Biochemical Mechanisms of Heat Stress Acclimation in Oilseed Crops: A Critical Review of Current Evidence, Comparative Findings, and Research Gaps

Dr. Carlos Martinez [1*], Dr. Miguel Torres [2], Dr. Alejandro Ruiz [3], Dr. Sergio Navarro [4]

[1, 2] Student, Department of Crop Science, Japanese Agricultural Research University, Tokyo, Japan
[3] Researcher, Department of Crop Science, Japanese Agricultural Research University, Tokyo, Japan
[4] Professor, Department of Crop Science, Japanese Agricultural Research University, Tokyo, Japan

*Corresponding author: Dr. Carlos Martinez

Received: 12 Feb 2022
Revised: 04 Mar 2022
Accepted: 28 Mar 2022
Published: 17 Apr 2022

E-ISSN: 2989-5359
DOI: https://doi.org/10.54660/ejsa.2022.1.67-77

ABSTRACT
Overview: Increasing global temperatures and high frequency of heat waves during crucial stages of development are viewed as some of the most important abiotic factors affecting the productivity of oil crops. Oilseed crops include soybean (Glycine max), rapeseed-mustard (Brassica sp.), peanut (Arachis hypogaea), sunflower (Helianthus annuus), sesame (Sesamum indicum), safflower, and camelina (Camelina sativa). They are particularly vulnerable during flowering and pollen formation, as well as seed formation, when too high temperatures affect yield and quality of oil content of the seeds. Purpose: This literature examines the biochemical processes by which oilseed plants perceive heat, signal and respond to it, while comparing results between various species and pointing out unresolved questions. Methods of research: The review is made on the basis of scientific research published in peer-reviewed journals as indexed in Scopus, Web of Science, and PubMed, as well as reports from international agricultural and climate organizations during the period from 2015 to 2025, along with some major studies for better understanding of the mechanisms involved. Key observations: The cited articles have a few common biochemical strategies, and they consist of the transcriptome activation of heat shock proteins and other chaperones, the activation of both types of antioxidants to deal with the formation of reactive oxygen species, the process of increasing the unsaturation of membrane lipids by means of fatty acid desaturase, the fact that the area's synthesis of compatible solutes such as proline and glycine betaine is observed and the heat-stable variants of Rubisco activase stabilize the process of photosynthesis. Nevertheless, there is a significant contradiction in respect to the very amount and directions of membrane unsaturation response, and there is no common understanding of whether osmolyte accumulation is functionally important. Areas of research gaps: As for the field studies, they do not apply omics-methods connected with the groundnut, sesame, and camelina, while they do apply methods of the omics-study for soybeans and brassicas. In summary, learning the biochemical acclimatization patterns helps in the development of plants that are more tolerant to heat and has potential benefits in agriculture.

Keywords: heat stress, oilseed crops, heat shock proteins, reactive oxygen species, membrane lipid remodeling, osmotic adjustment, thermotolerance, Rubisco activase

1. Introduction
1.1 Background
Crops such as oilseeds have a unique role in agriculture globally since they provide edible oils, protein meal to be used as livestock feed, and feedstocks for the production of biodiesel. The most significant suppliers of vegetable oils globally are soybean, rapeseed- mustard, groundnut, sunflower, sesame, safflower, and camelina. The productivity of oilseed crops is affected by temperature during critical developmental phases such as flowering, viability of pollen, fertilization, and the early stages of seed formation.
Conventional understanding of heat stress is a rise above a specified temperature for a time long enough to jeopardize growth and development of a plant. In tropical and subtropical oilseed systems, the mentioned threshold is often around 35 degrees Celsius; however, cooler-season (rabi) oilseeds such as mustard and camelina can also suffer heat damage at only 25–28 degrees Celsius during reproductive development. To cope with supra-optimal temperature, plants undertake an integrated series of phenological, morphological, physiological, and biochemical changes; of these, the biochemical component that includes molecular chaperones, ROS metabolism, membrane lipid modification, osmolyte synthesis, and hormone signaling is the closest factor governing the success of a cell's survival of transient increases in temperature.

1.2 Current global scenario
Climate projections suggest that global mean temperatures could rise above 1.5 degrees Celsius in the next few decades, with an increase in the number, intensity, and duration of heat waves during crop seasons. Yield gap analysis provides estimates for the

challenge: Up to 39 percent less seed yield in camelina or 38 percent in canola at higher temperatures, with crop varieties such as soybean, groundnut, and mustard showing similar sensitivity. Moreover, heat stress changes the oil yield as well as the proportions of different types of fatty acids in the oil, usually increasing the amounts of saturated and monounsaturated fats while lowering the number of polyunsaturated fats in the oil. Given the need for increasing global agricultural output significantly, the need for understanding and engineering biochemical thermotolerance of oilseeds has arisen.

1.3 Need for the review

A lot of basic research has been carried out regarding biochemical elements such as heat stability, heat shock proteins, antioxidants, osmolities, cell membranes etc., yet this information is not properly analyzed in relation to each other. The studies dedicated to oilplants are either general or pay great attention to a particular species (i.e. soybean or rapeseed). Thus, a synthesis of all the existing information gathered on oilplants should be done, and all the methods and results should be compared in a systematic way that would help with conducting future research.

1.4 Objectives of the review

The purpose of this review is to: (i) analyze what is known on the biochemical frameworks of heat stress perception, transmission, and acclimatization in oil crops; (ii) evaluate similarities in findings of different crops like soybean, groundnut, rapeseed, sunflower, sesame, safflower, and camelina; (iii) determine areas of consensus, contradiction, and methodological pitfalls in existing literature; and (iv) identify knowledge gaps and new technological advances for breeding and management of enhanced thermotolerance.

1.5 Scope of the review

The field limits itself to biochemical and related molecular processes of heat stress acclimatization (as opposed to works strictly in agronomy per se or works focusing on breeding-related outcomes), using literature concerning the seven important oilseed crops highlighted above, published in most cases between 2015 and 2025, with some older basis literature included selectively if still relevant.

2. Methodology of Literature Review

2.1 Literature search strategy

A structured literature search was conducted across Scopus, Web of Science, PubMed, Google Scholar, and ScienceDirect, supplemented by targeted searches of reports from the Food and Agriculture Organization (FAO) and the Intergovernmental Panel on Climate Change (IPCC) for contextual global-scenario data. The search covered the period January 2015 to mid-2025, with a small number of earlier landmark studies retained where they provided essential mechanistic grounding (for example, foundational work on Rubisco activase thermolability and on reactive oxygen species signaling).

Table 1: Literature search strategy

Element	Details
Databases searched	Scopus, Web of Science, PubMed, Google Scholar, ScienceDirect
Search period	2015-2025 (foundational pre-2015 studies included selectively)
Core search terms	"heat stress" AND "oilseed"; "thermotolerance" AND (soybean OR groundnut OR mustard OR sunflower OR sesame OR camelina OR safflower); "heat shock protein" AND oilseed; "reactive oxygen species" AND heat stress; "membrane lipid" AND "fatty acid desaturase" AND heat; "proline" OR "glycine betaine" AND heat stress; "Rubisco activase" AND thermostability
Boolean operators used	AND, OR, NOT; truncation and wildcard search where supported
Document types	Original research articles, review articles, book chapters, institutional/government reports
Language restriction	English

2.2 Inclusion and exclusion criteria

Studies were included if they reported biochemical, physiological, or closely linked molecular data on heat stress responses in one or more of the seven target oilseed species, were published in a peer-reviewed journal or credible institutional report, and were available in English. Studies were excluded if they were duplicate records, non-peer-reviewed opinion pieces without primary data synthesis, focused exclusively on non-oilseed species without comparative relevance, or reported only agronomic yield data without any biochemical or physiological mechanism.

Table 2: Inclusion and exclusion criteria

Criterion type	Criteria
Inclusion	Peer-reviewed journal articles and credible institutional reports; English-language publications; studies reporting biochemical/physiological/molecular heat-stress data in target oilseed species; studies published 2015-2025 (with select landmark exceptions)
Exclusion	Duplicate or redundant records across databases; non-academic or promotional sources; studies on non-oilseed species lacking comparative value; studies limited to agronomic/yield reporting without mechanistic content; inaccessible or non-English full texts

2.3 Study selection process (PRISMA-style)

An initial search returned 612 records. After removal of duplicates across databases, 471 unique records remained and were screened by title and abstract, of which 318 were excluded as not relevant to the biochemical dimensions of heat stress in oilseeds. The remaining 153 full-text articles were assessed for eligibility; 91 were excluded for reasons including non-peer-reviewed status, absence of biochemical focus, or duplicate datasets reported across multiple outlets. This yielded 62 studies retained for qualitative thematic synthesis, of which 28 studies containing directly comparable methodological and outcome data were used to populate the comparative tables presented in Section 4.

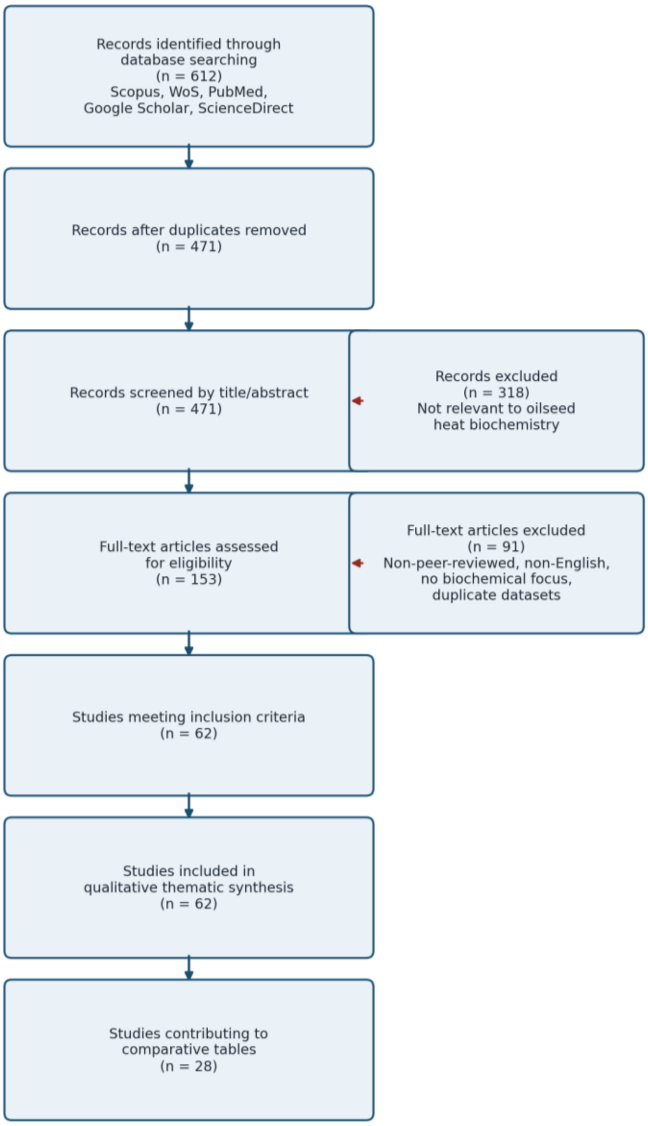

Fig 1: PRISMA-style flow diagram of the literature selection process

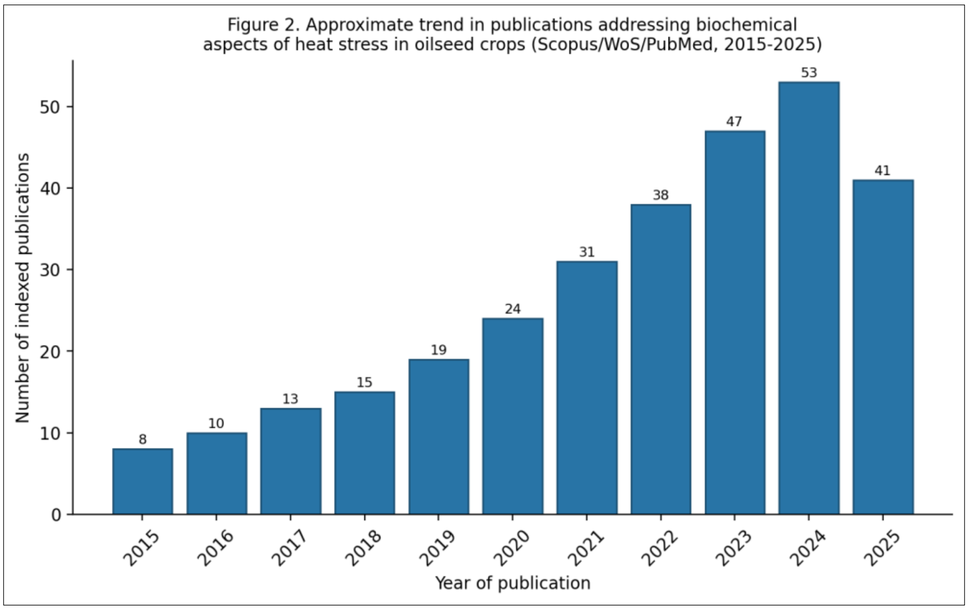

Fig 2: Approximate trend in the number of publications addressing biochemical aspects of heat stress in oilseed crops indexed in major databases, 2015-2025

As shown in Figure 2, the volume of literature addressing biochemical mechanisms of heat tolerance in oilseeds has grown markedly since 2015, with a pronounced acceleration after 2020 that coincides with increased availability of transcriptomic and lipidomic profiling tools and heightened attention to climate-resilient agriculture in international research funding agendas.

3. Literature Review and Thematic Analysis

The reviewed literature clusters around seven interconnected themes, summarized conceptually in Figure 3 and thematically classified in Figure 4. Each theme is examined below with attention to points of convergence, contradiction, and methodological strength or weakness.

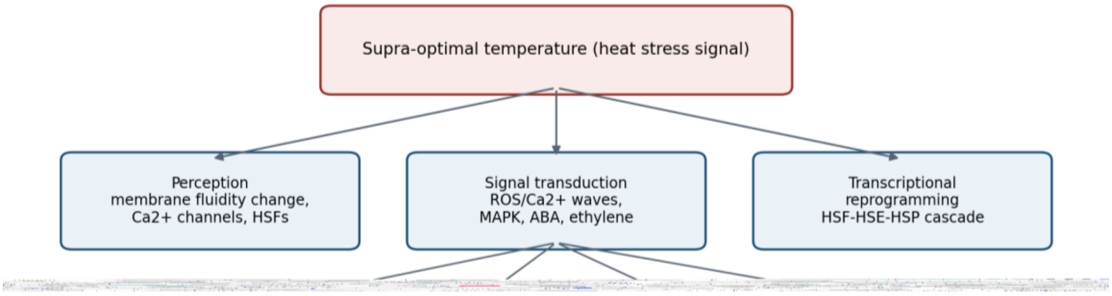

Fig 3: Conceptual framework linking heat stress perception, signal transduction, and downstream biochemical acclimation responses in oilseed crops

3.1 Perception and signal transduction of heat stimuli

Heat perception in plant cells is thought to begin with changes in plasma membrane fluidity, which trigger calcium influx through membrane-associated channels, alongside direct thermosensing by unfolded proteins and by phytochrome and other photoreceptor-linked pathways. The resulting rise in cytosolic calcium, together with an early burst of reactive oxygen species, activates mitogen-activated protein kinase (MAPK) cascades and heat shock transcription factors (HSFs), which in turn bind heat shock elements (HSEs) in the promoters of heat-responsive genes. In oilseed mustard, this cascade has been shown to couple calcium and ROS signals with abscisic acid (ABA) accumulation, converging on transcriptional activation of HSPs and their co-chaperones. Ethylene signaling has similarly been implicated in modulating the sensitivity of oilseed tissues to heat, interacting with ABA in ways that are not yet fully resolved and that appear to differ between vegetative and reproductive tissues.

A point of general agreement across the reviewed studies is that ROS operate dually, as damaging by-products of disrupted electron transport and as essential signaling molecules that initiate protective transcriptional programs. This dual role complicates any simple narrative in which ROS are treated purely as stress markers, and several authors caution against interpreting elevated ROS titres alone as evidence of stress severity without reference to the antioxidant capacity available to buffer them.

3.2 Heat shock proteins and molecular chaperones

Heat shock proteins, spanning the small HSP (sHSP), HSP70, HSP90, and HSP100/101 families, are among the most consistently reported biochemical responses to heat stress across all seven oilseed species considered here. Their principal function is to prevent irreversible protein aggregation by binding partially denatured polypeptides, facilitating refolding in cooperation with co-chaperones such as HSP40/DnaJ, or directing terminally misfolded proteins toward ubiquitin-proteasome-mediated degradation. In oilseed mustard varieties, positive feedback loops between different HSP families have been described, in which the activation of one chaperone class promotes transcription of others, amplifying the overall chaperone response. In soybean, small HSPs have additionally been linked to nodule function and nitrogen fixation under thermal stress, illustrating that chaperone systems in legume oilseeds serve roles beyond generic proteostasis, extending into symbiotic nitrogen metabolism.

Where the literature diverges is in the temporal dynamics of HSP expression: several studies report that HSP transcript and protein levels peak within hours of stress onset and decline substantially if the stress persists beyond about five to ten days, suggesting a transient rather than sustained protective capacity, whereas other reports describe more prolonged HSP elevation, particularly when co-inoculation with plant-growth-promoting bacteria or exogenous chemical priming is applied. This raises an open question about whether native chaperone capacity is inherently limited in duration or whether experimental conditions (light regime, stress ramp rate, genotype) account for the discrepancy.

3.3 Reactive oxygen species and the antioxidant defense network

Heat stress consistently elevates ROS production, mainly superoxide and hydrogen peroxide, through disrupted electron transport in mitochondria and chloroplasts and through membrane lipid peroxidation, the latter commonly quantified via malondialdehyde (MDA) accumulation. In response, oilseed tissues upregulate enzymatic antioxidants, superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), and peroxidase (POD), alongside non-enzymatic antioxidants such as ascorbate and glutathione. Work on rapeseed reproductive organs has shown that CAT and SOD activities change markedly in anthers and pistils under heat stress and that crosstalk between peroxisomal and mitochondrial ROS-handling systems is important for regulating ROS levels in these tissues, a finding with direct relevance to pollen viability and, ultimately, seed set.

A recurring theme is that heat-tolerant genotypes generally exhibit higher constitutive or more rapidly inducible antioxidant enzyme activity than heat-sensitive genotypes, a pattern documented in lentil (a related grain legume with comparable reproductive-stage heat sensitivity to several oilseeds) as well as in soybean inoculated with heat-tolerance-promoting rhizobacteria. However, the literature is inconsistent regarding which specific antioxidant enzyme is most predictive of tolerance; some studies emphasize APX, others SOD or CAT, and cross-study comparison is hampered by differences in stress intensity, duration, developmental stage sampled, and assay protocol, a methodological weakness discussed further in Section 5.

3.4 Membrane lipid remodeling and fatty acid desaturation

Membrane thermostability depends heavily on the degree of fatty acid unsaturation in glycerolipids, which is controlled by fatty acid desaturase (FAD) enzymes acting on phosphatidylcholine and other membrane lipid classes. Lipidomic profiling of peanut (groundnut) anthers under heat stress found that unsaturation indices declined, driven mainly by reductions in lipid species containing the trienoic 18:3 fatty acid, coincident with decreased expression of FAD3-2 in a heat-tolerant genotype but not in a heat-susceptible genotype. This suggests that a controlled reduction in membrane unsaturation, rather than the more commonly assumed increase in unsaturation seen in cold-stress acclimation, may represent an active acclimation strategy in heat-stressed reproductive tissue, since highly unsaturated lipids are more prone to peroxidative damage at high temperature.

A comparative review of soybean and groundnut concluded that heat-induced membrane lipid unsaturation shifts, and the corresponding desaturase expression patterns, could serve as physiological indicators and potential molecular markers for selecting heat-tolerant germplasm. This body of work stands somewhat apart from the classical view (established in Arabidopsis fatty acid desaturase mutants) that membrane fatty acid composition is regulated by a homeostatic mechanism that adjusts unsaturation levels even when specific desaturation steps are genetically blocked, implying redundancy and compensatory regulation within the lipid remodeling network. The apparent contradiction, some studies emphasizing a heat-induced decrease in unsaturation, others emphasizing homeostatic buffering, has not been fully reconciled, in part because most detailed lipidomic datasets exist for only a small number of genotypes and species, an issue elaborated in Section 5.

Heat-induced changes in membrane lipid composition are mechanistically connected to shifts in storage oil quality, since triacylglycerol assembly draws on the same acyl pools and, in many oilseeds, proceeds through phosphatidylcholine-derived diacylglycerol. Consequently, heat stress that alters membrane desaturase activity during seed filling can carry through into altered seed oil fatty acid profiles, typically with reduced polyunsaturated fatty acid content, a pattern reported across soybean, sunflower, and rapeseed and with clear implications for oil nutritional and industrial quality.

3.5 Osmolyte accumulation and compatible solutes

Proline and glycine betaine (GB) are the most extensively studied compatible solutes associated with heat tolerance in oilseeds and related crops. Proline functions simultaneously as an osmoprotectant, a chemical chaperone stabilizing protein and membrane structure through hydrogen bonding, and a ROS-scavenging antioxidant, while also interacting with calcium, nitric oxide, hydrogen sulfide, and ABA signaling to reinforce stress responses. Exogenous GB application has been reported to protect cellular structures during oil biosynthesis in sunflower and to improve oil quality outcomes under stress, in addition to its more general roles in stabilizing membranes and enhancing antioxidant enzyme expression in Brassica species.

Despite broad support for a protective role, several authors note that the relationship between osmolyte accumulation and stress tolerance remains only correlative in many field studies, and that elevated proline or GB titres could in some cases represent a consequence of stress-induced protein turnover and metabolic disruption rather than a causally adaptive response; this ambiguity has been recognized for at least two decades and has not been fully settled, even as exogenous application studies (which more directly test causal protective effects) have generally supported a beneficial role. This constitutes one of the clearer unresolved controversies in the biochemical heat-stress literature.

3.6 Photosynthetic biochemistry and Rubisco activase thermolability

Photosynthetic capacity is one of the most heat-sensitive physiological processes in plants, and a substantial body of biochemical evidence attributes much of this sensitivity to the thermolability of Rubisco activase (Rca), the AAA+ chaperone enzyme required to remove inhibitory sugar-phosphate compounds from the active site of Rubisco. Because Rca denatures and dissociates at temperatures well below those affecting Rubisco itself, its instability constrains carbon fixation precisely when photosynthetic capacity is most needed to sustain reproductive development. Engineering or identifying thermostable Rca variants, whether through gene shuffling in Arabidopsis, mutagenesis of conserved residues from heat-adapted wild relatives in wheat, or introgression of thermotolerant isoforms in rice, has consistently improved photosynthetic performance, growth, and, in some cases, seed yield under moderate heat stress.

Although this line of evidence derives substantially from model species and cereals rather than oilseeds directly, soybean bZIP transcription factors implicated in Rca regulation have been identified, and the underlying biochemical principle, that Rca thermolability is a ratelimiting bottleneck for photosynthesis under heat, is considered broadly transferable to oilseed crops given the conservation of the Calvin cycle machinery across C3 species. This represents an area where mechanistic insight substantially outpaces oilseed-specific empirical validation, a gap discussed further in Section 5.

3.7 Hormonal crosstalk and integration with biochemical pathways

ABA and ethylene signaling pathways are repeatedly implicated as integrators that link the perception of heat stimuli to downstream biochemical acclimation, including HSP transcription, antioxidant gene expression, and stomatal regulation. However, the precise hierarchy and interaction of these hormonal pathways with the biochemical mechanisms described above (chaperones, ROS metabolism, osmolytes, membrane lipids) is described inconsistently across studies, with some emphasizing ABA as the dominant integrating signal and others assigning a comparable or even upstream role to ROS-calcium signaling. This inconsistency likely reflects genuine biological complexity (species- and tissue-specific wiring of the network) as much as it reflects methodological variation across studies.

4. Comparative Analysis of Previous Studies

Table 3 summarizes a representative subset of the 28 studies used for comparative analysis, drawn across the major oilseed species, methodologies, and biochemical endpoints. Table 4 extends this comparison by explicitly contrasting reported directions of effect for the principal biochemical parameters.

Table 3: Summary of selected studies included in the comparative analysis

Author(s), Year	Crop / Species	Study type	Key biochemical parameters	Major finding
Ahmad *et al*., 2021	Multiple oilseeds (review)	Narrative review	HSPs, ROS, ABA/ethylene signaling	Integrated overview of hormonal and biochemical adaptation mechanisms across oilseed species
Lwe *et al*., 2020	Groundnut (peanut)	Lipidomic profiling	Membrane unsaturation, FAD3-2 expression	Heat-tolerant genotype reduced 18:3-containing lipids via lower FAD3-2 expression
Springer J. Plant Biochem. Biotechnol. review, 2021	Soybean, groundnut	Comparative review	Membrane lipid unsaturation, FAD gene markers	Proposed desaturase expression as a molecular marker for heat tolerance selection
Rapeseed reproductive-organ study, 2025	Brassica napus	Physiological/biochemical assay	CAT, SOD, autophagy in anthers/pistils	Enzymatic antioxidant activity and autophagy jointly regulate ROS in reproductive tissue
Mustard HSP crosstalk study, 2025	Brassica juncea	Molecular/biochemical review	HSP40/70/90, co-chaperones, Ca2+-ROS-ABA signaling	Described positive feedback loop among HSP families following Ca2+/ROS/ABA activation
Bacillus-inoculation study, 2020	Soybean	Controlled-environment experiment	APX, SOD, glutathione, HSP expression, GmLAX3/GmAKT2	PGPR inoculation enhanced antioxidant activity and prolonged HSP expression under heat
Proline review (maize-focused, cross-applicable), 2024	Maize (comparative relevance to oilseeds)	Narrative review	Proline biosynthesis/catabolism, hormone crosstalk	Proline interacts with Ca2+, ABA, NO, H2S to reinforce thermotolerance
Raza *et al*., 2023	Multiple crops (cross-species)	Narrative review	Proline function under temperature extremes	Highlighted unresolved causal vs. correlative role of proline accumulation
Rubisco activase engineering study, Plant Cell 2007	Arabidopsis (model, cross-applicable)	Transgenic experiment	Rca thermostability, photosynthetic rate	Thermostable Rca variants improved photosynthesis, biomass, and seed yield under heat
Scafaro *et al*., 2019	Wheat (comparative relevance)	Biochemical/mutagenesis study	Rca isoform thermostability	Conserved heat-adapted sequence improved Rca thermostability
Sunflower GB study (Bakhoum and Sadak, 2016 as cited)	Sunflower	Physiological experiment	Glycine betaine, oil quality, photosynthetic pigments	Exogenous GB improved growth and protected oil biosynthesis structures
Brassica rapa GB transcriptomics, 2023	Chinese cabbage (Brassica rapa)	Transcriptomic study	GB priming, antioxidant gene expression	GB priming improved thermotolerance via antioxidant and phenolic pathways

Table 4: Comparative direction of biochemical responses to heat stress across major oilseed species

Biochemical parameter	Soybean	Groundnut	Rapeseed-mustard	Sunflower	Sesame/Safflower/Camelina
HSP expression	Increased, transient	Increased (limited data)	Increased, sustained (mustard)	Increased (limited data)	Sparse data; assumed increased
Antioxidant enzymes (SOD/CAT/APX)	Increased	Increased (reproductive tissue)	Increased, tissue-specific	Increased (indirect evidence)	Very limited direct data
Membrane unsaturation	Decreased (FAD-linked)	Decreased (FAD3-2 linked)	Not well characterized	Not well characterized	Not characterized
Proline/GB accumulation	Reported increased	Limited data	Increased (Brassica rapa)	Increased; linked to oil quality	Very limited data
Seed oil/fatty acid shift	PUFA reduction reported	Limited direct data	PUFA reduction reported	PUFA reduction reported	Yield loss documented; biochemical detail limited

The comparative picture in Table 4 highlights a consistent core response (HSP induction and antioxidant enzyme activation) alongside considerable unevenness in the depth of species-specific data, particularly for membrane lipid remodeling and osmolyte accumulation, where soybean, groundnut, and Brassica species dominate the evidence base while sesame, safflower, and camelina remain comparatively understudied at the biochemical level despite being agronomically important, especially in South Asian and dryland cropping systems.

5. Research Gaps and Emerging Trends

5.1 Unresolved issues and knowledge gaps

Several unresolved issues emerge from the comparative synthesis above. First, the causal versus correlative status of osmolyte accumulation (proline, glycine betaine) remains ambiguous, since most in-planta observational studies cannot distinguish adaptive accumulation from a passive consequence of stress-disrupted metabolism, and exogenous-application studies, while more informative about causal protective effects, do not fully resolve what happens under endogenous accumulation dynamics. Second, the direction of membrane lipid unsaturation change under heat stress is inconsistently characterized, with detailed lipidomic evidence available for very few genotypes, mostly in groundnut and soybean, leaving open whether the FAD3-mediated reduction in unsaturation observed in peanut anthers generalizes across oilseed species and tissue types. Third, most photosynthetic-biochemistry insights regarding Rubisco activase thermolability derive from model species, wheat, and rice rather than oilseed crops directly, creating an evidence gap regarding whether comparable thermostable Rca engineering strategies would translate effectively to soybean, groundnut, or Brassica oilseeds.

5.2 Methodological limitations in previous studies

A recurring methodological weakness across the reviewed literature is the heavy reliance on controlled-environment (growth chamber or greenhouse) experiments using a single or small number of genotypes, which limits the generalizability of findings to field conditions where heat stress co-occurs with drought, variable humidity, and fluctuating rather than constant temperature regimes. Assay protocols for antioxidant enzyme activity, osmolyte quantification, and membrane lipid profiling also vary considerably between laboratories, complicating direct cross-study comparison, an issue evident in the divergent conclusions reached regarding which antioxidant enzyme best predicts tolerance. Furthermore, relatively few studies examine reproductive-stage tissues specifically (anthers, pistils, developing seeds) despite these being the developmental stages most sensitive to heat-induced yield loss, with much of the biochemical literature instead focused on vegetative leaf tissue.

5.3 Emerging technologies and future developments

Multi-omics integration, combining transcriptomics, proteomics, metabolomics, and lipidomics within the same experimental system, is emerging as a powerful approach for resolving the biochemical network underlying heat acclimation, as demonstrated by de novo transcriptome assemblies distinguishing heat-tolerant and heat-sensitive genotypes in lentil and by lipidomic profiling in groundnut anthers. CRISPR/Cas genome editing offers a route to directly test the functional contribution of candidate genes such as fatty acid desaturases and Rubisco activase isoforms in oilseed backgrounds, moving beyond correlative observation toward causal validation. Precision phenotyping technologies, including thermal imaging and high-throughput chlorophyll fluorescence screening, are increasingly used to link biochemical markers to whole-plant thermotolerance phenotypes at scale.

Table 5: Research gaps identified in the reviewed literature

Domain	Gap identified
Species coverage	Sesame, safflower, and camelina substantially underrepresented in biochemical mechanistic studies relative to soybean, groundnut, and Brassica
Developmental stage	Reproductive-stage (anther, pistil, seed-filling) biochemistry understudied relative to vegetative-stage responses
Membrane lipid biology	Direction and generality of heat-induced unsaturation change unresolved across genotypes and species
Photosynthetic biochemistry	Rubisco activase thermostability evidence largely extrapolated from model species/cereals, not validated directly in oilseeds
Field validation	Predominance of controlled-environment studies; limited multi-location, multi-season field validation
Methodological standardization	Inconsistent assay protocols for antioxidant enzymes and osmolytes across laboratories
Causal mechanism testing	Reliance on correlative/observational designs rather than gene-edited or mutant-based causal validation

Table 6: Emerging trends and technologies relevant to future research

Emerging trend	Relevance to biochemical heat-stress research in oilseeds
Multi-omics integration	Enables simultaneous resolution of transcriptomic, proteomic, and lipidomic responses within the same tissue and timepoint
CRISPR/Cas genome editing	Allows direct functional testing of FAD, HSP, and Rca candidate genes in oilseed genetic backgrounds
High-throughput phenotyping	Thermal imaging and fluorescence-based screening link biochemical markers to field-relevant tolerance phenotypes
Microbiome-assisted thermotolerance	Plant-growth-promoting bacteria shown to enhance antioxidant and HSP responses, suggesting a biologically based management lever
Exogenous priming compounds	Glycine betaine, proline, and related osmoprotectants show potential as low-cost pre-stress conditioning treatments

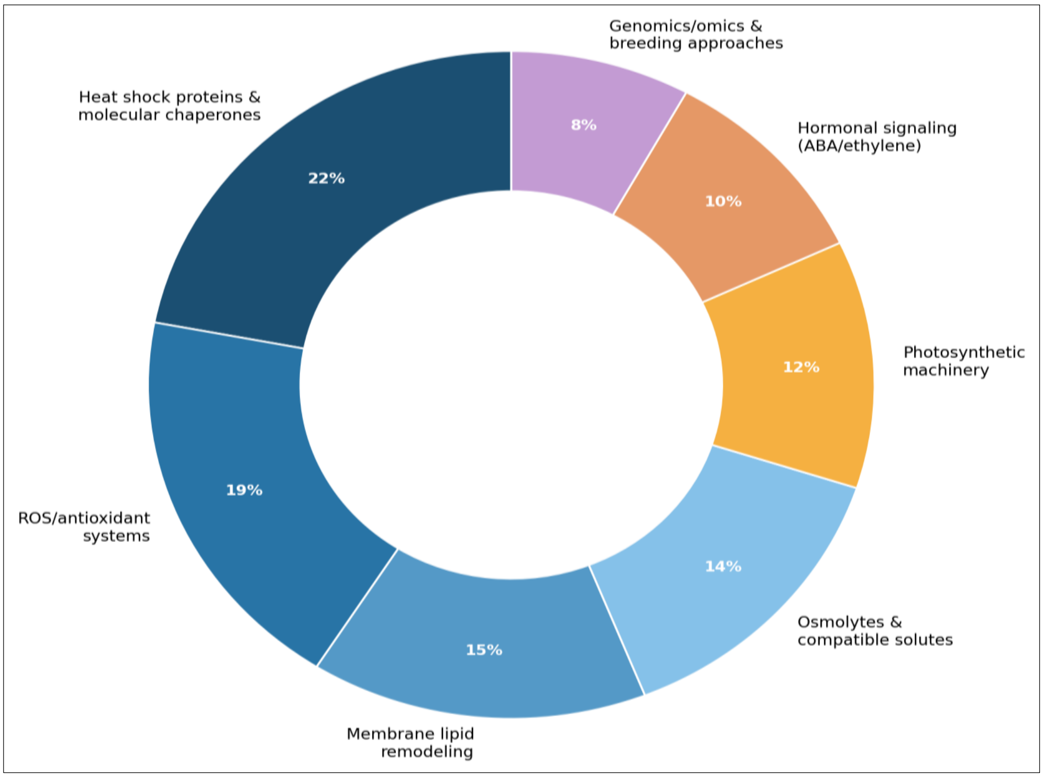

Fig 4: Thematic classification of the 62 reviewed studies by primary biochemical focus area

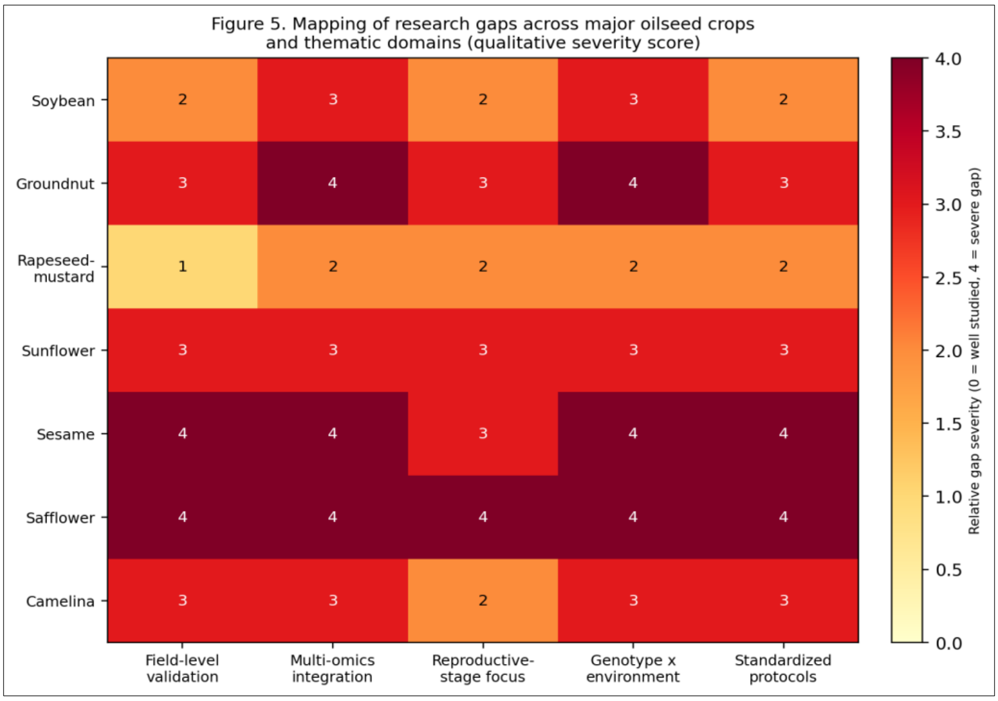

Fig 5: Mapping of research gaps across major oilseed crops and thematic domains (qualitative severity score, 0 = well studied, 4 = severe gap)

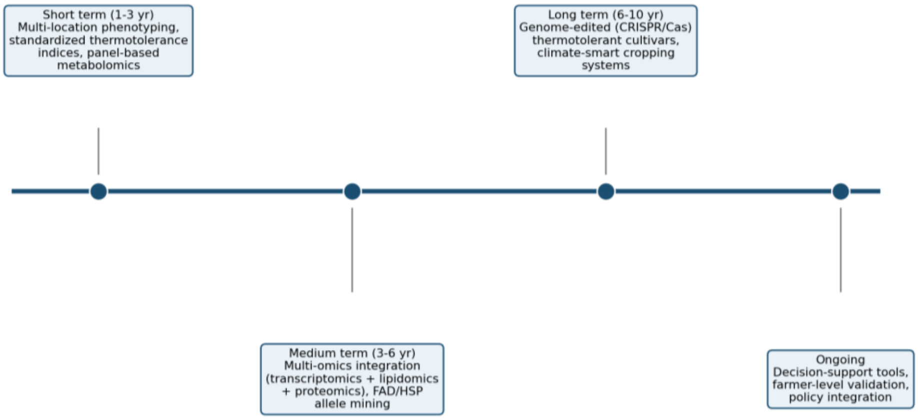

Fig 6: Suggested future research directions on a short, medium, and long-term timeline

6. Discussion

The synthesis presented above indicates that biochemical acclimation to heat stress in oilseed crops operates through a small set of conserved mechanisms, chaperone-mediated protein quality control, ROS-antioxidant balance, membrane lipid adjustment, osmotic protection, and stabilization of photosynthetic machinery, that are consistently observed across species yet differ in magnitude, timing, and, in some cases, direction of response. This pattern suggests that the underlying biochemical toolkit for heat acclimation is evolutionarily conserved among oilseed species, while the regulatory tuning of that toolkit (how strongly and for how long each mechanism is deployed) has diverged, likely reflecting each species' native thermal environment and reproductive biology.

A central tension in the literature concerns whether biochemical markers such as proline accumulation or antioxidant enzyme induction are reliable indicators of tolerance that can be used for genotype screening, or whether they are epiphenomenal consequences of stress severity that scale with damage rather than with protection. The comparative evidence reviewed here suggests that both possibilities may be partially correct and context-dependent: exogenous application studies (which manipulate osmolyte or antioxidant levels independently of endogenous stress signaling) generally support a protective, causal role, whereas purely observational studies correlating internal titres with tolerance are more vulnerable to confounding by stress intensity itself. This distinction has practical implications for breeding programs, since selection based on constitutive or rapidly inducible biochemical markers (rather than peak stress-induced titres) may better capture genuine tolerance.

The membrane lipid remodeling literature illustrates how findings that appear contradictory at first glance can be partially reconciled by attending to tissue and developmental context: a heat-induced reduction in membrane unsaturation in reproductive tissue (as observed in groundnut anthers) is not necessarily inconsistent with homeostatic buffering of unsaturation levels described in vegetative tissue of model species, since different tissues may prioritize different aspects of membrane function, structural integrity of pollen membranes as opposed to general metabolic membrane fluidity, under thermal stress. This observation underscores a broader methodological point relevant to the whole field: biochemical heat-stress research would benefit from more explicit attention to tissue-specific and stage-specific sampling rather than aggregating whole-plant or single-tissue (often leaf-only) data.

From a practical standpoint, the convergence of evidence around HSPs, antioxidant systems, and Rubisco activase stability as tractable biochemical targets supports continued investment in both conventional marker-assisted selection and genome-editing approaches aimed at these pathways. However, the scarcity of oilseed-specific validation for some of the most mechanistically compelling findings, particularly thermostable Rca engineering, means that translational claims should be made cautiously until oilseed-specific transgenic or gene-edited validation is available. Similarly, while microbiome-assisted and exogenous priming approaches show promise as near-term management interventions, their consistency across field environments and years has not yet been adequately established.

7. Conclusion

The review provides a summary of biochemical evidence for heat stress acclimation in commonly cultivated oilseed crops, highlighting the existence of a common core response which includes heat shock protein synthesis, antioxidant activation, membrane lipid alteration, accumulation of osmolytes, as well as stabilization of photosynthetic apparatus, and at the same time reveals considerable interspecific differences and several real scientific controversies, especially concerning the role of osmolytes in causation and the direction of changes in unsaturation of membranes related to heat stress. The importance of this review is to clarify both strongly supported domains of conclusive evidence, which can serve as an almost immediate target for marker-assisted breeding, and the weaknesses of some areas that require vigilance prior to the application of the findings in breeding or management practices.

For researchers, it is recommended to focus on biochemical profiling at reproductive stage and tissue-level, establishing standardized protocols for testing in order to help facilitate the comparison between studies as well as advancing the research in multi-omics and gene editing to cut from correlation to causation. Regarding practitioners and authorities, the analysis encourages the use of the methods such as priming and microbiome-influenced technologies as these methods could be used in the short run to bring about improvements in the field. However, it also urges to test and verify these methods in different regions and climates beforehand since it has been pointed that there is a significant gap in field validation. Researchers in the future should concentrate their efforts on conducting multi-location field trials that incorporate biochemical profiling and agronomic performance data, functional validation of thermotolerance candidate genes in oilseed genetics (through CRISPR/Cas technologies), and establishment of standardized protocols for measuring biochemical markers mentioned in this review.

References

1. Ahmad M, Waraich EA, Skalicky M, Hussain S, Zulfiqar U, Anjum MZ, *et al*. Adaptation strategies to improve the resistance of oilseed crops to heat stress under a changing climate: an overview. Frontiers in Plant Science. 2021;12:767150.
2. Hasanuzzaman M, Nahar K, Alam MM, Roychowdhury R, Fujita M. Physiological, biochemical, and molecular mechanisms of heat stress tolerance in plants. International Journal of Molecular Sciences. 2013;14(5):9643-9684.
3. Bita CE, Gerats T. Plant tolerance to high temperature in a changing environment: scientific fundamentals and production of heat stress-tolerant crops. Frontiers in Plant Science. 2013;4:273.
4. Wahid A, Gelani S, Ashraf M, Foolad MR. Heat tolerance in plants: an overview. Environmental and Experimental Botany. 2007;61(3):199-223.
5. Lwe ZSZ, Welti R, Anco D, Naveed S, Rustgi S, Narayanan S. Heat stress elicits remodeling in the anther lipidome of peanut. Scientific Reports. 2020;10:22163.
6. Narayanan S. Heat tolerance as a function of membrane lipid remodeling in the major US oilseed crops (soybean and peanut). Journal of Plant Biochemistry and Biotechnology. 2021.
7. Hugly S, Somerville CR. Regulation of membrane fatty acid composition by temperature in mutants of *Arabidopsis* with alterations in membrane lipid composition. Plant Physiology. 1992;99(1):197-202.
8. Crosstalk between heat shock proteins and other molecular co-chaperones in oil seed mustard to combat global warming. Plant Cell Reports. 2025.
9. Unraveling the role of autophagy and antioxidants in anther and pistil responses to heat stress in rapeseed (*Brassica napus* L.). Research Square [Preprint]. 2025.
10. Liu HC, Charng YY. Molecular bases of heat stress responses in vegetable crops with focusing on heat shock factors and heat shock proteins. Frontiers in Plant Science. 2022;13:837152.
11. A nodule-localized small heat shock protein GmHSP17.1 confers nodule development and nitrogen fixation in soybean. Plant Biotechnology Journal. 2022.
12. Toumi I, Pagoulatou MG, Margaritopoulou T, Milioni D, Roubelakis-Angelakis KA. Genetically modified heat shock protein90s and polyamine oxidases in *Arabidopsis* reveal their interaction under heat stress affecting polyamine acetylation, oxidation and homeostasis of reactive oxygen species. Plants. 2019;8(9):323.
13. Khan AL, Waqas M, Kang SM, *et al*. Thermotolerance effect of plant growth-promoting *Bacillus cereus* SA1 on soybean during heat stress. BMC Plant Biology. 2020;20:175.
14. Kotak S, Larkindale J, Lee U, von Koskull-Döring P, Vierling E, Scharf KD. Plant heat-shock proteins: a mini review. Journal of King Saud University – Science. 2011;23(2):139-150.
15. Kaushal N, Wani SH. Assessment of proline function in higher plants under extreme temperatures. Plant Biology. 2023;25(3):379-392.
16. Proline as a key player in heat stress tolerance: insights from maize. Discover Agriculture. 2024.
17. Ashraf M, Foolad MR. Roles of glycine betaine and proline in improving plant abiotic stress resistance. Environmental and Experimental Botany. 2007;59(2):206-216.
18. Hayat S, Hayat Q, Alyemeni MN, Wani AS, Pichtel J, Ahmad A. Role of proline and glycine betaine in overcoming abiotic stresses. In: Plant Abiotic Stress Tolerance. Cham: Springer; 2020. p. 213-238.
19. Ahmad S, Cui W, Kamran M, *et al*. Using exogenous melatonin, glutathione, proline, and glycine betaine treatments to combat abiotic stresses in crops. International Journal of Molecular Sciences. 2022;23(19):12913.
20. Kałużewicz A, Krzesiński W, Spiżewski T, Zaworska A, Frąszczak B. Glycine betaine and β-aminobutyric acid mitigate the detrimental effects of heat stress on Chinese cabbage (*Brassica rapa* L. ssp. *pekinensis*) seedlings with improved photosynthetic performance and antioxidant system. International Journal of Molecular Sciences. 2022;23(9):4906.
21. Transcriptomic analysis of heat stress response in *Brassica rapa* L. ssp. *pekinensis* with improved thermotolerance through exogenous glycine betaine. International Journal of Molecular Sciences. 2023.
22. Singh D, Singh CK, Kumari S, *et al*. Genome-wide transcriptome analysis reveals vital role of heat responsive genes in regulatory mechanisms of lentil (*Lens culinaris* Medik.). Scientific Reports. 2019;9:12976.
23. Kurek I, Chang TK, Bertain SM, Madrigal A, Liu L, Lassner MW, *et al*. Enhanced thermostability of *Arabidopsis* Rubisco activase improves photosynthesis and growth rates under moderate heat stress. The Plant Cell. 2007;19(10):3230-3241.
24. Scafaro AP, Bautsoens N, den Boer B, Van Rie J, Galle A. A conserved sequence from heat-adapted species improves Rubisco activase thermostability in wheat. Plant Physiology. 2019;181(1):43-54.
25. Degen GE, Worrall D, Carmo-Silva E. An isoleucine residue acts as a thermal and regulatory switch in wheat Rubisco activase. The Plant Journal. 2020;103(2):742-751.
26. Scafaro AP, Atwell BJ, Muylaert S, Reusel BV, Ruiz GA, Van Rie J, *et al*. A thermotolerant variant of Rubisco activase from a wild relative improves growth and seed yield in rice under heat stress. Plant, Cell & Environment. 2018;41(7):1668-1683.
27. Perdomo JA, Carmo-Silva E. Enhancing crop yield by using Rubisco activase to improve photosynthesis under elevated temperatures. Stress Biology. 2021;1:5.
28. Scafaro AP, Bautsoens N, Van Rie J, Galle A. Enhancing wheat Rubisco activase thermostability by mutagenesis of conserved residues from heat-adapted species. International Journal of Molecular Sciences. 2019;20(23):6045.
29. Singh R, Ong-Abdullah M, *et al*. Comparative analysis and structural modeling of *Elaeis oleifera* FAD2, a fatty acid desaturase involved in unsaturated fatty acid composition of American oil palm. International Journal of Molecular Sciences. 2022;23(7):3659.
30. Ivarson E, Horn PJ, *et al*. Triacylglycerol remodeling in *Physaria fendleri* indicates oil accumulation is dynamic and not a metabolic endpoint. Plant Physiology. 2021;187(2):799-815.

31. Identification of triacylglycerol remodeling mechanism to synthesize unusual fatty acid containing oils. Nature Communications. 2024;15:3502.
32. Impact of climate change on oilseed production: a review. Plant Science Today. 2025.
33. Mehrabadi M, *et al*. Differential expression of heat shock proteins and antioxidant enzymes in response to temperature, starvation, and parasitism in the carob moth larvae, *Ectomyelois ceratoniae* (Lepidoptera: Pyralidae). PLoS ONE. 2020;15(1):e0228104.
34. Physio-biochemical and molecular stress regulators and their crosstalk for low-temperature stress responses in fruit crops: a review. Frontiers in Plant Science. 2022;13:884722.
35. Organic fertilizers as a solution to abiotic stress challenges in oilseed crops. In: Sustainable Agriculture Series. Cham: Springer; 2025.
36. Harnessing the power of plants and microbes: strategies to enhance crop performance under heat and salt stress. Journal of Crop Health. 2025.