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Overexpression of Heat Shock Proteins in Orchids: Molecular Mechanisms, Evidence, Applications, and Future Prospects

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Abstract
Orchids are among the most economically important ornamental plants and represent one of the largest and most diverse families of flowering plants. However, their growth, flowering, reproduction, and postharvest quality are strongly affected by elevated temperature. Heat stress disrupts membrane integrity, photosynthesis, water relations, redox homeostasis, protein folding, and reproductive development. Heat shock proteins (HSPs), also known as molecular chaperones, are central components of the plant heat-stress response because they prevent protein aggregation, assist refolding, stabilize membranes and organelles, and coordinate antioxidant and stress-signaling systems. In orchids, studies on *Dendrobium*, *Oncidium*, *Phalaenopsis*, and related genera have demonstrated heat-responsive expression of HSP20, HSP40, HSP70, HSP90, and HSP101 genes. Genome-wide analysis of *Dendrobium officinale*, for example, identified 37 HSP20, 43 HSP70, and four HSP90 genes, many of which responded to environmental or hormone-related stress. Short-term heat treatment in *Oncidium* also rapidly induced HSF and HSP transcripts, whereas recent comparative work has associated thermotolerant cultivars with strong expression of small HSPs, HSP70, HSP90, antioxidant genes, and chloroplast-protective genes. Although direct, stable overexpression studies in orchids remain limited, evidence from orchids and heterologous plant systems strongly supports HSP overexpression as a promising strategy for improving thermotolerance. This review examines HSP families and their regulation in orchids, evaluates current evidence for HSP overexpression, discusses transformation and gene-editing approaches, and identifies biological, technical, ecological, and regulatory challenges. Future orchid improvement will likely depend on inducible, tissue-specific, and multiplex manipulation of HSPs combined with heat shock transcription factors, antioxidant pathways, osmoprotectants, and precise phenotyping.

Keywords: orchids, heat stress, heat shock proteins, HSP20, HSP70, HSP90, HSP101, heat shock factors, thermotolerance, genetic engineering

Introduction

Orchids belong to Orchidaceae, one of the largest and most evolutionarily diverse families of angiosperms. They are cultivated worldwide for cut flowers, potted plants, landscaping, medicinal products, fragrances, and breeding materials. Important horticultural genera include *Phalaenopsis*, *Dendrobium*, *Oncidium*, *Cattleya*, *Cymbidium*, *Vanda*, and *Paphiopedilum*. Commercial orchid production depends on precise regulation of temperature, light, humidity, nutrition, and irrigation. Among these factors, temperature is particularly important because orchids often possess narrow environmental requirements for vegetative growth, flower initiation, floral development, and postharvest longevity.

High temperature affects orchids at several levels. At the cellular level, heat increases membrane fluidity, destabilizes protein structure, accelerates reactive oxygen species formation, and impairs the function of photosystem II. At the whole-plant level, it causes leaf yellowing, reduced chlorophyll content, impaired root growth, flower abortion, delayed or abnormal flowering, reduced pollen viability, and shortened vase life. In Phalaenopsis, temperatures above approximately 27–29°C can maintain plants in a vegetative state, while short exposure to 32–35°C may produce heat-stress symptoms [23, 2]. Such responses are important commercially because temperature management directly influences the timing and quality of flowering.

Plants cannot escape unfavorable temperatures. Instead, they rely on physiological acclimation and molecular defense systems. The heat shock response is one of the most conserved stress responses in biology. It involves rapid transcriptional activation of heat shock genes, synthesis of HSPs, modification of metabolism, production of antioxidants and osmolytes, and restoration of cellular proteostasis. HSPs are not induced only by heat; they also respond to drought, salinity, oxidative stress, intense light, heavy metals, pathogen attack, wounding, and chemical treatments [30, 33, 13].

The term “overexpression” may refer to natural stress-induced accumulation, constitutive transgene expression, inducible expression, or targeted upregulation through transcription-factor manipulation. In orchids, much of the available literature documents heat-induced or stress-induced expression rather than stable genetic overexpression. Therefore, it is important to distinguish between correlation and functional proof. Increased HSP transcripts in a heat-tolerant orchid may indicate that HSPs contribute to tolerance, but it does not alone establish that a particular HSP is sufficient to improve thermotolerance. Stronger evidence comes from transgenic complementation, gene silencing, protein-function assays, and controlled comparisons of overexpression lines.

This review evaluates the role of HSP overexpression in orchids and places orchid research within the broader plant molecular-biology literature. It focuses on the principal HSP families, their regulation through heat shock factors (HSFs), their relationship with photosynthesis and antioxidant metabolism, available evidence from orchid species, lessons from heterologous overexpression studies, and strategies for future orchid biotechnology.

Heat Stress in Orchids

Physiological Effects

Heat stress is commonly defined as a temperature condition sufficiently high to cause irreversible or potentially damaging changes in plant growth and development. The threshold is species-, genotype-, tissue-, and developmental-stage dependent. Orchids are particularly sensitive during reproductive development because flowers and pollen often have lower thermal tolerance than vegetative tissues.

One immediate effect of heat is increased membrane fluidity. Excessive fluidity alters the function of transporters, receptors, membrane-bound enzymes, and chloroplast and mitochondrial membranes. Plants compensate by changing lipid composition, increasing saturated fatty acids, and producing protective proteins. In orchids, membrane injury can appear as leaf chlorosis, necrosis, tissue water loss, and reduced flower quality.

Photosynthesis is another major target. Heat can inhibit chlorophyll biosynthesis, damage the oxygen-evolving complex, disturb thylakoid membranes, and reduce the activity or stability of Rubisco. Photosystem II, especially the D1 reaction-center protein, is highly sensitive to thermal injury. Heat-tolerant plants generally preserve higher chlorophyll content, photosystem II efficiency, stomatal regulation, and electron-transport activity than sensitive plants.

Elevated temperature also increases the production of reactive oxygen species (ROS), including superoxide radicals, hydrogen peroxide, hydroxyl radicals, and singlet oxygen. At moderate levels, ROS act as signaling molecules; at excessive levels, they oxidize proteins, lipids, nucleic acids, and pigments. Antioxidant enzymes such as superoxide dismutase, catalase, ascorbate peroxidase, glutathione peroxidase, and glutathione reductase are therefore closely associated with heat tolerance.

Water relations are also affected. Heat increases transpiration demand and may cause dehydration even when soil moisture is adequate. Orchids possess specialized water-conservation traits, including thick cuticles, velamen-covered roots, pseudobulbs in some genera, and, in several epiphytic lineages, crassulacean acid metabolism. Nevertheless, prolonged high temperature can disturb stomatal behavior, reduce relative water content, and inhibit root function.

Reproductive Consequences

Heat stress can reduce pollen viability, impair pollen germination, disrupt pollen-tube growth, and alter ovule development. In addition, high temperature can interfere with floral induction and floral organ development. In Oncidium, prolonged exposure to elevated temperature can promote floral transition through changes involving cytosolic ascorbate peroxidase and cellular redox status [15]. This observation demonstrates that temperature is not merely a stress factor but also a developmental signal.

Because commercial orchid production depends on flower number, color, symmetry, shelf life, and timing, heat-related reproductive damage has substantial economic consequences. A molecular strategy that protects reproductive tissues, rather than only leaves, would therefore be especially valuable.

HSP Families and Functions

HSPs are grouped according to approximate molecular mass, sequence relationships, subcellular localization, and chaperone activity. The principal plant families are small HSPs, HSP40, HSP60, HSP70, HSP90, HSP100, and HSP110. Their functions overlap but are not interchangeable.

Small Heat Shock Proteins

Small HSPs, also called HSP20s or sHSPs, generally range from approximately 12 to 40 kDa. They contain a conserved α-crystallin domain and variable N- and C-terminal regions. Most plant sHSPs function as ATP-independent holdases. They bind partially unfolded proteins during stress and prevent irreversible aggregation. The captured proteins can later be refolded by ATP-dependent chaperones such as HSP70 and HSP100 [34, 10].

Plant sHSPs are divided into cytosolic/nuclear and organelle-targeted groups, including chloroplast, mitochondrial, endoplasmic-reticulum, and peroxisomal forms. Their compartment-specific distribution is highly relevant to

orchids because heat simultaneously affects photosynthetic, respiratory, secretory, and reproductive tissues.

Small HSPs are often among the earliest and most strongly induced heat-responsive proteins. Their abundance may correlate with membrane stability, chlorophyll retention, improved photosystem II efficiency, and reduced protein aggregation. Recent transcriptomic work in Oncidium found that thermotolerance was particularly associated with strong induction of HSP20 genes, sometimes more prominently than canonical HSP70 and HSP90 genes (Luo *et al*., 2025) [5]. This finding supports the view that sHSPs may be especially suitable targets for orchid improvement.

HSP40 and HSP70

HSP40 proteins, also called J-domain proteins, act as cochaperones for HSP70. They recognize unfolded or misfolded substrates and stimulate the ATPase activity of HSP70. HSP70 proteins bind nascent, unfolded, or damaged polypeptides and participate in protein folding, intracellular transport, assembly, disaggregation, and degradation.

The HSP70 system is divided broadly into cytosolic, endoplasmic-reticulum, mitochondrial, and chloroplast forms. Cytosolic HSP70s are commonly induced by heat, whereas HSC70 proteins are expressed constitutively. HSP70 collaborates with HSP40, nucleotide-exchange factors, HSP90, and HSP100 to maintain proteostasis.

In orchids, heat-induced expression of HSP40 and HSP70 has been reported in Oncidium. Lin *et al*. (2014) [15] observed rapid induction of HsfA genes together with Hsp40 and Hsp70 during short-term exposure to 30 and 35°C. These results indicate that the HSP70 machinery is activated early during thermal stress and may participate in both stress protection and temperature-dependent developmental responses.

HSP90

HSP90 is an ATP-dependent chaperone that stabilizes signaling proteins, kinases, transcription factors, receptors, and other "client" proteins. It operates with cochaperones such as HSP70, HSP40, HSP90-organizing protein, p23, and immunophilins. HSP90 is present in the cytosol, nucleus, chloroplasts, and mitochondria.

The importance of HSP90 extends beyond protein folding. It regulates developmental signaling, hormone responses, immunity, chromatin remodeling, and the activity of HSFs. Under normal conditions, HSP90 and HSP70 can maintain HSFs in inactive complexes. Heat-induced protein damage diverts these chaperones toward unfolded proteins, allowing HSF activation and transcription of stress genes (Yoshida *et al*., 2011; Scharf *et al*., 2012) [36, 25].

In Dendrobium nobile, heat-stress studies identified strong upregulation of HSP90A and HSP-associated genes, particularly when heat stress was alleviated by exogenous calcium (Zhang *et al*., 2023) [38]. These observations suggest that HSP90 participates in a broader network involving calcium signaling, antioxidant metabolism, endoplasmic-reticulum protein processing, and autophagy.

HSP100 and HSP101

HSP100 proteins are ATP-dependent disaggregases. They cooperate primarily with HSP70 and HSP40 to reactivate aggregated proteins. HSP101 is especially important for acquired thermotolerance in plants. A brief priming heat treatment can induce HSP101 and related factors, enabling plants to survive a later, more severe heat episode.

The HSP100 system is important because prevention of aggregation is not always sufficient. Once proteins have formed aggregates, the plant must either refold or degrade them. HSP101 helps dissolve aggregates and return proteins to functional pathways. Although orchid-specific functional studies of HSP101 are scarce, evidence from other plants makes it a strong candidate for future work.

Regulation by Heat Shock Factors

HSP overexpression is normally controlled by HSFs. In plants, HSFs form a complex family with class A, B, and C groups. Class A factors generally act as positive regulators of heat-responsive genes, whereas class B factors can act as co-regulators or repressors depending on context. HSFs bind heat shock elements in promoters of HSP genes and activate transcription.

Under non-stress conditions, HSFs are maintained in relatively inactive states through interactions with HSP90, HSP70, and other regulatory proteins. Heat-induced protein unfolding increases the demand for chaperones and changes these interactions. HSFs then oligomerize, enter the nucleus, bind heat shock elements, and recruit transcriptional machinery.

The response is controlled by feedback. Newly synthesized HSP70 and HSP90 can suppress HSF activity after proteostasis is restored. This feedback prevents a costly and potentially harmful prolonged heat-shock program. Consequently, constitutive overexpression of a single HSP may not always produce continuous protection; it may also alter growth, development, and stress signaling.

In Oncidium, HsfA-1 and HsfA-2 were rapidly induced by moderate high-temperature treatment, together with Hsp40 and Hsp70 (Lin *et al*., 2014) [15]. Recent cultivar comparisons have also implicated HSFA2-centered regulatory networks in thermotolerance (Luo *et al*., 2025) [5]. These findings support two possible engineering approaches: direct overexpression of selected HSP genes or manipulation of upstream HSFs to activate a coordinated group of HSPs.

Direct HSP overexpression may be more predictable when a specific cellular compartment or developmental stage is targeted. HSF overexpression may produce broader protection but could also cause undesirable constitutive stress signaling. A rational strategy would therefore use tissue-specific or heat-inducible promoters.

Evidence from Orchid Species

Dendrobium Officinale

Dendrobium officinale is important as an ornamental and medicinal orchid. Its genome-wide HSP analysis identified 37 HSP20 genes, 43 HSP70 genes, and four HSP90 genes (Zhang *et al*., 2022) [37]. The identified genes were distributed among several subfamilies and predicted cellular compartments, indicating considerable functional specialization.

Many Dendrobium HSP genes responded to stress treatments. Although the study emphasized methyl jasmonate responses, the results are relevant to overexpression research because they demonstrate that HSP genes are responsive to signaling molecules in addition to heat. Several HSP20 genes showed especially strong induction, suggesting that they may be useful candidates for functional validation.

A limitation is that high transcript abundance does not necessarily mean high protein abundance or improved

thermotolerance. Post-transcriptional regulation, translation efficiency, protein stability, and subcellular localization must be examined. Nevertheless, the large number of HSP genes provides a valuable resource for selecting organelle-specific and stress-responsive candidates.

Dendrobium Nobile

Dendrobium nobile is sensitive to heat stress and has been used to investigate physiological and transcriptomic responses. Exogenous calcium improved growth, pigment retention, antioxidant capacity, and other indicators of heat tolerance. Transcriptomic analysis identified increased expression of HSPA1s, HSP90A, HSPBP1, and genes related to protein processing in the endoplasmic reticulum (Zhang *et al*., 2023) [38].

These findings are important for interpreting HSP overexpression. HSPs do not operate in isolation. Calcium treatment affected signaling, redox balance, protein processing, and autophagy, while HSP genes formed part of this integrated network. An orchid engineered to overexpress an HSP may therefore show the greatest benefit when combined with enhanced calcium signaling, antioxidant capacity, or protein-degradation pathways.

The study also illustrates the distinction between adaptive expression and engineered overexpression. Heat or calcium can naturally increase HSP transcript abundance, whereas a transgene may produce expression in tissues or developmental stages that would not normally activate the gene.

Oncidium

Oncidium orchids are useful models for studying the interaction between temperature and flowering. Short-term heat treatment rapidly induced HSF and HSP genes, including Hsp40 and Hsp70 (Lin *et al*., 2014) [15]. Prolonged elevated temperature also affected flowering-related redox pathways, particularly cytosolic ascorbate peroxidase.

A recent physiological and transcriptomic comparison of Oncidium cultivars showed that a thermotolerant cultivar had stronger upregulation of HSP20, HSP70, HSP90, antioxidant enzymes, and chloroplast-stabilizing genes than a heat-sensitive cultivar (Luo *et al*., 2025) [5]. The analysis highlighted HSP20 as a particularly prominent component of the tolerant response, with numerous small-HSP transcripts associated with improved physiological performance.

The Oncidium results support a model in which thermotolerance depends not on one “master” HSP but on coordinated expression of several HSP classes. Small HSPs may act rapidly to prevent aggregation, HSP70 may mediate folding and transport, HSP90 may stabilize signaling proteins, and HSP100 may reactivate aggregates. Together, these systems can protect chloroplasts, mitochondria, membranes, and the nucleus.

Phalaenopsis and Other Orchids

Phalaenopsis is one of the most commercially important orchids, but its temperature response is complex because heat affects both vegetative growth and flowering. Studies of orchid environmental physiology have established temperature ranges associated with vegetative maintenance and heat injury (Sakanishi *et al*., 1980; Baker & Baker, 1991) [23, 2]. However, direct stable overexpression experiments in Phalaenopsis HSP genes remain less developed than the physiological literature.

Other orchid genera also offer valuable genetic resources. Cymbidium, Vanda, Cattleya, and Paphiopedilum differ in habitat, photosynthetic physiology, leaf anatomy, and temperature preference. Comparative analysis of HSP promoters and alleles across these genera may identify natural variants associated with heat tolerance. Tropical orchids may possess HSP systems adapted to warmer environments, whereas cool-growing orchids may provide alleles useful for understanding stress sensitivity.

Functional Basis of HSP Overexpression

Protein Protection

The most direct benefit of HSP overexpression is stabilization of proteins under heat. Elevated temperature increases the probability that proteins will unfold or expose hydrophobic regions. HSP20s bind these intermediates and prevent aggregation, while HSP70 and HSP90 support refolding. HSP100 assists in disaggregation.

Overexpression can increase the capacity of this system. If a heat-sensitive orchid accumulates HSPs too slowly, a transgene with a strong heat-inducible promoter could produce protective chaperones before extensive damage occurs. This approach is particularly attractive for sudden heatwaves, during which rapid response is essential.

Protection of Photosynthesis

Heat-tolerant plants often maintain higher chlorophyll content and photosystem II efficiency. Chloroplast-localized sHSPs can stabilize thylakoid membranes and protect photosynthetic complexes. HSP70 and HSP90 also assist the import, folding, and assembly of chloroplast proteins.

In orchids, protection of photosynthesis is likely to influence carbohydrate availability, root growth, flower development, and recovery after stress. However, HSP overexpression should be evaluated alongside gas exchange, chlorophyll fluorescence, pigment composition, and photosynthetic recovery rather than inferred from transcript data alone.

Redox Regulation

Heat stress leads to excessive ROS production. HSPs can reduce oxidative damage indirectly by protecting antioxidant enzymes and directly by interacting with signaling components. HSP overexpression has been associated in various plants with increased superoxide dismutase, catalase, ascorbate peroxidase, and glutathione-related activity (Hasanuzzaman *et al*., 2013; Bita & Gerats, 2013) [9, 3].

The relationship is bidirectional. ROS can induce HSP expression, and HSPs can modify ROS accumulation. A successful orchid HSP line should therefore be assessed for hydrogen peroxide, malondialdehyde, electrolyte leakage, antioxidant enzyme activity, and reduced-to-oxidized glutathione balance.

Membrane and Water Relations

HSP20s may associate with membranes and help preserve their structure during heat. Stabilized membranes reduce ion leakage and maintain cellular compartmentalization. HSP overexpression may also improve water retention indirectly by protecting aquaporins, stomatal regulators, and metabolic enzymes.

For epiphytic orchids, water relations are particularly important. Heat combined with low humidity creates a compound stress that may not be solved by chaperone accumulation alone. HSP overexpression should therefore be

evaluated under realistic combinations of high temperature, vapor-pressure deficit, light intensity, and water availability.

Recovery and Acquired Thermotolerance

Heat tolerance includes both basal thermotolerance and acquired thermotolerance. Basal thermotolerance is the ability to withstand heat without prior acclimation. Acquired thermotolerance develops after exposure to a mild priming temperature.

HSP101, HSP70, HSP90, and sHSPs contribute to acquired thermotolerance. An orchid engineered to express an HSP constitutively may show improved survival during acute heat, but inducible expression may be more efficient for repeated or seasonal stress. Priming treatments involving mild heat, calcium, salicylic acid, jasmonates, or beneficial microorganisms could be combined with genetic enhancement.

Lessons from Heterologous Overexpression

Direct orchid transformation is technically challenging and relatively slow. Consequently, evidence from other plants helps identify promising genes and mechanisms.

Constitutive expression of AtHSP101 improved heat tolerance in plant systems and protected reproductive tissues in transgenic crops (Agarwal *et al*., 2003; Queitsch *et al*., 2000) [1, 21]. Similarly, overexpression of rice HSP70 enhanced heat tolerance, while expression of rice HSP20 increased heat and salt tolerance in multiple organisms (Qi *et al*., 2011; Guo *et al*., 2020) [22, 8]. A tea small HSP, CsHSP17.2, improved thermotolerance when expressed in Arabidopsis, increasing survival, photosynthetic performance, compatible solutes, and antioxidant responses (Sarkar *et al*., 2017) [24].

These studies provide several principles for orchids:

- HSPs can function across species boundaries because their chaperone activities are evolutionarily conserved.
- Small HSPs may provide strong protection with relatively simple molecular architecture.
- HSP overexpression can improve heat tolerance partly by activating secondary antioxidant and stress-response genes.
- The promoter is as important as the coding sequence.
- Constitutive expression may cause growth or developmental costs.
- Functional validation should include both acute heat and prolonged, fluctuating heat regimes.

Heterologous studies cannot, however, prove that an HSP will function identically in orchids. Orchids differ in genome structure, epigenetic regulation, tissue culture behavior, photosynthetic physiology, and developmental timing. An HSP that is effective in *Arabidopsis* may be poorly expressed, mislocalized, or metabolically costly in an orchid.

Strategies for Orchid HSP Engineering

Candidate Selection

Candidate genes should be selected using multiple criteria:

1. Strong and reproducible heat induction.
2. Association with naturally heat-tolerant orchid cultivars.
3. Appropriate subcellular targeting.
4. Demonstrated chaperone activity.
5. Low expression cost under non-stress conditions.
6. Compatibility with orchid transformation systems.
7. Conservation of regulatory motifs and protein domains.

HSP20 candidates are particularly attractive because they are often strongly heat induced and can act as first-line aggregation preventers. HSP70 and HSP90 candidates may provide broader proteostasis and signaling functions. HSP101 is a logical target for recovery from severe stress.

Promoter Choice

Constitutive promoters such as CaMV 35S are widely used, but they may produce unnecessary expression during normal growth. A heat-inducible promoter could reduce fitness costs and increase expression only when needed. Orchid-specific promoters could further restrict expression to leaves, roots, meristems, pollen, or developing flowers.

Tissue-specific expression may be especially valuable. For example, reproductive-tissue expression could protect pollen and floral organs while avoiding excessive expression in vegetative tissues. Chloroplast-targeted HSPs could be expressed primarily in photosynthetic organs, whereas cytosolic HSPs may be useful throughout the plant.

Transformation Systems

Orchid transformation commonly involves *Agrobacterium*-mediated transformation of protocorm-like bodies, embryogenic callus, or other regenerable tissues. Particle bombardment and transient expression systems may also be used. The major challenges are genotype dependence, low transformation efficiency, somaclonal variation, prolonged regeneration, and difficulty maintaining stable transgene expression.

For commercially valuable orchids, transformation protocols should include molecular confirmation, copy-number analysis, expression profiling, phenotypic stability, and multi-generation evaluation. Because orchids may be clonally propagated, a stable elite line could potentially be multiplied efficiently, but regulatory and market acceptance issues remain important.

Genome Editing

CRISPR/Cas systems could be used to alter HSP promoters, activate endogenous HSP genes, remove negative regulatory elements, or modify HSF-HSP feedback. Gene activation systems such as CRISPRa may offer advantages over random transgene insertion because they can increase expression of native genes while preserving endogenous targeting sequences.

Editing should be designed carefully. Complete loss of an HSP may be lethal or produce broad developmental effects. Promoter editing or partial modulation may be more appropriate than gene knockout. Multiplex editing could target several HSP20 genes, but redundancy and off-target effects must be evaluated.

Limitations and Risks

Correlation is Not Causation

Many orchid studies measure HSP transcript changes after heat treatment. Such changes are useful indicators but do not establish that HSP expression causes tolerance. The induced transcript may be a marker of injury rather than a protective factor. Functional tests should include overexpression, silencing, protein localization, chaperone assays, and complementation.

Growth Penalties

HSPs require substantial cellular resources. Constitutive

accumulation may divert energy and amino acids from growth, flowering, fragrance production, or storage metabolism. It may also interfere with normal protein turnover and signaling. For orchids grown under non-stress conditions, these penalties could outweigh the benefit of improved heat resistance.

Network Effects

HSPs interact with HSFs, hormone pathways, calcium signaling, ROS, autophagy, ubiquitin-mediated degradation, and the unfolded-protein response. Excess HSP90, for example, may influence developmental and immune signaling. HSP overexpression should therefore be evaluated using transcriptomics, proteomics, metabolomics, and physiological phenotyping.

Environmental Complexity

Commercial greenhouses expose orchids to changing temperature, humidity, light, irrigation, nutrient supply, and pathogens. A gene that improves tolerance to constant high temperature may not improve performance during fluctuating heat and drought. Testing should include realistic environmental scenarios and long-term flowering trials.

Biosafety and regulation

Genetically modified orchids may raise concerns about pollen flow, persistence, ecological interactions, and consumer acceptance. Many commercial orchids are propagated clonally, which may reduce gene flow but does not eliminate ecological considerations. Genome-edited plants may be regulated differently across jurisdictions, and regulatory status can change over time.

Future Directions

Future research should move from descriptive expression studies toward mechanistic and translational work.

First, complete orchid pangenomes should be used to identify natural HSP alleles, promoter variants, copy-number differences, and structural changes associated with heat tolerance. Comparing tolerant and sensitive cultivars within the same species will help distinguish adaptive variation from species-specific background effects.

Second, protein-level studies are needed. Transcript abundance does not reliably predict HSP accumulation or activity. Quantitative proteomics, immunoblotting, thermal proteome profiling, and protein-aggregation assays should be combined with gene-expression measurements.

Third, subcellular localization should receive greater attention. Chloroplast, mitochondrial, endoplasmic-reticulum, nuclear, and cytosolic HSPs may have different effects. A highly expressed HSP that is incorrectly localized may provide little protection.

Fourth, inducible and combinatorial approaches should be developed. A promising design might combine a heat-inducible small HSP with HSP101 or HSP70, while simultaneously enhancing antioxidant capacity. Another option would be to activate HSFA2 only during heat exposure, thereby inducing the plant's endogenous HSP network.

Fifth, reproductive thermotolerance should become a major target. Experiments should measure pollen viability, pollen-tube growth, capsule formation, seed germination, floral symmetry, and postharvest longevity. Vegetative survival alone is insufficient for evaluating commercial value.

Sixth, researchers should test HSP-engineered orchids under compound stress. Heat frequently occurs with drought, high light, salinity, or pathogen pressure. The most useful genotype will be one that maintains growth and flowering under combined stress rather than one that survives a short laboratory heat shock.

Finally, non-transgenic approaches should be integrated with molecular breeding. Heat priming, calcium nutrition, beneficial microbes, controlled irrigation, shade management, and selection of naturally tolerant cultivars may complement HSP-based improvement. In Dendrobium nobile, calcium treatment was associated with improved physiological performance and increased expression of HSP-related genes, illustrating the potential of combining cultural and molecular approaches (Zhang *et al*., 2023) [38].

Conclusion

HSPs are central components of orchid thermotolerance. They protect proteins from unfolding and aggregation, support recovery, stabilize membranes and photosynthetic machinery, and interact with antioxidant, calcium, hormone, autophagy, and developmental pathways. Orchid research has established stress-responsive HSP repertoires in Dendrobium, rapid HSP activation in Oncidium, and strong associations between small HSP expression and cultivar-level heat tolerance. However, direct stable overexpression studies in orchids remain limited, and much of the current evidence is based on transcript accumulation rather than functional genetic validation. The strongest future strategy is unlikely to be constitutive overexpression of one HSP. Instead, orchid improvement should emphasize inducible, tissue-specific, and carefully balanced manipulation of HSP20, HSP70, HSP90, HSP101, and their HSF regulators. Functional studies must measure protein abundance, localization, chaperone activity, photosynthesis, oxidative damage, growth, flowering, reproduction, and recovery under realistic environmental conditions. With advances in orchid genomics, transformation, genome editing, and controlled-environment phenotyping, HSP engineering could become an important component of climate-resilient orchid production.

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