerance are heritable and correspond to the same biological processes identified by physiological research.

There are multiple patterns in the literature, so we must be cautious about interpreting a mechanistic model as fully resolved at this point. For example, much of the hormone-crosstalk evidence regarding pollen thermotolerance comes primarily from studies conducted on Arabidopsis, tomato, rice, and cotton rather than being directly tested on *Cicer arietinum*, so while we have a very solid understanding regarding the general signal transduction or logic of plant hormone function overall, we still have yet to explicitly verify

and quantify the specific parameters being used in chickpea anthers (Barnabas *et al*, 2008; Chaturvedi *et al*, 2021) [26, 16]. Additionally, although enzyme activity and osmolyte levels can indeed differentiate between heat-tolerant and heat-sensitive plant genotypes (when measured at the whole leaf level), very few studies to date have actually measured these enzyme activities or osmolyte levels in the anther or pollen tissues of any genotype, thus creating an opportunity to potentially interpret whole-plant levels of oxidative status as a correlate of ROS damage at the pollen level rather than as an actual cause of damage (Bhandari *et al*, 2020; Devi *et al*, 2022) [4, 2].

Another point of nuance is whether or not genotyping is consistent across studies (or genotype ranking). Despite the f act that there are several genotypes that are consistently used as heat-tolerant reference lines in multiple independent research projects (examples include ICCV 92944, GNG1969, and PantG186), each study does not necessarily report the same physiological "key" trait associated with heat tolerance. For example, 2 studies identified pollen viability (or germination) as the main discriminating (or "key") trait, while 4 studies identified ROS-related biochemical traits and membrane stability indices as primary discriminators (Devi *et al*, 2022; Bhandari *et al*, 2020) [2, 4]. It is important to understand that it is not contradictory to describe the traits above as physiologically connected (as the synthesis in this review suggests). The lack of one agreed upon (or "gold standard") heat tolerant trait for screening chickpea genotypes limits the ability of breeding programs to establish standardized selection criteria (Chaudhary *et al*, 2020; Gaur *et al*, 2019) [6, 8].

This synthesis has both practical and theoretical implications. On the one hand, the consistent, strong correlation of pollen viability or germination with pod set or yield as shown in independent studies [1,2,4,5] supports continued investment in phenotyping pollen as the most tractable way to assess heat tolerance traits across large areas using high-throughput imaging technology and would therefore represent a more practical solution than trying to directly measure hormonal changes or reactive oxygen species profile from field observations (Devi *et al*, 2022; Bheemanahalli *et al*, 2019) [2, 25]. On the other hand, the evidence for convergence of independently mapped quantitative trait loci (QTLs) on pollen-specific and heat-shock-transcription factor genes (Jha *et al*, 2021; Kumar *et al*, 2023) [12, 13] suggests that future research into the heat tolerance of chickpeas should concentrate on conducting physiological studies that exclusively utilize candidate genes identified through genome mapping, rather than assaying physiology and genomics as related but uncoordinated bodies of literature as has been characteristic of most studies to date. This conclusion is consistent with views expressed by very recent syntheses in the area (Jha *et al*, 2025) [19], which have explicitly recommended that integration of multi-omics approaches will be the next methodological phase in the study of heat stress in chickpeas.

7. Conclusion

Of the 58 peer-reviewed studies synthesizing chickpea (C. arietinum) studies on pollen viability, reactive oxygen species (ROS) scavenging, and hormonal cross talk under terminal heat stress, 58 were confirmed to be peer-reviewed literature to synthesize within this spectrum of physiological research and with accompanying genomic and breeding research. Evidence suggests that heat produced reproductive failure in chickpeas is due to a disruption of anther carbohydrate metabolism, ROS-antioxidant balance, and phytohormones; this ultimately contributes to the failure of pollen viability, reduction in pod set, and a reduction in seed yield. Of the genotypes of chickpeas that have been identified as heat tolerant, there is a consistent finding of a smaller decrease in pollen viability and germination, greater levels of antioxidant enzymes and osmolytes present, and more substantial heat shock protein induction across many independent studies, and these findings are supported approximately, by recent QTL and meta-QTL studies indicating that part of the heat tolerance mapped to a genetic locus associated with pollen and heat shock transcription factors.

The significance of this synthesis is to make direct the linkages between physiological mechanisms and genomic architecture that have, until now, been studied in isolation by disparate literatures. The synthesis recommends to researchers that they confirm hormonal-crosstalk mechanisms previously inferred from other species specifically in chickpeas and design physiological experiments using candidate genes identified through QTL and meta-QTL studies rather than conducting physiological experiments and genomic analyses independently. Additionally, it suggests that whenever possible, researchers should measure pollen- and anther-level (as opposed to only leaf-level) ROS and hormonal markers.

Regarding practitioners and breeding programs, the synthesis recommends that they use standardized, field-deployable pollen viability and germination assays as the primary screening trait for heat tolerance. The literature indicates a consistently strong correlation between these traits and yield. In terms of complementary secondary screens, membrane stability and antioxidant-enzyme assays should be used. In addition, practitioners should conduct marker-assisted introgression of the reduced-size meta-QTL regions recently identified to close the translational gap from genomic discovery to released cultivars.

Policymakers and research funders addressing the issue of developing heat-tolerant chickpeas specifically through marker-assisted selection should focus on closing the translational breeding gap rather than conducting more gene discoveries because of the consistent, low rate of development for released heat-tolerant chickpea cultivars.

Going forward, research needs to focus on: 1) the combined and single-use studies of pollen viability, reactive oxygen species (ROS) and hormone content in the anthers from chickpea plants grown in both controlled and uncontrolled environments with multiple locations for maximum reliability; 2) the functional validation of candidate genes identified through QTL mapping, meta-QTL mapping and studies of the HSP gene family using gene editing or transgenic technology; and, 3) the establishment of a clear relationship between the agronomic mitigation strategies (sowing date optimization, the application of exogenous protectants and the use of stress priming) and the physiological processes discussed in this review article so that agronomic recommendations are based upon proven physiological mechanisms rather than yield outcomes alone. If the three research directions are pursued together, they provide a feasible pathway for the development of heat resilient chickpea varieties with sustained reproductive success, and yields, as global cropping systems continue to experience increased heat stress in the future.

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