International
Journal of Agriculture Innovations and Cutting-Edge Research

Biochemical and Physiological Insights into Heat and Drought Stress Tolerance in Chickpea Pollens and Yield

Muhammad Abu Bakar Ghalib[1](Corresponding Author) , Zain Ul Abideen[2], Muhammad Talha Ramzan[3], Muhammad Shaban[4], Muhammad Bilal[5]

[1,] Department of Plant Breeding and Genetics, University of Agriculture Faisalabad, Pakistan, Email: abubakarghalibpbg@gmail.com
[2,] Department of Agronomy, Faculty of Agriculture & Environment, Islamia University of Bahawalpur, Pakistan, Email: zainulabideen4121@gmail.com
[3,] Department of Agronomy, University of Agriculture Faisalabad, Pakistan, Email: 2019ag6226@uaf.edu.pk
[4,] Department of Plant Breeding and Genetics, University of Agriculture Faisalabad (Burewala Campus), Punjab, Pakistan, Email: drshaban@uaf.edu.pk
[5,] State Key Laboratory of Herbage Improvement and Grassland Agro-ecosystems, College of Pastoral Agriculture Science and Technology, Lanzhou University, China, Email: bilalm4muhammad@gmail.com

Abstract

This study aimed to investigate the impact of temperature extremes (heat and cold) and salinity stress on chickpea (Cicer arietinum L.) production, particularly focusing on yield losses and physiological responses under these abiotic stresses. A review of recent experimental and field studies was conducted, incorporating statistical analyses of yield reductions and stress tolerance traits. Data synthesis included quantitative assessments of yield loss percentages and evaluations of antioxidant enzyme activity levels in different chickpea genotypes. Findings revealed that heat stress during the reproductive phase could reduce chickpea yield potential by 30-40%, while combined heat and drought stress might cause yield declines of 40-45%. Stress during pod filling adversely affected pollen viability and pod set, leading to shrivelled pods. The analysis also highlighted that certain chickpea genotypes exhibited higher activities of proline and antioxidant enzymes (linked to the ascorbate-glutathione cycle), which play key roles in heat tolerance. Temperature stress during early growth and reproductive phases significantly diminishes chickpea production by disrupting physiological and reproductive processes. The results underscore the urgent need to develop climate-resilient and stress-tolerant chickpea cultivars tailored for different agroecological regions. To address these challenges, the study recommends leveraging modern genetic tools such as CRISPR and genome-wide association studies (GWASs) to breed chickpea cultivars with superior stress tolerance. Sustained investment in biotechnology and targeted breeding programs will be essential for securing chickpea productivity under changing climate conditions.

Keywords: Chickpea, Physiological trait, Genotypes, QTLs, Heat stress, Climate resilience.

DOI:	https://zenodo.org/records/16516001
Journal Link:	https://jai.bwo-researches.com/index.php/jwr/index
Paper Link:	https://jai.bwo-researches.com/index.php/jwr/article/view/135
Publication Process	Received: 09 Jun 2025/ Revised: 22 Jul 2025/ Accepted: 26 Jul 2025/ Published: 29 Jul 2025
ISSN:	Online [3007-0929], Print [3007-0910]

| *Indexing:* | Academia.edu, DRJI, doi, zenodo, Google Scholar, INDEX COPERNICUS INTERNATIONAL |
| *Publisher:* | BWO Research International (15162394 Canada Inc.) https://www.bwo-researches.com |

Introduction

Chickpea (*Cicer arietinum* L.) is an annual leguminous crop that belongs to the *Faboideae* sub-family of the *Fabaceae*. The two primary categories of crops are Rabi crops and Kharif crops. Chickpea belongs to the Rabi season. Chickpeas are mostly grown in semi-arid and arid regions in over fifty nations worldwide (Pareek *et al*., 2019). India is the greatest producer, contributing approximately 70% of global output. Major producers include India, Australia, Turkey, Myanmar, and Pakistan, with a global production of 12-14 million metric tons (MMTs) per year (FAO, 2021). Chickpea has a great portion of protein, dietary fibre, vitamins, and macro and micronutrients to feed the expanding population of the world. Protein and carbohydrates account for 80% of the total dry mass of chickpeas (Devi *et al*., 2022). However chickpea production can be considerably increased by progressing high-growth, climate-resilient, resistant in disease, and cultivars, as well as improving agronomic techniques (Jukanti *et al*., 2012; Kumari *et al*., 2018). Chickpeas are vulnerable to abiotic conditions such as heat and drought at different growth stages during the season of productivity, resulting in reduced production (Yadav *et al*., 2021).

Global warming is a major threat to crop productivity as the agricultural sector has been negatively impacted by the unexpected temperature rise in decades, which can also affect crop plants (Pareek *et al*., 2019). Excessive temperatures disrupt the respiration mechanism, membrane function, enzyme functions, hormone functions, and primary and secondary metabolite functions and reduce crop yield (Devi *et al*., 2022). Reportedly, greater reductions are seen in chickpea yield due to a rise in 1°C beyond the high conditions. Cool-season crops, such as chickpeas, are negatively impacted by rising temperatures, particularly during the reproductive stage of plant growth (Devi *et al*., 2022). Chickpea production ranked third in the world. Between 2006 and 2009, approximately 11.3 million hectares of chickpeas were grown worldwide, with a yield of 849 kg/ha, with production of 9.6 mm/t (Jukanti *et al*., 2012).

According to Abscisic acid, ABA is a hormone that is present in plants that plays a pivotal part in controlling and moderating plant responses to different environmental stressors, including drought, cold, and flooding. Chickpeas are grown extensively in a variety of climates due to their nod factor. Changing climate at different crop development (CD) stages may affect the sowing time at various locations, which also differ in yield/ha. As a result, the most crucial environmental elements for chickpea growth, production, and adaptation are low and high temperatures. According to the characteristics of chickpea lines for drought-tolerant varieties, ICCs 4958 and ICC 1882 have been calculated. ICC 1882 (264 RILs) and ICC 4958 (large root) are the RIL mapping populations. Tiny Root for the experiment has been developed. ICC 1882and ICC 4958 have been thoroughly studied for drought tolerance to combat the abiotic stress.

Under open field conditions, a mixture of clay and sand was filled into PVC cylinders reaching a maximum diameter of 18 cm and a maximum height of 120 cm for the root phenotyping study. Thirty-five days later, plants were sampled. Several measurements and the sowing data were noted, and 10 RILs based on phenotypic evolution were chosen (Deokar *et al*., 2011). The phenotypes of low and high root biomass have been extensively utilized for comparing SSH patterns for the traits being measured. The gene expression in various

tissues under various circumstances is calculated. Gene expression is not applied to factors that are variously expressed in chickpeas in response to drought stress (Deokar *et al*., 2011). Water pressure was used in all investigations, and the study identifies the genetic variation in drought tolerance among chickpea genotypes. ICC 4958 was more effective in coping with drought stress as compared to ICC 1882. Overall, the combination of these elements has the potential to enhance chickpea yields by 40-60% in the future. The findings underscore the importance of root traits aimed to improve drought tolerance (DT) in chickpeas (Ceylan *et al*., 2013). **Table 1: Analysis of the physiological parameters in two chickpea genotypes under drought stress.**

Physiological parameter	Drought tolerant Pusa362	Drought susceptible SBD377	References
Relative Water Content (%) Control Stress	72.18 ± 1.69 55.96 ± 0.60	70.166 ± 1.32 51.82 ± 0.75	(Singh *et al*., 2021)
Soil Moisture Content (%) Control Stress	28.59±1.69 12.48 ± 0.60	27.29 ± 1.32 9.733 ± 0.75	(Singh *et al*., 2021)

This describes the physiological parameters of genotype Pusa362 and Sbd377 for drought-tolerant and drought-susceptible parameters.

Effects of heat stress on chickpea growth

Chickpea thrives in an annual rainfall of 600–1000 mm, the optimal temperature range for chickpea development is 18–26 °C during the day and 21–29 °C at night (Kumari *et al*., 2018). 90% of the chickpea land is in Asia. East Africa grows most chickpeas, accounting for 4.7%, which includes Ethiopia, Malawi, and Tanzania. The world's largest producer of chickpeas is India, including the sub-continent. According to (Singh *et al*., 2014). Other significant chickpea-growing nations in the area are Pakistan and Iran. Between 2008 and 2010, these two nations made up roughly 11% and 5%of Asia's chickpea-growing region. A chickpea is a very nutrient-dense grain legume crop. According to (Singh *et al*., 2014), it is a significant source of energy, protein, minerals, vitamins, fibre, and other potentially health-beneficial substances. Gram comes in two varieties: one is Kabali, which has white or grey seeds, and the other is Desi, which has light to dark brown seeds. Approximately 85% of the gram area is covered by the native variety. Despite being a crop native to temperate climates, chickpeas are being grown in tropical and subtropical areas due to their higher demand. Production of chickpeas is usually encouraged in the hot, short-season tropical regions. Delayed and late sowing in chickpeas results in exposure to different stresses, including heat stress.

The study aimed to evaluate five different genotypes of chickpeas at early growth stages' thermotolerance behaviour in five different genotypes of chickpeas to examines study aimed to evaluate five different genotypes of chickpeas at early growth stages' thermotolerance behavior in five different genotypes of chickpeas to examine the plant's reaction to heat stress. Plants are cultivated without heat stress in their early stages. To determine whether five chickpea genotypes were sensitive to heat or tolerant to high temperatures, three different temperatures were selected to study their effects on the growth parameters. In this study, the seeds were cultivated for 50 days under stress and control conditions, then they were moved to a recovery period, which was for 10 days (Kumari *et al*., 2018). Analysis of thermotolerance behaviour of five chickpea

genotypes at early growth stages. Results have shown that heat stress during the early growth stages can adversely affect the physiological and biochemical processes in chickpeas, leading to a reduction in crop production. However, certain enzymes, like proline and antioxidant enzymes, activate their activities in stress response, which indicates their role in heat tolerance. Exposure to stress during the reproductive stage is way more hazardous as it directly affects the chickpea yield. It is believed that flowers and pods are more sensitive to changes surrounding temperature. Exposure to high temperatures (35 °C) leads to reduced seed production and pod formation (Singh *et al*., 2014).

High temperature during grain filling possibly reduces the dough and baking quality in grain crops, including chickpeas. Climate change negatively affects grain cultivation in hot, dry regions due to global warming. Heat and drought will put strains on its output in the future. Furthermore, a plant's stress response can differ significantly between the seedling and reproductive stages, and the latter is a crucial phase that determines yield in grams (Deokar *et al*., 2011). Usually, heat stress can cause some synthesis of proteins called heat shock proteins. Heat shock proteins (HSPs) are unique proteins that are produced in stressful environments. By preventing the aggregation of foreign proteins, these proteins preserve cellular homeostasis. Under the stressful circumstances, polypeptides are formed (Yadav *et al*., 2021). HSPs belong to a class of special proteins. There are tiny HSPs with a wide molecular mass range from 15 to 104 kDa. Together with HSP70, these proteins prevent cellular proteins from clumping together. To develop tolerance against high temperatures, various stressors are necessary (Kumari *et al*., 2018). The entire plant life cycle, including morphological, reproductive, and developmental activities, is impacted by changes in the intensity and duration of high temperatures because they cause the cellular machinery to break down. Grain and legume output is limited by heat stress during the reproductive and seed-filling stages, which can occasionally prove disastrous (Sita *et al*., 2018).

High temperatures can lead to damage to the membrane thermos ability, and over the chickpea plants' leaf water content, chlorophyll content, and photosynthetic efficiency to disrupt cellular processes (Devi *et al*., 2022). Plant responses to abiotic stresses can fluctuate depending on their growth and developmental stage, harshness, incidence, and exposure to the stress (Dresselhaus and Huckelhoven, 2018). At ICRISAT, a successful field screening method for chickpea heat tolerance has been developed (Sita *et al*., 2018). 18 heat-tolerant genotypes have recently been discovered (Krishnamurthy *et al*. 2011) by field screening, including ICC 1205, ICC 637, and ICC 15618. Here is the detail of the effects of heat stress on various aspects of chickpea plants, including pollen germination, pollen viability, seed germination, plant growth, and reproductive growth.

Pollen Germination

Selection and collection of pollen grains. According to the study included five blooms of the genotype. These flowers yielded three sets of pollen grains. The pollen grains were carefully collected to avoid contamination and to ensure their vitality. In vitro solution is prepared as:

Sucrose Solution: To produce a 10% sucrose solution, dissolve 10 grams of sucrose in 100 millilitres of distilled water.

Potassium Nitrate Solution: To prepare 990 mM potassium nitrate, dissolve the appropriate amount in distilled water and

adjust the pH to 6.5 with diluted nitric acid or sodium hydroxide.

Calcium Nitrate Solution: To prepare 1.3 mM calcium nitrate, dissolve the appropriate amount of calcium nitrate in distilled water.

Boric Acid Solution: 1.64 mM boric acid was made by dissolving the required amount in distilled water.

Magnesium Sulphate Solution: To prepare 812 mM magnesium sulphate, dissolve the necessary amount of magnesium sulphate in distilled water. These solutions were combined to make the final germination media, with thorough mixing to ensure a homogenous solution.

Germination Test Procedure

Pollen grains were equally distributed on a glass slide coated with the prepared germination media to stimulate germination; the slides were placed in a temperature-controlled incubator. Pollen grains were regarded as having germinated when the diameter of the tube exceeded that of the pollen grain. Each replication contained 100 pollen grains to compute the pollen germination percentage (PGP) (Devi *et al.*, 2022). Pollen grains from flowers blooming on the same day were collected and mixed for pollen viability percentage (PVP) analysis to determine the influence of heat stress.

Identification of viable pollen grains

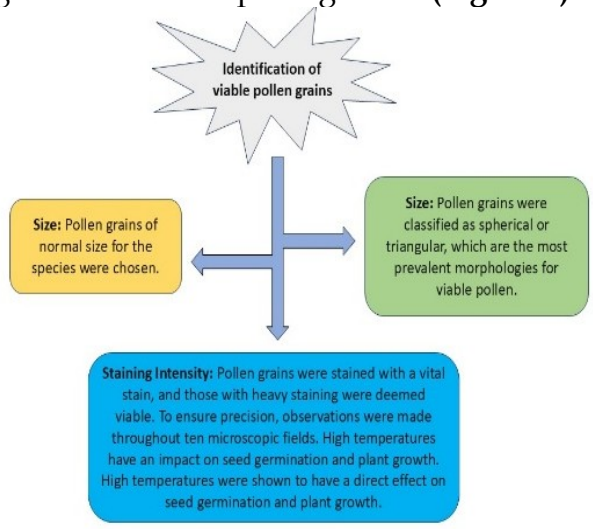
Viable pollen grains were identified using three criteria:

Size: Pollen grains of normal size for the species were chosen.

Shape: Pollen grains were classified as spherical or triangular, which are the most prevalent morphologies for viable pollen.

Staining Intensity: Pollen grains were stained with a vital stain, and those with heavy staining were deemed viable.

To ensure precision, observations were made throughout ten microscopic fields (Jukanti *et al.*, 2012). High temperatures have an impact on seed germination and plant growth. High temperatures were shown to have a direct effect on seed germination and plant growth **(Figure 1)**.



Seed Germination Tolerance

Seed germination can tolerate temperatures as high as 45 degrees Celsius. Beyond this temperature, there was a significant decline in seed growth and seedling death (Ramakrishnan *et al.*, 2024).

To assess the temperature variation, experiments were carried out at two temperatures, 35/25°C and 40/30°C, to observe biomass changes. At 35/25°C, biomass increased in both tolerant and susceptible types, but declined at 40/30°C (Ramakrishnan *et al.*, 2024).

Reproductive growth of chickpea under heat stress

Following is the response of chickpeas.

Heat stress during reproduction typically gives a better yield. Decreased flowering results in a decrease in the number of pods. Causes pollen sterility with inadequate pollination, resulting in loss of vigour. Germination lessens stigma, and fertilization leads to sterility. Results in irregular ovulation and slows down the healing of chickpeas. Decreases seed number, seeding weight, and seed yield through photosynthesis in seeds (Rani *et al.*, 2020). Heat stress has been shown to limit

phenological development and vigour at all phases, with the reproductive stage being most sensitive. Chickpeas responded to heat stress during reproduction as follows:

- Yield Impact: Reduced flowering resulted in fewer pods.
- Pollen Sterility: Increased pollen sterility led to insufficient pollination and reduced vigour.
- Stigma Receptivity: Reduced stigma receptivity and fertilization resulted in infertility.
- Irregular ovulation and sluggish recovery in chickpeas have been noted.
- Seed Development: Reduced photosynthesis resulted in fewer seeds, heavier seeds, and lower yields.

Physiological effects on chickpea

According to Kumari et al. (2018), heat stress reduces leaf photosynthesis and increases oxidative stress. This resulted in a decrease in soluble carbohydrates and ATP in the pistil, which reduced yield by limiting nutrient transport from the style into the pollen tube. This slowed pollen tube development and ovary elongation, reducing seed output. When chickpea genotypes were tested at high temperatures, there was significant genetic heterogeneity in heat sensitivity, which highlights the stress tolerance among varieties (Rani *et al*., 2020).

Variability in the effect of heat stress on seed yield

SSI stands for the stress susceptibility index. Genotypes with an SSI greater than one are considered heat tolerant. MP stands for mean productivity. A high MP value indicates that the genotype is suitable for late sowing conditions.

GMP stands for Geometric Mean Productivity. Heat-tolerant genotypes have high geometric mean productivity levels.

HM stands for Harmonic Mean. Genotypes with a high harmonic mean are excellent for both timely and late-sown circumstances.

HTI stands for heat tolerance. The variability for heat tolerance index among chickpea genotypes was found to be large, and lines with high HTI values are preferable for high-temperature tolerance.

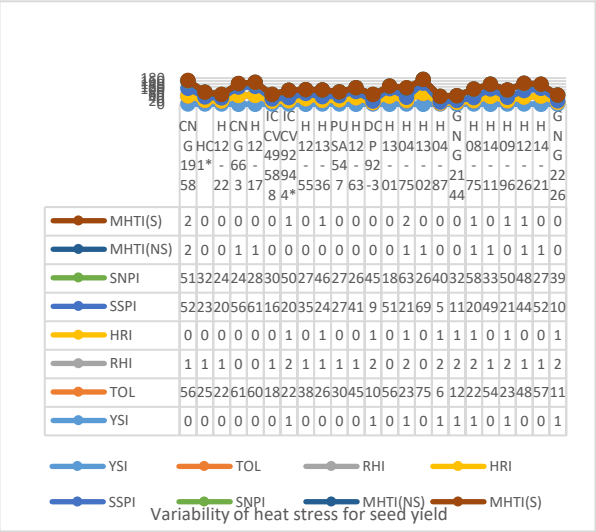
YI stands for Yield Index. Genotypes with YI values greater than 1 are considered vulnerable, while YI values less than 1 indicate a tolerance nature.

YSI stands for yield stability. Genotypes with high YSI values are ideal for late-sown situations. TOL stands for Tolerance Index. The tolerance index is indicated for assessing genotypes appropriate for high-temperature stress. Lines with low TOL values are more stable under various growing situations (timely and late seeded).

RHI stands for Relative Heat Index. RHI is a positive measure used to investigate the nature of stress tolerance in crop plants. Genotypes having a high RHI are suited for growth in stressful settings.

HRI stands for heat resistance. It was claimed that grouping genotypes based on multiple stress indices is a useful method for selecting genotypes with stable performance and high potential yield under varying environmental conditions.

SSPI stands for Stress Susceptibility Percentage. Lower SSPI values indicate that genotypes have a stronger ability to endure stress situations. SNPI stands for Stress Non-Stress Production. The stress index, SNPI, is suitable for selecting breeding materials for economic purposes in both stress and non-stress conditions in chickpea. MHTL stands for Modified Heat Tolerance. Genotypes with high MHTI values are preferred.

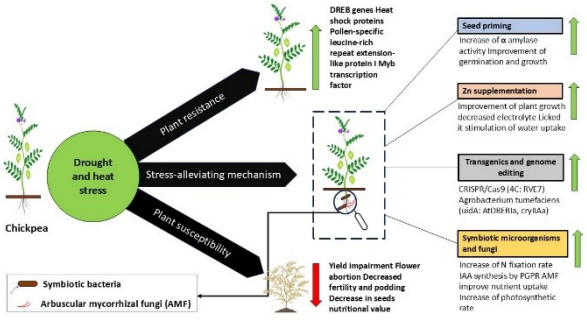


(Figure 2).

Response of biochemicals alleviates drought and heat stress.

Under water stress, plants, especially chickpeas, retain their turgor pressure and cell wall flexibility by enlisting an osmotic adjustment process that enables the accumulation of vital biochemical substances, such as proline, glutathione, trehalose, molecular chaperones, and other antioxidant enzymes (Farooq *et al.*, 2009). Proline is still an essential amino acid that plants make in response to stress, among the many chemical compounds that respond to it. Researchers have studied how different genotypes of chickpea that are drought-tolerant and drought-sensitive respond to water stress in terms of the expression pattern of the proline synthesis enzyme (D1-pyrroline-carboxylate synthetase) and the catabolism of proline by proline dehydrogenase at different vegetative and reproductive stages (Kaur & Prasad, 2021). To withstand drought stress more than the Bitall-2016 desi genotype, the desi Bakhar2011 chickpea genotype acquired more proline, trehalose, and non-reducing sugars.

This was achieved by minimizing the negative impacts of oxidative stress and retaining greater carbon absorption (Farooq *et al.*, 2009). The biochemical that allow chickpeas to adapt to drought and heat stress also include several ROS-scavenging antioxidant enzymes, such as super oxidase dismutase, catalase, and glutathione peroxidase, which help to detoxify and protect cells from damage caused by reactive oxygen species (ROS), such as superoxide radicals and singlet oxygen that accumulates during drought and heat stress (Kaur & Prasad, 2021). It has recently been shown that providing a zinc-based diet could help boost antioxidant activities and lessen the negative impacts of heat stress and drought on chickpea. These processes provide partial drought tolerance and are effective in moderately drying environments (Ullah *et al.*, 2019). A comprehensive strategy that includes plant physiological methods, genomics resources, and cutting-edge breeding methods to create chickpea cultivars that are resistant to drought and high temperatures. A comprehensive strategy is shown in **Figure 3.**



Abiotic common constraints in chickpea

Stressful growing conditions require plants to employ a range of coping mechanisms; the most efficient is to escape the stress, avoidance is the next best tactic, and tolerance is the last option because it hurts production (Bueckert & Clarke, 2013; Shunmugam et al., 2018). Major abiotic factors that restrict chickpea production in most regions are high and low temperatures, as well as moisture stress caused by drought. These stimuli impact different characteristics, metabolic, and

physiological systems, and plants respond differently (Table 1). Depending on the growth/developmental stage, the intensity, frequency, and duration of the stress exposure, plants react differently to abiotic stresses. Chickpeas are largely responsive during the reproductive phase, although they are also somewhat sensitive to abiotic stress in the early phases of vegetative growth, which can result in fewer seeds (Shunmugam *et al*., 2018). The impact of these abiotic stressors on flower set, pollen viability, pod set/abortion, and retention of which are important factors that determine seed number-largely reduces output**(Table 2)**.

Table 2: The primary abiotic stressors affecting chickpea characteristics and processes.

Abiotic Stress	Key process affected	References
Drought	Crop duration, rate, and growth Reproductive organs Activity of enzymes	(Devasirvatham *et al*., 2015; Devasirvatham *et al*., 2012; Kaushal *et al*., 2013)
Heat	Reproductive organs Enzymatic activity and Membrane integrity Germination and/or establishment Photosynthesis Crop growth duration and rate	(Berger *et al*., 2012; Kumar *et al*., 2011; Ramamoorthy *et al*., 2016; Ramamoorthy *et al*., 2017)
Cold	Duration of plant growth Reproductive growth Abscisic acid (ABA) accumulation in the pod or seed	(Pang *et al*., 2017; Pushpavalli *et al*., 2014; Ramamoorthy *et al*., 2017)

Heat shock proteins

A variety of proteins are included in the dataset, including information on their molecular mass, biological roles, cellular location, and fold change values. V5UP77 and A0A067XTG8, which are found in membrane structures, are disease resistance proteins that aid in defence responses and signal transmission, whereas other heat shock proteins (A0A076L224 and A0A076L2J9) are involved in ATP binding. The glycine decarboxylase complex subunit (A0A076L2J9) is linked to glycine catabolism, while protein LEA 4 (E7BSD7) is involved in embryonic development. A chromosomal condensation regulator fragment (Q8H6W4) is involved in sucrose synthase activity, whereas a cytochrome P450 monooxygenase fragment (Q9SML1) does both heme-binding and monooxygenase activity. The metabolism of sucrose in chloroplasts is associated with sucrose synthase (I1SUZ1). Several proteins, such as ATP synthase subunit alpha (B5LMN1), which promotes ATP production and proton transport in chloroplast thylakoid membranes, and ribulose-1,5-bisphosphate carboxylase/oxygenase (Q8WJD8, B5LMK8), are involved in photosynthesis and carbon fixation. For example, phenylalanine ammonia-lyase 2 (Q9SMK9) is essential to produce cinnamic acid, while ethylene-related proteins (A0A076L4S6, E7D235) support signalling pathways and receptor activation. Both beta-galactosidase (O82670) and glycosyltransferase (A0A067XTB3) are membrane-associated transferases involved in the metabolism of carbohydrates. An unnamed protein (Q9LEN6) is also mentioned. Their considerable differential expression is indicated by the wide range of fold change values, with the highest values found for ribulose bisphosphate carboxylase large

subunit (B5LMK8) at 29.6 and beta-galactosidase (O82670) at 43.3.
(Table 3).

Table 3: Tolerance of Chickpea genotype (JG14) in comparison with sensitive genotype (ICC 16374) under heat stress (Parankusam *et al*., 2017).

Sr. No.	Protein Names	Biological process	Cellular components	Molecular function
1	Heat shock protein	Morphogenesis of cells	-	ATP building
2	Disease resistance protein TIR-NBS-LRR	Defence reaction: transduction of signals	Essential element of the membrane	ADP building
3	Protein LEA 4	Development of the embryo	-	-
4	Heat shock protein	-	-	ATP building
5	Subunit T of the Glycine Decarboxylase Complex (Fragment)	Catabolic reaction of glycine	-	Activity of Aminomethyl (AM) transferase
6	Disease resistance protein CC-NBS-LRR	Defence response	Essential element of the membrane	ADP building
7	Fragment of cytochrome P450 monooxygenase	-	-	Monooxygenase activity; Heme binding; iron ion binding; Oxidoreductase activity, acting on paired donors, with Incorporation or reduction of molecular oxygen; metal ion binding
8	Protein called chromosomal condensation regulator (Fragment)	-	-	Activity of Sucrose Synthase
9	(EC 2.4.1.13) Sucrose synthase	The process of Sucrose metabolism	Chloroplast	Magnesium ion binding; ribulose-bisphosphate Carboxylase activity
10	Large subunit of ribulose-1,5-bisphosphate carboxylase/oxygenase (Fragment)	Photosynthesis; Carbon fixation	-	-

11	excess protein in late embryo genesis	-	Endoplasmic reticulum (ER)	Ethylene binding; ethylene receptor activity;
12	Protein similar to the ethylene receptor	Ethylene-activated signalling pathway negative regulation	membrane; a crucial part of a membrane	Phosphorelay sensor kinase activity
13	(EC 4.3.1.24) Phenylalanine ammonia-lyase 2	The catabolic process of L-phenylalanine and the biosynthesis of Cinnamic acid	Cytoplasm	Phenylalanine ammonia-lyase activity
14	(EC 2.4.1.-) Glycosyltransferase	-	membrane; a crucial part of a membrane	Transferase activity, transferring hexosyl groups
15	Potentially unidentified protein		-	-
16	Chroplastic (EC 3.6.3.14), ATP synthase F1 sector subunit alpha, ATP synthase subunit alpha, and F-ATPase subunit alpha	Transportation of protons related to ATP hydrolysis and ATP synthesis	Proton-transporting ATP synthase complex, catalytic core F(1), and chloroplast thylakoid membrane	Binding of ATP; rotational mechanism, transporting of proton, ATPase activity, transporting of proton & synthase of ATP activity
17	Big chain of ribulose bisphosphate carboxylase (Rubisco large subunit) (EC 4.1.1.39)	Photosynthesis and Fixation of Carbon	Chloroplast	binding of magnesium ions; activity of ribulose-bisphosphate carboxylase
18	EC 3.2.1.23, or beta-galactosidase	The metabolic process of carbohydrate	-	Activity of Beta- Beta-Beta-Beta-Beta-Beta-galactosidase

Table 3: The expression ratio of JG14/ICC16374 determined by quantitative proteomics during heat stress was computed as a fold increase or reduction. Here is a list of the proteins that were either up- or down-regulated by five times in JG-14 alone. The average of two biological and three experimental replicates is represented by each value. Every ratio displayed is $p < 0.05$, meaning it is statistically significant. Analysis of the ontology was done using the online www.uniprot.org.

Strategies to overcome abiotic stresses

Biotechnological strategies for improved tolerance to abiotic stress

Plants can recognize abiotic stress and respond appropriately by changing their growth, development, and metabolism. Through gene transfer, abiotic stress tolerance can be acquired at the molecular level by changing processes such as Osmo protectant accumulation, chaperone synthesis, superoxide radical scavenging mechanisms, and ion exclusion or compartmentation by effective transporter and symporter systems(Chinnusamy et al., 2004; Valliyodan & Nguyen, 2006). There has been a rise in knowledge of the molecular mechanisms of genes linked to various cellular pathways that regulate the complex trait of abiotic stress tolerance, with an understanding of the function of stress-inducible genes serving as a means of deciphering potential mechanisms of stress tolerance (Shinozaki *et al.*, 2003). Many approaches have been used in the last 10 years to identify the genes responsible for the stress response and comprehend the underpinnings of stress tolerance (Vij & Tyagi, 2007). They include the following:

- Producing a distinct transcript-specific short sequence of 9–17 bp using serial gene analysis appears to prove crucial for analyzing the global expression of genes (Shah *et al.*, 2006).
- Massively parallel signature sequencing (MPSS) is an additional potent method for genome-wide transcription profiling. For three plant species—grapes, rice, and Arabidopsis-the MPSS resource is accessible in a public database http://mpps.udel.edu (Nakano *et al.*, 2006).
- Global gene expression profiling is now transformed by microarray technology, which makes it possible to examine every gene in the genome in a single experiment (Duggan *et al.*, 1999).

To improve stress tolerance without causing growth retardation, stress-inducible promoters with low background expression under normal growth conditions have recently been used in conjunction with transgenes (Bhatnagar-Mathur *et al.*, 2007). The selection of an appropriate promoter is essential for developing clever genetic engineering tactics because powerful abiotic stress-inducible promoters are needed for transgene expression responsible for varying abiotic-stress tolerance at different stages of growth (Singhal *et al.*, 2016).

Biochemical and physiological basis of tolerance

The external application of Abscisic acid (ABA) causes fatty acid desaturation in the plasma membrane and resulting in low cell lysis at low temperature, as evidenced by the double bond index (DBI) (Bakht *et al.*, 2013). Applying glycine betaine during the budding stage can reduce cold stress by enhancing the viability, germination, growth, stigma receptivity, and ovule viability of the pollen grains. However, treatment during the podding stage raises RWC, seed output, and the number of seeds per pod (Nayyar *et al.*, 2005). When compared to cold-stressed plants, chickpeas exhibit fundamentally different responses to external ABA, such as the retention of chlorophyll, increased pollen viability, germination, flower retention, and pod set, as well as an increase in seed weight, single-seeded pods, and a decrease in infertile pods. Additionally, ABA inhibits oxidative damage by boosting plant proline and antioxidant activity (Kumar *et al.*, 2008). In the same way, demonstrated that exogenous ABA administration facilitates adaptation to freezing conditions. Antioxidative enzymes like catalase, ascorbate

peroxidase, glutathione reductase, and sucrose synthase have also been shown to shield seeds and pod walls from cold stress, which can be extremely helpful in the development of cold-tolerant chickpea lines (Kaur *et al*., 2009).

Improving breeding strategies to tolerate extreme climate events

Investigating genetic diversity for features that affect yield requires breeding for heat and drought tolerance. Various genotypes are currently available for breeding programs designed to improve drought and heat resistance (Upadhyaya *et al*., 2011). After separating populations from A1 x ICC4958, ICCV2 x ICC4958 were assessed for physiological characteristics that contributed to drought tolerance (grain yield, root biomass) (Mannur *et al*., 2009). Several markers, including Diversity Arrays Technology (DArT), Single Nucleotide Polymorphism (SNP), and Simple Sequence Repeats (SSR), have been used to create high-density genetic maps. In two mapping populations (ICC4958 x ICC1882; ICC283 x ICC8261), main effect quantitative trait loci (QTLs) and epistatic QTLs for various drought tolerance traits were identified. These identified QTL-hotspots, or genomic regions, regulate 12 drought tolerance traits, including root length density, root surface area, shoot dry weight, plant height, days to 50% flowering, days to maturity, harvest index, 100 seed weight, biomass, yield, pods per plant, and seeds per pod (Varshney *et al*., 2014). This is regarded as a potential drought-tolerant genetic area. Similarly, using RILs derived from the desi chickpea cross ICC4567 x ICC15614, four QTLs for the number of filled pods, total seeds, grain yield, and percentage of pod set were discovered under heat stress (Paul *et al*., 2018). Additionally, ICRISAT created multiparent advanced generation inter-cross populations (MAGIC) utilizing a set of eight drought-tolerant, well-adapted lines, including ICC4958, ICCV10, JAKI9218, JG11, JG130, JG16, ICCV97105, and ICCV00108. This method enables the identification of genes and the comprehension of complicated characteristics that cause drought. Marker-assisted selection (MAS), which is being developed for pulses, will result from these efforts. Breeders of chickpeas must create trait-specific mapping populations and map the QTLs to get MAS (Copeland, 2020). It will make it easier to generate improved chickpea cultivars that can withstand heat and drought in the future.

Conclusion

This review consolidates current biochemical and physiological insights into the impact of heat and drought stress on chickpea (*Cicer arietinum L.*) growth, reproduction, and yield. It highlights that temperature extremes, particularly heat stress during critical reproductive phases, can severely compromise pollen viability, germination, and pod set, resulting in yield losses estimated at 30-40%. When heat stress coincides with drought, losses may escalate to 40-45%, posing a substantial threat to chickpea cultivation, especially in hot and arid regions. The review also emphasizes the physiological variability among chickpea genotypes, where certain lines exhibit enhanced tolerance due to elevated activities of antioxidant enzymes like proline and components of the ascorbate-glutathione cycle. These biochemical adaptations play a vital role in mitigating oxidative damage during stress episodes. Furthermore, the analysis underscores the importance of understanding pollen biology under thermal stress, including germination tests and the identification of viable pollen grains, as these are critical determinants of successful reproduction and seed set. Variability in heat stress response at the

seed yield level further highlights the complex interplay between genetic, biochemical, and environmental factors influencing stress tolerance. Beyond physiological adaptations, the role of heat shock proteins and other stress-responsive molecules offers promising avenues for enhancing resilience.

Objectives

To examine the effects of heat and drought stress on chickpea reproductive growth, seed yield, and physiological processes.

To analyze the role of biochemical responses, including antioxidant enzyme activity and heat shock proteins, in mitigating abiotic stress.

To explore modern breeding and biotechnological strategies aimed at developing stress-tolerant chickpea cultivars suitable for diverse agroecological regions.

Future Prospective

Future research should prioritize integrating advanced biotechnological tools, such as CRISPR and GWAS, to identify and introduce heat and drought tolerance traits in chickpea cultivars. Emphasis should be placed on dissecting the biochemical and physiological pathways-particularly those involving antioxidant enzymes, proline accumulation, and heat shock proteins-that underpin resilience to extreme temperatures. Developing climate-resilient genotypes tailored to diverse agroecological zones, combined with improved breeding strategies, will be essential for stabilizing chickpea yields under rising global temperatures. Further exploration of pollen viability, seed germination tolerance, and reproductive physiology under stress conditions will also enhance our understanding of sustaining productivity amid climate change.

Author declaration

Ethics approval

Not needed for this review

Funding

Non-funded review

Conflict of interest

The authors declare that they don't have any personal relationships that can affect the work reported in this review.

Data availability

All data supporting the findings of this study are included within the article and its supplementary materials. No additional datasets were generated or analyzed beyond what is presented in this manuscript.

Author Contributions

All authors have equal contributions.

References

Bakht, J., Bano, A., Shafi, M., & Dominy, P. (2013). Effect of abscisic acid applications on cold tolerance in chickpea (*Cicer arietinum* L.). *European Journal of Agronomy*, *44*, 10-21.

Bhatnagar-Mathur, P., Devi, M. J., Reddy, D. S., Lavanya, M., Vadez, V., Serraj, R., Yamaguchi-Shinozaki, K., & Sharma, K. K. (2007). Stress-inducible expression of At DREB1A in transgenic peanut (Arachis hypogaea L.) increases transpiration efficiency under water-limiting conditions. *Plant Cell Reports*, *26*, 2071-2082.

Brewbaker, J. L., & Kwack, B. H. (1963). The essential role of calcium ions in pollen germination and pollen tube growth. *American journal of botany*, *50*(9), 859-865.

Bueckert, R. A., & Clarke, J. M. (2013). Annual crop adaptation to abiotic stress on the Canadian prairies: Six case studies. *Canadian Journal of Plant Science*, *93*(3), 375-385.

Ceylan, H. A., Türkan, I., & Sekmen, A. H. (2013). Effect of coronatine on antioxidant enzyme response of chickpea roots to a combination of PEG-induced osmotic stress and heat stress. *Journal of plant growth regulation*, *32*, 72-82.

Chinnusamy, V., Schumaker, K., & Zhu, J. K. (2004). Molecular genetic perspectives on cross-talk and specificity in abiotic stress signalling in plants. *Journal of Experimental Botany*, *55*(395), 225-236.

Copeland, L. (2020). A century of agriculture at the University of Sydney. *Agricultural science*, *31*(2), 58-65.

Deokar, A. A., Kondawar, V., Jain, P. K., Karuppayil, S. M., Raju, N., Vadez, V., Varshney, R. K., & Srinivasan, R. (2011). Comparative analysis of expressed sequence tags (ESTs) between drought-tolerant and susceptible genotypes of chickpea under terminal drought stress. *BMC plant biology*, *11*, 1-20.

Devi, P., Jha, U. C., Prakash, V., Kumar, S., Parida, S.K., Paul, P. J., Prasad, P. V., Sharma, K. D., Siddique, K. H., & Nayyar, H. (2022). Response of physiological, reproductive function, and yield traits in cultivated chickpea (*Cicer arietinum* L.) under heat stress. *Frontiers in Plant Science*, *13*, 880519.

Duggan, D. J., Bittner, M., Chen, Y., Meltzer, P., & Trent, J. M. (1999). Expression profiling using cDNA microarrays. *Nature Genetics*, *21*(1), 10-14.

Farooq, M., Wahid, A., Kobayashi, N., Fujita, D., & Basra, S. M. (2009). Plant drought stress: effects, mechanisms and management. *Sustainable agriculture*, 153-188.

Jukanti, A. K., Gaur, P. M., Gowda, C., & Chibbar, R. N. (2012). Nutritional quality and health benefits of chickpea (*Cicer arietinum* L.): a review. *British Journal of Nutrition*, *108*(S1), S11-S26.

Kaur, R., & Prasad, K. (2021). Nutritional characteristics and value-added products of Chickpea (*Cicer arietinum*)—A review. *Journal of Postharvest Technology*, *9*(2), 1-13.

Kaur, S., Gupta, A., Kaur, N., Sandhu, J., & Gupta, S. (2009). Antioxidative enzymes and sucrose synthase contribute to cold stress tolerance in chickpea. *Journal of Agronomy and Crop Science*, *195*(5), 393-397.

Kumar, S., Kaur, G., & Nayyar, H. (2008). Exogenous application of abscisic acid improves cold tolerance in chickpea (*Cicer arietinum* L.). *Journal of Agronomy and Crop Science*, *194*(6), 449-456.

Kumar, S., Kaushal, N., Nayyar, H., & Gaur, P. (2012). Abscisic acid induces heat tolerance in chickpea (*Cicer arietinum* L.) seedlings by facilitating the accumulation of osmoprotectants. *Acta physiologiae plantarum*, *34*, 1651-1658.

Kumari, P., Singh, S., & Yadav, S. (2018). Analysis of thermotolerance behaviour of five chickpea genotypes at early growth stages. *Brazilian Journal of Botany*, *41*(3), 551-565.

Mannur, D., Salimath, P., & Mishra, M. (2009). Evaluation of segregating populations for drought-related morphological and physiological traits in chickpea. *Journal of Food Legumes*, *22*(4), 233-238.

Nakano, M., Nobuta, K., Vemaraju, K., Tej, S. S., Skogen, J. W., & Meyers, B. C. (2006). Plant MPSS databases: signature-based transcriptional resources for analyses of mRNA and small RNA. *Nucleic Acids Research*, *34*(suppl_1), D731-D735.

Nayyar, H., Chander, K., Kumar, S., & Bains, T. (2005). Glycine betaine mitigates cold stress damage in chickpea. *Agronomy for sustainable development*, *25*(3), 381-388.

Pareek, A., Rathi, D., Mishra, D., Chakraborty, S., & Chakraborty, N. (2019). Physiological plasticity to high temperature stress in chickpea: Adaptive responses and variable tolerance. *Plant Science*, *289*, 110258.

Paul, P. J., Samineni, S., Thudi, M., Sajja, S. B., Rathore, A., Das, R. R., Khan, A. W., Chaturvedi, S. K., Lavanya, G. R., & Varshney, R. K. (2018). Molecular mapping of QTLs for heat tolerance in chickpea. *International journal of molecular sciences*, *19*(8), 2166.

Ramakrishnan, R. S., Nagre, S., Kumar, A., Sharma, R., Upadhyay, A., & Samaiya, R. (2024). Seed germination and seed vigour induction through foliar application of plant growth regulators and nutrients under drought stress in chickpea (*Cicer arietinum* L.). *Archives of Current Research International*, *24*(1), 13-23.

Rani, A., Devi, P., Jha, U. C., Sharma, K. D., Siddique, K. H., & Nayyar, H. (2020). Developing climate-resilient chickpea involving physiological and molecular approaches with a focus on temperature and drought stresses. *Frontiers in Plant Science*, *10*, 1759.

Shah, T., de Villiers, E., Nene, V., Hass, B., Taracha, E., Gardner, M. J., Sansom, C., Pelle, R., & Bishop, R. (2006). Using the transcriptome to annotate the genome revisited: application of massively parallel signature sequencing (MPSS). *Gene*, *366*(1), 104-108.

Shinozaki, K., Yamaguchi-Shinozaki, K., & Seki, M. (2003). Regulatory network of gene expression in the drought and cold stress responses. *Current opinion in plant biology*, *6*(5), 410-417.

Shunmugam, A. S., Kannan, U., Jiang, Y., Daba, K. A., & Gorim, L. Y. (2018). Physiology-based approaches for breeding of next-generation food legumes. *Plants*, *7*(3), 72.

Singh, P., Nedumaran, S., Boote, K. J., Gaur, P., Srinivas, K., & Bantilan, M. (2014). Climate change impacts and potential benefits of drought and heat tolerance in chickpea in South Asia and East Africa. *European Journal of Agronomy*, *52*, 123-137.

Singhal, P., Jan, A. T., Azam, M., & Haq, Q. M. R. (2016). Plant abiotic stress: a prospective strategy of exploiting promoters as an alternative to overcome the escalating burden. *Frontiers in Life Science*, *9*(1), 52-63.

Sita, K., Sehgal, A., Bhandari, K., Kumar, J., Kumar, S., Singh, S., Siddique, K. H., & Nayyar, H. (2018). Impact of heat stress during seed filling on seed quality and seed yield in lentil (Lens culinaris Medikus) genotypes. *Journal of the Science of Food and Agriculture*, *98*(13), 5134-5141.

Thudi, M., Gaur, P. M., Krishnamurthy, L., Mir, R. R., Kudapa, H., Fikre, A., Kimurto, P., Tripathi, S., Soren, K. R., & Mulwa, R. (2014). Genomics-assisted breeding for drought tolerance in chickpea. *Functional Plant Biology*, *41*(11), 1178-1190.

Ullah, A., Romdhane, L., Rehman, A., & Farooq, M. (2019). Adequate zinc nutrition improves the tolerance against drought and heat stresses in chickpea. *Plant Physiology and Biochemistry*, *143*, 11-18.

Upadhyaya, H. D., Dronavalli, N., Gowda, C., & Singh, S. (2011). Identification and evaluation of chickpea germplasm for tolerance to heat stress. *Crop Science*, *51*(5), 2079-2094.

Valliyodan, B., & Nguyen, H. T. (2006). Understanding regulatory networks and engineering for enhanced drought tolerance in plants. *Current opinion in plant biology*, *9*(2), 189-195.

Varshney, R. K., Thudi, M., Nayak, S. N., Gaur, P. M., Kashiwagi, J., Krishnamurthy, L., Jaganathan, D., Koppolu, J., Bohra, A., & Tripathi, S. (2014). Genetic dissection of drought tolerance in chickpea (*Cicer arietinum* L.). *Theoretical and Applied Genetics*, *127*, 445-462.

Vij, S., & Tyagi, A. K. (2007). Emerging trends in the functional genomics of the abiotic stress response in crop plants. *Plant biotechnology journal*, *5*(3), 361-380.

Yadav, R., Juneja, S., & Kumar, S. (2021). Cross priming with drought improves heat-tolerance in chickpea (*Cicer arietinum* L.) by stimulating small heat shock proteins and antioxidative defence. *Environmental Sustainability*, *4*(1), 171-182.

Berger, J., Kumar, S., Nayyar, H., Street, K., Sandhu, J. S., Henzell, J., Kaur, J., & Clarke, H. (2012). Temperature-stratified screening of chickpea (Cicer arietinum L.) genetic resource collections reveals very limited reproductive chilling tolerance compared to its annual wild relatives. *Field Crops Research*, *126*, 119-129.

Devasirvatham, V., Gaur, P., Raju, T., Trethowan, R., & Tan, D. (2015). Field response of chickpea (Cicer arietinum L.) to high temperature. *Field Crops Research*, *172*, 59-71.

Devasirvatham, V., Tan, D., Gaur, P., Raju, T., & Trethowan, R. (2012). High temperature tolerance in chickpea and its implications for plant improvement. *Crop and Pasture Science*, *63*(5), 419-428.

Kaushal, N., Awasthi, R., Gupta, K., Gaur, P., Siddique, K. H., & Nayyar, H. (2013). Heat-stress-induced reproductive failures in chickpea (Cicer arietinum) are associated with impaired sucrose metabolism in leaves and anthers. *Functional Plant Biology*, *40*(12), 1334-1349.

Kumar, S., Malik, J., Thakur, P., Kaistha, S., Sharma, K. D., Upadhyaya, H., Berger, J., & Nayyar, H. (2011). Growth and metabolic responses of contrasting chickpea (Cicer arietinum L.) genotypes to chilling stress at the reproductive phase. *Acta physiologiae plantarum*, *33*, 779-787.

Pang, J., Turner, N. C., Khan, T., Du, Y.-L., Xiong, J.-L., Colmer, T. D., Devilla, R., Stefanova, K., & Siddique, K. H. (2017). Response of chickpea (Cicer arietinum L.) to terminal drought: leaf stomatal conductance, pod abscisic acid concentration, and seed set. *Journal of Experimental Botany*, *68*(8), 1973-1985.

Parankusam, S., Bhatnagar-Mathur, P., & Sharma, K. K. (2017). Heat-responsive proteome changes reveal molecular mechanisms underlying heat tolerance in chickpea. *Environmental and experimental botany*, *141*, 132-144.

Pushpavalli, R., Zaman-Allah, M., Turner, N. C., Baddam, R., Rao, M. V., & Vadez, V. (2014). Higher flower and seed number leads to higher yield under water stress conditions imposed during reproduction in chickpea. *Functional Plant Biology*, *42*(2), 162-174.

Ramamoorthy, P., Lakshmanan, K., Upadhyaya, H. D., Vadez, V., & Varshney, R. K. (2016). Shoot traits and their relevance in terminal drought tolerance of chickpea (Cicer arietinum L.). *Field Crops Research*, *197*, 10-27.

Ramamoorthy, P., Lakshmanan, K., Upadhyaya, H. D., Vadez, V., & Varshney, R. K. (2017). Root traits confer grain yield advantages under terminal drought in chickpea (Cicer arietinum L.). *Field Crops Research*, *201*, 146-161.

Singh, L., Kohli, D., Gaikwad, K., Kansal, R., Dahuja, A., Paul, V., Bharadwaj, C., & Jain, P. K. (2021). Effect of drought stress on morphological, biochemical, physiological traits and expression analysis of microRNAs in drought-tolerant and sensitive genotypes of chickpea. *Indian Journal of Genetics and Plant Breeding*, *81*(02), 266-276.