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Review article

Heat stress phenotyping: A key for plant selection in a warming climate

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ABSTRACT

Climate change, through rising temperatures and more frequent heat waves, threatens food security and agricultural sustainability. Heat stress is a major abiotic factor that negatively affects plant function and crop yields. Advanced phenotyping strategies are critical for developing heat-resilient crops and mitigating the impacts of climate change. This review offers an updated perspective on plant phenotyping under heat stress, providing practical guidance for researchers. It discusses key trial sites, modern phenotyping techniques, and the physiological and molecular mechanisms that plants employ in response to heat stress. Special emphasis is placed on plant plasticity and its role in adaptive responses to stress. The review further examines advanced phenotyping technologies and sensors used across various environments, both field-based and controlled, and at multiple scales, from cellular to aerial. It highlights a range of approaches, from high-tech to cost-effective solutions, aimed at enhancing accessibility. Additionally, it underscores the importance of improved data management, standardized protocols, and alignment with international best practices in heat stress phenotyping. Finally, the review presents global initiatives focused on advancing plant phenotyping for heat stress research, emphasizing the critical role of international collaboration in addressing this urgent challenge.

1. How hot is the last decade, the effects of heat stress on plants

Climate change has led to a significant increase in global average temperatures, rising by about 1.1°C since pre-industrial times (https://climate.ec.europa.eu/climate-change/causes-climate-change_en). The intensity, frequency, and duration of heatwaves have been rapidly increasing around the globe (Perkins-Kirkpatrick and Lewis, 2020). Nineteen of the 20 warmest years on record have occurred since 2000. It is predicted that the trend of rising temperatures, along with more frequent, prolonged, and intense extreme heat events, will continue in the future (IPCC, 2023).

Summer 2024 recorded some of the highest temperatures in recent years. The average land temperature for Europe in August 2024 was 1.57°C above the 1991–2020 average for August, making it the second warmest August on record for Europe, after August 2022, which was 1.73°C above average (https://climate.copernicus.eu/copernicus-summer-2024-hottest-record-globally-and-europe).

According to the JRC MRS Bulletin-Crop Monitoring in Europe, released in July 2023, the forecasted yield for most crops in Europe remains at or slightly above the five-year average. In several regions, negative impacts of limited soil-water availability for crops were exacerbated by unusually warm temperatures and high radiation. Southern parts of the Iberian Peninsula and northern Italy experienced intense heatwaves, increasing the risk of heat-induced sterility for flowering summer crops, with impacts on yields that may be irreversible. Rainfall surplus delayed harvesting, and potentially reduced grain quality in western parts of Bulgaria, Romania, Slovenia, Croatia, and Hungary (Baruth et al., 2023).

The increasing number of hot days and intensified soil evaporation will lead to the expansion of arid and semi-arid areas, causing agricultural food production to rely more heavily on irrigation (Palmgren and Shabala, 2024).

Heat stress significantly affects crop production through a variety of complex physiological mechanisms and the temporal dynamics of high-temperature events, as well as their interactions with specific phenological stages (Janni et al., 2020; Sadok and Jagadish, 2020; Sadok et al.,

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2021; Palermo et al., 2024). Key physiological mechanisms are strongly hampered by heat stress.

Among the most impacted processes, photosynthesis is significantly reduced due to impaired stomatal ~~opening~~ closure, a process controlled by specialized guard cells that regulate gas exchange in plants. This leads to a disconnect between stomatal conductance and photosynthetic assimilation of CO_2 (Pankasem et al., 2024). If heat stress occurs during the reproductive stage, it can impair pollen viability and reduce fertilization rates, leading to reduced grain or fruit set. This is particularly detrimental in cereals like wheat and maize, where grain yield can drastically decline (Zenda et al., 2022), but also in tomatoes (Ouonkap et al., 2024). Furthermore, prolonged exposure to high temperatures can lead to protein denaturation and enzyme inactivation, which affects cellular metabolism. This results in physiological disorders and reduced growth (Bokszczanin et al., 2013; Mishra et al., 2023). Elevated temperatures can accelerate leaf senescence, which in turn reduces the photosynthetic capacity of crops and limits biomass production. This has a direct impact on yields, especially in crops that rely on extended growth periods for yield formation, such as rice and wheat (Farhad et al., 2023). Moreover, heat stress can negatively affect the nutritional quality of crops. Protein content in cereals like wheat tends to decrease under heat stress, while oil crops may produce lower-quality oil (Kashyap et al., 2024).

Under normal growth conditions, the synthesis and degradation of chlorophyll (Chl) are balanced, and its levels remain stable (Rossi et al., 2017; Wang et al., 2018; Hu et al., 2020). The decline in Chl levels is largely driven by the activation of chlorophyllase and Chl-degrading peroxidase, which accelerate Chl degradation under heat stress (Wang et al., 2018). Interestingly, Chl degradation can also serve as a protective mechanism, as excessive light energy absorbed by Chl can threaten photosynthesis. In contrast, maintaining Chl content during post-flowering heat stress has been shown to mitigate yield losses (Fu et al., 2022).

Further emphasizing the importance of Chl retention, delayed senescence has been identified as a key strategy to prolong the photosynthetic capacity of leaves under stress. Roy et al. (2021) demonstrated that delaying leaf senescence enhances the longevity of uppermost leaves, enabling extended translocation of photosynthates into grains and maintaining greenness for longer durations under heat stress. These findings highlight the interconnectedness of physiological processes such as Chl stability and plant resilience under adverse conditions.

It is also well established that transpiration is strongly increased by heat stress (Sadok et al., 2021; Huang et al., 2022; Palermo et al., 2024). However, recent studies have described a strong link between transpiration, vapor pressure deficit (VPD) and stomatal closure (Sadok et al., 2021), along with insights into the dynamics and relationship between evapotranspiration and transpiration (Yang et al., 2023). The temporal dynamics of water availability for crop use are largely dictated by stomatal sensitivity to atmospheric VPD, which reflects a change in the slope of the transpiration rate response to increasing VPD (Sadok et al., 2021). High-temperature stress increases the transpiration rate through processes that are generally distinct from those governing responses to VPD. While the underlying mechanisms are complex and not yet fully understood, they appear to converge on increasing transpiration through either purely physical processes (e.g., changes in water viscosity or melting of cuticular waxes) or regulatory physiological mechanisms that enable stomatal opening, such as transmembrane water fluxes mediated by K^+ transport, aquaporin activity, or membrane fluidity and selectivity (Cochard et al., 2000; Sadok et al., 2021).

Moreover, Yang et al. 2023 demonstrated that varying durations of heat stress at different growth stages had different effects on transpiration and evapotranspiration. In particular, the authors hypothesize that heat stress negatively affects transpiration due to its adverse impact on plant growth and productivity, while it enhances evaporation due to the increased vapor pressure deficit. Short-term heat stress affects hourly transpiration, which decreases significantly in the middle hours of the day due to limitations in stomatal amplitude and conductance. When plants experience heat stress, it triggers a water-saving mechanism, leading to partial stomatal closure and reduced stomatal conductance (Asseng et al., 2015), thereby preventing excessive water loss. Heat stress also imposes profound effects on plant reproduction, contributing to yield reduction. Elevated temperatures disrupt floral development, resulting in smaller flowers, structural abnormalities, or even sterility (Resentini et al., 2023; Tushabe et al., 2023). Fertilization and embryo development are particularly vulnerable, often resulting in reduced seed production. Resentini et al. (2023) Resentini et al., 2023 provided insights into the molecular mechanisms of heat stress resilience during reproductive development, comparing Arabidopsis and *Oryza sativa* as model systems. Among these mechanisms, heat stress has been shown to modulate the activity of calcium (Ca^{2+})-permeable channels, inducing transient cytosolic Ca^{2+} increases that activate heat shock factors and other proteins involved in the heat stress responses (Kour et al., 2023; Kang et al., 2024).Together, these findings highlight the multifaceted impacts of heat stress on plant physiology, spanning Chl stability, water dynamics, and reproductive success. Addressing these challenges requires an integrated understanding of molecular, physiological, and ecological processes to develop strategies for enhancing crop resilience under rising global temperatures. High temperatures cause the production and accumulation of reactive oxygen species (ROS), which are reactive forms of molecular oxygen (O_2), including singlet oxygen ($^1O_2$), superoxide anion ($O_2\bullet^-$), hydrogen peroxide (H_2O_2), and hydroxyl radical ($HO\bullet$). ROS accumulation induces oxidative damage to biomolecules such as pigments, proteins, lipids, carbohydrates, and DNA, ultimately resulting in cellular injury and cell death (Fortunato et al., 2023). Palermo et al., 2024 described a set of natural durum wheat small Heat Shock Protein 26 (*TdHsp26)* mutants, in response to short-term heat stress at two different phenological stages: seedling and tillering. In this study, two contrasting genotypes were identified. One of them, SSD69, exhibited altered accumulation of H_2O_2 and malondialdehyde (MDA) under both conditions, providing new insights into the oxidative response to heat stress. Heat stress causes oxidation of lipids and proteins due to reactive oxygen species (ROS) generation during early plant responses to abiotic stresses. Lipid peroxidation leads to the continuous production of MDA, a key marker of oxidative damage and, consequently, of heat stress.

In Palermo et al. (2024), MDA was used as an index to assess the tolerability and susceptibility to heat stress of various durum wheat genotypes from a germplasm collection. Specifically, MDA levels were analyzed at the seedling and tillering stages under short-term heat stress at four time points: before stress (23°C), four hours after stress (38°C), one hour after recovery (23°C) and one week post stress (Palermo et al., 2024). In addition, continuous heat stress was applied from the seedling stage until the end of the vegetative cycle, with MDA measured one week after flowering (Palermo et al., under submission).

All experiments demonstrated that MDA exhibited distinct accumulation patterns, varying according to both the genotype and the specific plant organ analyzed (ear or leaf). Notably, stress-tolerant genotypes consistently accumulated lower MDA levels than susceptible ones. Heat stress can also significantly affect the primary and secondary metabolism of plants (Dorado et al., 2023). A clear example is observed in chestnut seedlings, where alterations in primary metabolism were found mainly above ground, including starch depletion and increases in soluble sugars, nitrogen, and proline. In contrast, changes in the secondary metabolism were observed below ground and included elevated levels of total phenolic, ortho-phenolic, and flavonoid content (Dorado et al., 2023). These data support the importance of understanding plant plasticity and the mechanisms triggered during adaptation to varying weather conditions across different climate zones, as these responses can vary significantly across crops species (Heino et al., 2023). A key factor in developing heat-tolerant varieties is plant phenotyping, which, despite significant advances in plant genomics, remains a major bottleneck in breeding programs (Yeh et al., 2012; Janni and

Pieruschka, 2022).

Heat stress in plants triggers a complex array of molecular responses that disrupt cellular homeostasis, leading to significant physiological impairments. These responses, including the activation of heat shock proteins, changes in metabolite profiles, and modifications in gene expression, contribute to reduced growth, diminished photosynthetic efficiency, and overall declines in plant productivity. Early detection of heat stress symptoms is therefore critical for mitigating adverse effects and for identifying novel sources of stress tolerance. in this context, the development of high-throughput and precise phenotyping technologies is essential for monitoring heat stress-related traits, thereby supporting pre-breeding strategies aimed at enhancing stress resilience under future climate extremes.

2. Where to conduct heat stress phenotyping experiments

The increased demand for plant phenotyping was assessed through a survey conducted in 2018 by the pan-European Research infrastructure EMPHASIS (*European Infrastructure for multi-scale Plant Phenomics and Simulation for food security in a changing climate*), aimed at better understanding the need of phenotyping users. Among 320 respondents from across the globe, 49 % reported applying heat stress in their research, followed by drought stress (63 %), nutrient stress (47 %), biotic stress (39 %), light stress (24 %), and chemicals stress (22 %) (https://zenodo.org/api/records/4723409/files-archive).

The survey also highlighted the most commonly used phenotyping techniques. Imaging methods, such as RGB, spectral and thermal imaging, were the most utilized, accounting for 53 % of cases. Low-cost techniques were employed in 19 % of cases, followed by free software analysis (13 %), and custom-built systems (11 %).

To effectively address user needs, it is important to identify where medium-scale heat stress phenotyping experiments can be conducted. However, it remains challenging to locate facilities that are well-equipped for heat stress experimentation. In this context, we highlight key infrastructures that specialize in heat stress phenotyping.

EMPHASIS has mapped and made available a database of field and controlled environment facilities across Europe (https://emphasis.plant-phenotyping.eu/phenotyping-landscape/infrastructure-map). However, the platform does not currently support targeted searches for facilities dedicated specifically to heat stress experiments. The AgroServ Catalogue of Services, an EU-funded initiative offering infrastructural support in agriculture and agroecology, which also includes EMPHASIS services, enables refined searches for phenotyping infrastructures and platform offering dedicated abiotic and biotic stresses (https://catalog.isia.cnrs.fr/catalog/2/services).

3. The phenotyping scale of measurements

Over the last decades, with the breakthrough in information and communication technologies, digital techniques for heat stress plant phenotyping have been significantly optimized to ensure high-resolution and precise monitoring of plant phenotypic parameters (Sun et al., 2022). Phenotypic sensors are primarily used to obtain crop phenotypic characteristics and are often installed on Unmanned Aerial Vehicles (UAVs). These sensors may include RGB, multispectral, hyperspectral, thermal infrared imaging camera, fluorescence sensor, and LIDAR (Feng et al., 2021).

These sensors serve various applications, which can vary depending on the scale of analysis required (Tripodi et al., 2018).

Several phenotyping scales, defined by the distance between the sensor and the plant, can be identified in heat stress plant monitoring (Tripodi et al., 2018; Vurro et al., 2023a). Based on the classification proposed by Dhondt et al., 2013, Tripodi and colleagues introduced a framework based on two criteria: depth (resolution) and processivity

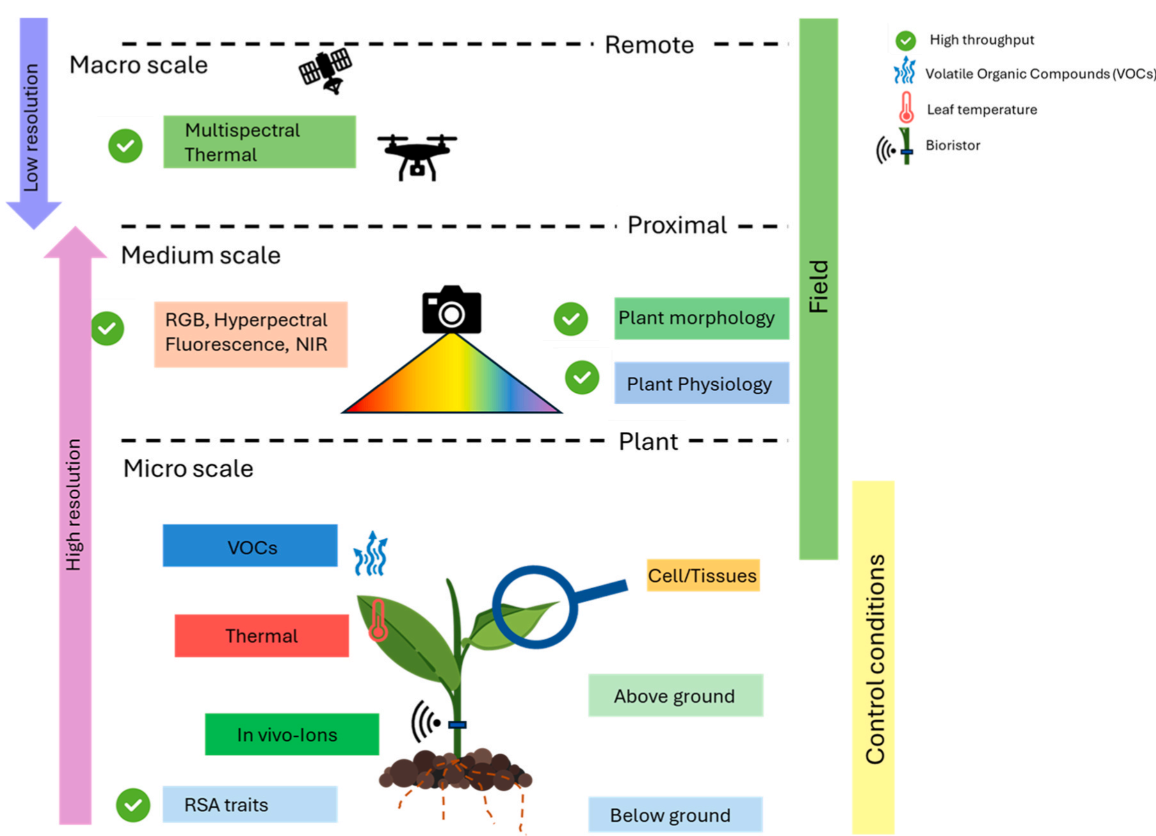

Fig. 1. Scheme of the phenotyping scale in heat stress phenotyping according to their resolution and field of application. RGB, Red, Green and Blue imaging; VOCs, Volatile Organic Compounds; RSA, Root System Architecture.

(throughput) (Fig. 1). High-throughput techniques generally involve whole-plant analysis at medium-to-low resolution. This is typical of field phenotyping platform used for resource management and crop monitoring (e.g., nutrient and water distribution, weed infestation, disease spread) allowing the screening of hundreds of plants per day in a non-destructive and automated manner (Dhondt et al., 2013). In contrast, high-resolution techniques are applied at organ, whole plant, or cellular levels, with the latter typically requiring invasive methods (e.g., metabolomics) which are not fully automatable due to extensive sample preparation and inherently low throughput (D'hondt et al., 2018).

An alternative classification of phenotyping scale, also applicable to heat stress monitoring, was proposed by Vurro et al., 2023a. They described three key scales: 1. Micro-plant scale, applicable in open fields, employing *in vivo* sensors such as the Bioristor (Janni et al., 2019; Vurro et al., 2023a,b); 2. Medium-proximal scale, which uses image-based phenotyping techniques close to the plant canopy (approximately 60 cm above), typically involving low-cost imaging solutions (Gracia-Romero et al., 2017, 2019; Vurro et al., 2023b); 3. Macro scale, which involves remote, multispectral image-based analysis (Fig. 1).

In addition, molecular phenotyping has been introduced as a distinct phenotyping scale in crops such as grapes and wheat. This includes approaches like transcriptomics, proteomics, metabolomics, and lipidomics, and may be further refined to single-cell phenotyping, where the effects of mutations are examined at the individual cell level (Schiefelbein, 2015; Carvalho et al., 2021; Palermo et al., 2024).

4. Cellular Phenotyping: tools and insights in plant tissue analysis

Recent advances in cellular-level phenotyping have largely focused on epidermal tissues of vegetative shoots, owing to their accessibility and relatively simple structure. These characteristics make surface area a practical and quantifiable metric for assessing cell dimensions (Zuch et al., 2022). Traditional techniques, such as leaf epidermal peels, varnish imprints, and tissue clearing, combined with microscopy, remain widely used for measuring cell size and number. However, modern image analysis algorithms now allow for the automated identification of epidermal cell types and precise quantification of individual cell dimensions, based on both microscopic images and computational models (Mele and Gargiulo, 2020; Zubairova et al., 2024). Integrating these methods with computational modeling has uncovered patterns of cell division and expansion and revealed correlations between cellular features and organ-level traits, as demonstrated in recent plant imaging studies (Trozzi et al., 2023, Preprint; Maraveas, 2024).

Advancements in imaging technologies have also expanded phenotyping capabilities beyond epidermal tissue, particularly using confocal microscopy. In *Arabidopsis thaliana* roots, non-invasive imaging provides high-resolution analysis of cellular features across various focal planes, enabling studies beyond surface layers (Ovečka et al., 2022). When combined with fluorescent protein markers that target subcellular structures, live imaging allows real–time insights into dynamic processes such as cell division, elongation and expansion (Ovečka et al., 2022). Beyond roots, these methods have significantly contributed to understanding shoot meristem development and anisotropic cell expansion. In particular, this approach proved effective in elucidating *Arabidopsis* root growth dynamics and cell cycle behavior under combined iron deficiency and transient heat stress, through time-lapse tracking of CYCLINB1;1-GFP, a marker for mitotic entry (Buckner et al., 2019). However, the application of these methods to thicker tissues, like leaves and crop roots, remains challenging due to increased tissue density. Nonetheless, they offer valuable insights into tissue-specific responses to heat stress.

For deeper structures, such as the mesophyll and vascular layers, rigorous tissue fixation, clearing, and staining protocols are required to enable imaging with confocal or multiphoton microscopy (Wuyts et al., 2010).

Recent software advancements have made it possible to generate 3D reconstructions of leaf tissues, facilitating volumetric analysis of cell expansion across multiple layers (Zhang and Ambrose, 2024).

Despite these technological gains, sample preparation, measurement accuracy, and imaging throughput remain major bottlenecks. Emerging technologies, including advanced sensors and novel experimental platforms, aim to overcome these limitations by increasing throughput and enhancing data dimensionality. A particularly promising strategy involves the isolation of specific cell types for high-resolution molecular profiling (Cuperus, 2022). This method has proven valuable in identifying genetic and environmental influences on cellular phenotypes, even in cases where no macroscopic changes are observable at the organ level (Lim et al., 2024). This cell-type–specific molecular analysis strengthens forward genetic studies and gene function characterization. For example, it enables deeper insights into Ca^{2+} signaling pathways, which play a pivotal role in regulating plant responses to environmental stimuli and developmental cues (Grenzi et al., 2021; Resentini et al., 2021).

5. Medium scale, controlled conditions

High throughput phenotyping infrastructures under controlled conditions are classified as medium-scale phenotyping facilities.

At the medium scale, RGB-based imaging, multispectral and near-infrared (NIR)-imaging, plays a significant role in monitoring crops such as wheat and maize under heat stress (Fig. 1) (Ryu et al., 2023). These imaging techniques have demonstrated performance that is comparable to, or even exceeds, traditional multispectral indices such as the Normalized Difference Vegetation Index (NDVI) for predicting key crop traits, particularly grain yield, under varying environmental conditions, including differences in water availability (Gracia-Romero et al., 2017, 2019; Fernandez-Gallego et al., 2019; Vurro et al., 2023a).

Soil monitoring sensors also play a central role in the phenotyping sensor landscape due to the critical role of soil in plant development, crop yield, and water resource optimization, all of which are directly influenced by heat stress (Polinova et al., 2019; Yin et al., 2021). High-throughput technologies have been widely applied to study heat-induced changes in both plant morphology and physiology, in model species such as *Arabidopsis thaliana* (Gao et al., 2020) and in agronomically important crops (Correia et al., 2022; Bhardwaj et al., 2024; Kundu et al., 2024; Zhao et al., 2024).

Additional studies on plant phenotyping under heat stress conditions are summarized in Table 1.

6. Macro scale, field phenomics

The field of heat stress plant phenotyping has seen significant advancements in recent years, driven by breakthroughs in high-throughput technologies, sensor innovations, and advanced data analytics (Fig. 1). These developments are crucial for breeding climate-resilient crops capable of maintaining productivity under increasingly warmer climates. Addressing the ongoing challenges posed by heat stress in agriculture will require sustained interdisciplinary research and continued technological innovation (Reynolds et al., 2020; Araus et al., 2022; Morisse et al., 2022; Vurro et al., 2023a).

Applications of digital phenotyping range from monitoring individual plant cells in controlled environments to satellite-based remote sensing at the canopy level. These efforts are supported by a variety of ground-based and mobile platforms, including gantries, agricultural vehicles, drones, and sensor technologies such as LIDAR, RGB cameras, and hyperspectral or multispectral devices. Proximal and remote sensing (PRS) techniques are increasingly used due to their efficiency in acquiring and analyzing multidimensional datasets (Tao et al., 2022).

At the macro scale, rapid advancements in sensor technologies, UAVs, image-processing algorithms, and computational capacity have

Table 1
List of the European Infrastructure performing heat stress phenotyping (Agro-Serv catalogue, https://agroserv.eu/research-services/explore-our-catalogue).

Platform/Faciity	European Research Infrastructure	National node, country
High-throughput phenotyping capability for plant research - PHENOPlant	Euro-BioImaging	Vienna BioCenter Core Facilities GmbH - Plant Sciences Facility, Austria
Integrated Plant Phenotyping for Agricultural Solutions (IPPAS)	EMPHASIS	VIB Agro-Incubator, Belgium
Phenoarch	EMPHASIS	INRAE Montpellier, France
PlantArray	EMPHASIS	Hebrew University of Jerusalem, Israel
Scanalyzer	EMPHASIS	Alsia, Matera, Italy
INFRA-VOL	EMPHASIS	University of Naples Federico II, Italy
FieldPhen	EMPHASIS	CREA-Foggia, Italy
PhenoCroP	EMPHASIS	ITQB NOVA, Portugal
Plant&Soil Pheno	EMPHASIS	Institute of Field and Vegetable Crops, Novi Sad, Serbia
JKI-FACE and Rainout-Shelter	EMPHASIS	Julius Kühn Institute (JKI), Germany
BreedFACE	EMPHASIS	Forschungzentrum Jülich (FZJ)
PhotoTraits	EMPHASIS	Forschungzentrum Jülich (FZJ)
ENEA Calliope - Gamma irradiation tests	METROFOOD-RI	ENEA, Italy
Monitoring and manipulation of environmental conditions to study plant responses	AnaEE-ERIC	CIHEAM-Bari, Italy
Optimal Agricultural Management: The Power of Drones in Precision Agriculture	AnaEE-ERIC	CIHEAM-Bari, Italy
Free Air Temperature Increase (FATI)	AnaEE-ERIC	University of Antwerp, Belgium

enabled diverse applications in aerial precision agriculture. These include measuring vegetation indices to assess heat stress severity in crops (Gill et al., 2022). Examples of such indices include those used to evaluate stress responses, as demonstrated in recent studies (Park et al., 2021; Fumia et al., 2023; Sharma et al., 2024).

7. Sensors and technologies for plant phenotyping

High-throughput phenotyping enables rapid assessment of large plant populations to identify traits linked to heat tolerance. Recent advances in imaging technologies, robotics, and automated data processing have significantly boosted the efficiency and accuracy of phenotypic evaluations. Sensor technologies have revolutionized phenotyping by enabling precise measurements of critical physiological traits, such as chlorophyll fluorescence and leaf temperature, that serve as early indicators of heat stress responses.

The integration of machine learning and advanced data analytics with phenotypic datasets has further enhanced the ability to interpret complex trait patterns and predict plant performance under heat stress conditions. These methodologies are particularly effective for managing the vast amount of data generated by high-throughput, precise, and automated approach to assessing phenotypic traits associated with heat tolerance (Tripodi et al., 2018; Pettenuzzo et al., 2022) (Fig. 2).

Heat stress phenotyping has greatly benefited from the integration of various cutting-edge technologies, as detailed in Fig. 2 and Fig. 3. These tools enable accurate, high-throughput assessment of heat tolerance traits and advance our understanding of plant responses to abiotic stress.

8. RGB imaging

It remains a cornerstone of high-throughput phenotyping (HTP) due to its accessibility and efficiency in capturing high-resolution images (Solimani et al., 2024). This approach is highly effective for measuring growth-related traits, such as canopy size, in one- and two-dimensional space, offering an accurate assessment of plant growth status (Rossi et al., 2022). Public repositories, such as *Quantitative Plant* (https://quantitative-plant.org), provide access to extensive datasets for further analysis.

8.1. Thermal cameras and infrared imaging systems

They are widely used to monitor plant temperature as a proxy for stomatal conductance and transpiration rates, key indicators of heat stress. Lower leaf temperatures often indicate improved cooling through transpiration, a trait associated with heat tolerance (Wen et al., 2023; Gano et al., 2024; Palermo et al., 2024). These technologies provide valuable insights into plant water-use efficiency under heat-stress conditions.

8.2. Hyperspectral sensors

They capture a broad spectrum of wavelengths, allowing detailed analysis of plant pigments, water content, and structural traits. This technology is especially valuable for detecting early signs of heat stress through subtle changes in leaf reflectance and chlorophyll fluorescence. Hyperspectral imaging has been applied in diverse environments to assess abiotic and biotic stress traits and plant quality (Costa et al., 2021; Faqeerzada et al., 2023; Sherstneva et al., 2024).

8.3. Light Detection and Ranging (LiDAR)

LiDAR provides precise 3D structural information about plant architecture. It is commonly used to measure morphological changes, such as leaf angles and plant height, in response to heat stress. These insights contribute to quantifying heat stress effects on growth patterns, canopy size, and overall productivity (Jin et al., 2021; Forero et al., 2022; Rincón et al., 2022).

8.4. Fluorescence imaging

It measures Chl fluorescence, a key indicator of photosynthetic efficiency. Heat stress often disrupts photosynthesis, particularly in photosystem II (PSII), and fluorescence imaging enables the detection of such impairments at an early stage (Cheng et al., 2025).

Automation has revolutionized large-scale phenotyping. Robotic platforms and field phenotyping systems integrate sensors (thermal, hyperspectral, fluorescence) to collect data from thousands of plants efficiently, either in controlled environments or field conditions (Milella et al., 2014, 2024; Xu and Li, 2022).

Recent advances in high throughput phenotyping allowed the collection of large datasets following plant growth and development over time. Machine Learning (ML) and Artificial Intelligence (AI) are pivotal in phenotyping, enabling the efficient analysis of large datasets from imaging, sensors, and environmental measurements, making inferring phenotypic plant traits from such datasets possible (Khanna et al., 2019). These tools enhance the prediction of plant performance under heat stress and assist in genomic selection by identifying markers linked to heat tolerance traits. AI-driven systems optimize phenotyping workflows, improve trait accuracy, and accelerate breeding programs (Rossi et al., 2022; Bettelli et al., 2023; Das Choudhury et al., 2024; Panella et al., 2024; Pecori et al., 2024; Sharma et al., 2024).

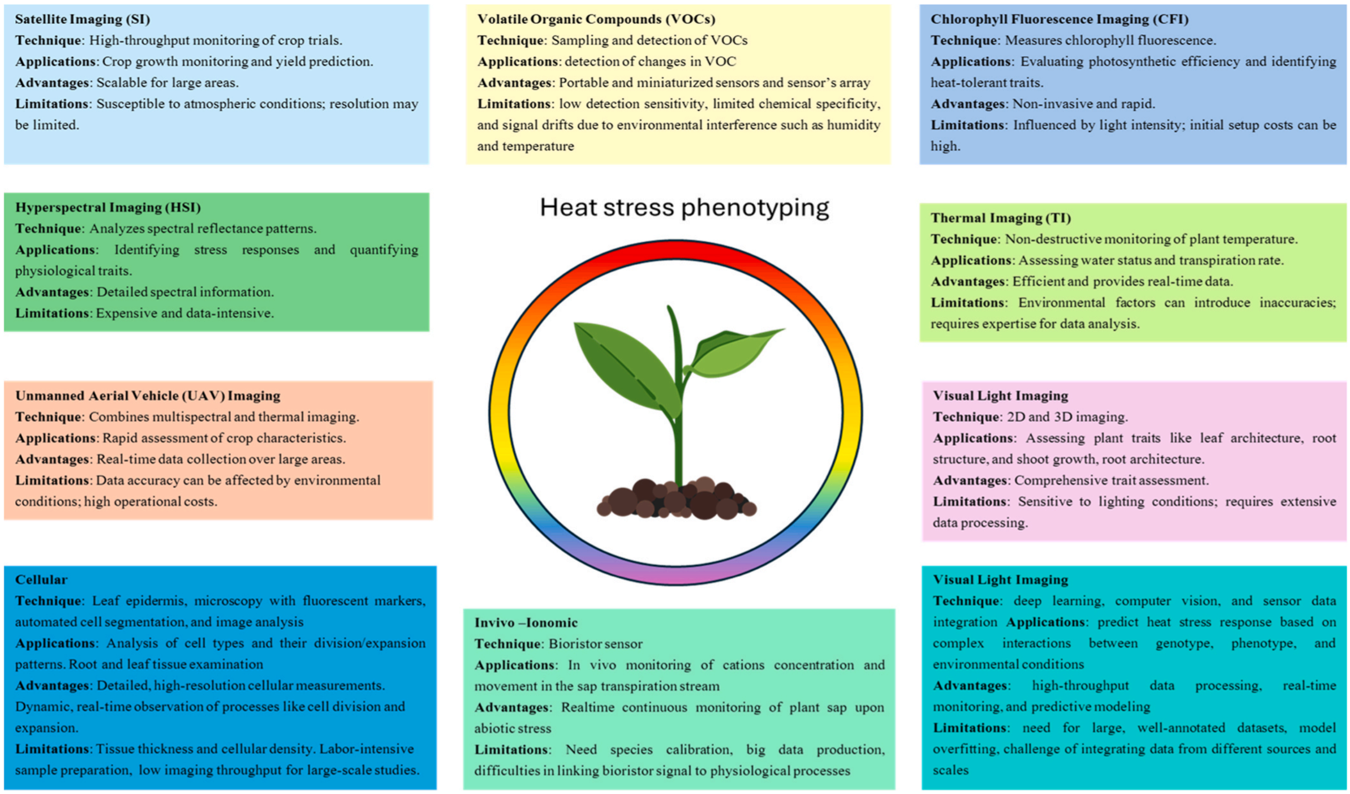

Fig. 2. Maps of key enabling technologies applied in heat stress phenotyping.

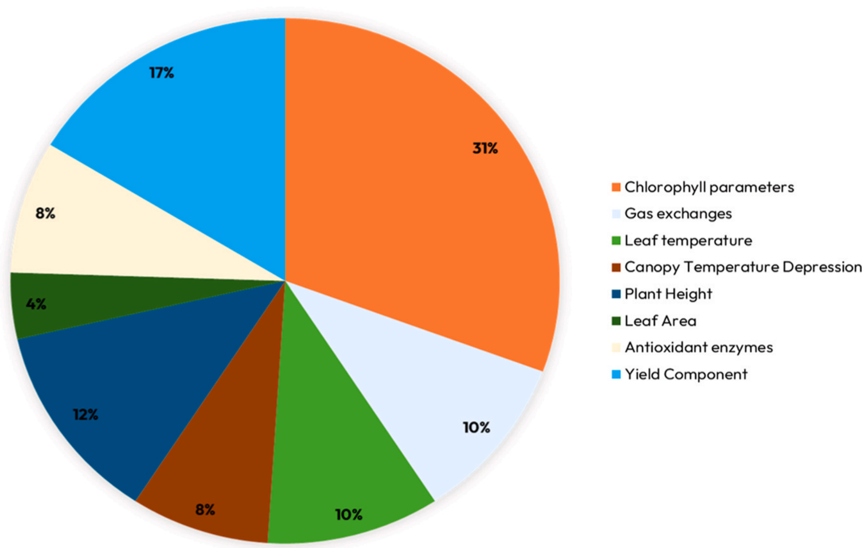

Fig. 3. Plot of the most analyzed indices in heat stress experiments. Data representative of 20 peer review articles published since 2020.

8.5. Real-Time Functional Phenotyping

Despite technological advances, real-time functional phenotyping remains underutilized. New platforms, such as plant arrays, allow simultaneous monitoring of soil–plant–atmosphere interactions under dynamic environmental conditions (Halperin et al., 2017; Lupo and Moshelion, 2023; Lupo and Moshelion, 2024). Additionally, the Bioristor, a novel Organic Electrochemical Transistor (OECT) sensor, enables continuous real-time monitoring of ion concentrations in the plant sap, indirectly detecting stress responses, including those associated with heat stress (Coppedè et al., 2017; Janni et al., 2021). Bioristor enables continuous monitoring of plant responses to drought, changes in vapor pressure deficit (VPD), and saline stress (Janni et al., 2019). It effectively detects variations in ion content within the transpiration stream, providing indirect insights into plant physiological responses (Vurro et al., 2023b). Consequently, Bioristor can potentially identify the onset of heat stress, which significantly impacts plant transpiration and stomatal behavior, making it a valuable tool for real-time phenotyping under dynamic environmental conditions (see also www.plantbit.it).

8.6. VOCs sampling and detection

VOC-SCREEN is a plant phenotyping platform to measure the emission of volatile organic compounds (VOCs), photosynthetic gas exchange and transpiration under ambient, or abiotic and biotic stress conditions. Jud and coworkers in 2018 nicely reviewed the available volatile sampling and detection/sensing methods (Jud et al., 2018) and ongoing efforts are being made to miniaturize VOC sampling devices (Lan et al., 2020). In addition, portable sensors applied in the field and composed of an array of four chemoresistive Metal-Oxides sensors (MOXs) were successfully applied by Fabbri and coworkers (Fabbri et al., 2020) and further improved through printed organic semiconductor gas sensors (Zappa et al., 2018; Gaiardo et al., 2023; Pecunia et al., 2024). However, funding access can strongly hamper the use of sensor technologies and access to phenotyping facilities, thus the review explores also low-cost phenotyping technologies.

8.7. Low-cost technologies for phenotyping

The analysis of the user demand performed in 2018 highlights the increasing use and need of cheap affordable phenotyping methods, together with custom-developed methods (33 % of the users) followed by portable devices (24 %), and UAVs (15 %). Deep phenotyping systems, such as conveyor and gantry-based systems, are used by only 5 % of respondents, likely due to high costs and limited access to infrastructure. In this study, we explored low-cost phenotyping technologies employed in heat stress experiments. Most existing low-cost phenotyping technologies are based on smartphone applications. Notable examples include PhenoTiki (Bontpart et al., 2020) and PYM, which relies on top-view imaging (Minervini et al. 2017; Valle et al., 2017). These approaches are particularly well-suited for plants growing on a horizontal plane or under field conditions. In contrast, the PhenoBox setup uses side-view imaging, making it more appropriate for plants with complex architectures (Czedik-Eysenberg et al., 2018). However, many custom-built solutions remain prohibitively expensive, posing a barrier for numerous laboratories. While low-cost tools significantly broaden access to image-based phenotyping, they often demand substantial engineering expertise, specialized equipment, and advanced skills in programming and data analysis.

As a result, there is a growing need for affordable, user-friendly phenotyping platforms that integrate low-cost hardware with intuitive software and clear documentation. Such tools would make advanced phenotyping methods more accessible to a wider range of researchers, including those with limited technical backgrounds or budgets (Yu et al., 2024).

9. International initiatives on plant phenotyping

On a global scale, the International Plant Phenotyping Network (IPPN, https://www.plant-phenotyping.org/) was established in 2016 by leading research institutions to promote and support state-of-the-art plant phenotyping. The network brings together numerous organizations from both academia and industry to collaborate in organizing major symposia, workshops, and training events across all areas of plant phenotyping (Pieruschka and Schurr, 2019). At the European level, several national plant phenotyping initiatives have been launched to develop and provide access to facilities capable of addressing multiscale plant phenotyping across diverse agro-climatic conditions. Most of these national initiatives serve as EMPHASIS national nodes, as listed on the EMPHASIS website (https://emphasis.plant-phenotyping.eu/phenotyping-landscape/national-communities).

At the international level, the Wheat Initiative fosters a collaborative community focused on improving wheat productivity, quality, and sustainable production. It includes an Expert Working Group on Wheat Phenotyping dedicated to sharing resources, capabilities, data, knowledge, and ideas (https://www.wheatinitiative.org/ewg-phenotyping). In parallel, the Global Wheat Consortium (https://www.global-wheat.com/) aims to enhance precision phenotyping under field conditions by assembling large, diverse, and well-annotated image datasets. A notable achievement has been the development of deep learning approaches for phenotypic analysis, made possible through extensive collaboration and in-kind contributions from multiple researchers (David et al., 2020, 2021).

10. Data management, a hot topic

Although plant phenotyping has emerged as a crucial field ~~of~~ in plant research, its rapid technological development brings a significant challenge: the risk of becoming overwhelmed by the massive amounts of data generated by automated phenotyping systems. The primary bottleneck today lies in effective data management, specifically in data annotation, proper metadata collection, and in fostering synergy across data collection and analysis (Coppens et al., 2017).

Data management encompasses the collection, description, processing, sharing, and reuse of data. As the volume and complexity of traits and descriptors increase, driven by high-throughput phenotyping and novel sensor technologies, There is a growing need for improved and harmonized metadata to ensure data interoperability and reproducibility (Krajewski et al., 2015). To address this, the Minimal Information About a Plant Phenotyping Experiment (MIAPPE, http://www.miappe.org) was developed by the international community. MIAPPE is an open, community-driven standard aimed at harmonizing the description of plant phenotyping experiments. It includes both a checklist and a metadata model to ensure the adequate and consistent documentation of phenotyping data (Coppens et al., 2017).

MIAPPE originated within the transPLANT project, funded under the European Commission's 7th Framework Programme for Research (Infrastructures theme), in collaboration with the European Plant Phenotyping Network (EPPN). It has been maintained, extended and implemented in projects such as the ELIXIR-EXCELERATE, EPPN2020, and EMPHASIS, all of which aim to develop and support infrastructures for plant phenotyping and life science informatics.

In parallel, the adoption of the FAIR principles (Findability, Accessibility, Interoperability, and Reusability) has become mandatory for long-term data stewardship (Mayer et al., 2011; Pommier et al., 2019). These principles extend beyond datasets to include the algorithms, tools, and workflows that generate and process data, ensuring full transparency, reproducibility, and reuse of scientific outputs (Wilkinson et al., 2016).

Phenomics datasets, at the intersection of disciplines such as plant physiology, genetics, ecology, and crop modeling, are managed within specialized data repositories and information systems tailored to each field. However, to enable cross-disciplinary research, phenomic information systems must support comprehensive data tracking. This includes temporal and spatial environmental variability, trait dynamics, and detailed links between samples, organs, genotypes, plants, experimental events, and their physical location (x-y coordinates). Semantic web technologies are increasingly used to automate the generation of complex metadata from minimal user input, enhancing both interoperability and the capacity for sophisticated cross-study analyses (Neveu et al., 2019), https://zenodo.org/records/3766987#.XqXrQ_gzY2w).

To promote interoperability, the EMPHASIS infrastructure has initiated collaboration with other European research infrastructures, ELIXIR (genomics), AnaEE (ecology), and AgMIP (crop modeling), through the establishment of dedicated working groups. These efforts aim to identify shared goals and streamline data integration across communities.

Although creating a unified data system across these diverse disciplines remains a complex challenge, enabling communication and interoperability among existing systems is both realistic and critical. Achieving this would facilitate seamless data sharing, support integrated workflows, and empower interdisciplinary research, particularly in addressing complex phenomena such as heat stress responses in plants.

10.1. Heat stress phenotyping, where do we stand? From 2020-to date

Integrating genomic data with phenotypic assessments has significantly advanced the identification of genetic markers and quantitative trait loci (QTL) associated with heat tolerance. Techniques such as genome-wide association studies (GWAS) and QTL mapping are essential for linking genetic variation to phenotypic traits. A deeper understanding of the molecular mechanisms underlying heat stress responses, focusing on heat shock proteins (HSPs), ROS scavenging enzymes, and transcription factors, has furthered our insight into plant resilience (Palermo et al., 2024).

Epigenetic modifications, including DNA methylation and histone changes, also regulate gene expression in response to heat stress. These epigenetic marks can confer transgenerational memory, enhancing progeny tolerance to subsequent heat events.

A recent analysis of heat stress phenotyping publications over the past four years shows morphometric traits dominate the research focus (Fig. 3). Plant height emerges as the most frequently measured trait, often assessed by traditional, non-imaging methods (El-Remaly, 2023; Molero et al., 2023; Sharma et al., 2024; Singh et al., 2022; Tene et al., 2023, Table 2).

Gas exchange parameters, including stomatal conductance, transpiration rate, and net photosynthesis, have been widely studies in wheat, rice, and potato under heat stress (Redhu et al., 2024, Karwa et al., 2020, Lazarević et al., 2022 and Lv et al., 2024) (Fig. 3). For istance, Redhu et al., (2024), Redhu et al., 2024 observed significant reductions in stomatal conductance in wheat, hindering photosynthate translocation and reducing yield. In rice, Karwa et al. 2020 found decreased photosynthesis but increased stomatal conductance and transpiration, indicative of thermal cooling through evapotranspiration under adequate water supply.

Contrastingly, Lazarević et al., 2022 reported an increase in photosynthetic rate in heat-stressed potatoes. Leaf temperature, a key indicator of heat stress, is often measured via thermal imaging using cameras and aerial platforms (Gao et al., 2020; Karwa et al., 2020; Abdelhakim et al., 2024; Molero et al., 2023), with rare use of infrared radiometers. Singh et al., 2022.

Leaf temperature reflects short-term thermal changes and is effective for distinguishing tolerant from susceptible genotypes (Gao et al., 2020; Karwa et al., 2020), (Table 2, Figs. 2,3)

Yield components are among the most analyzed trait in heat stress studies (Karwa et al., 2020; Mikołajczak et al., 2023; Molero et al., 2023; Redhu et al., 2024; Sharma et al., 2024; Singh et al., 2022; Thistlethwaite et al., 2020), (Table 2). These components are critical because heat stress has a significant impact on them, causing a reduction in grain yield (Table 2, Fig. 3).

NDVI reflects the chlorophyll content in the plant. NDVI can be collected by aerial platforms (Molero et al., 2023) and multispectral images (Lazarević et al., 2022).

Seed phenotyping has become essential for developing thermotolerant plant species. This multidisciplinary approach integrates advanced spectroscopy, imaging, and machine learning technologies to decode the physiological and biochemical traits that underpin resilience to heat stress. Spectroscopic methods such as NIR, Raman, and Terahertz (THz) spectroscopy enable non-destructive analysis of seed properties, offering insights into moisture content, protein stability, lipid integrity, and metabolic adaptations to heat. Raman spectroscopy is particularly effective for detecting molecular vibrations that signal stress-induced biochemical changes, while THz spectroscopy can detect alterations in the structural organization of seeds under thermal stress (Liu et al., 2024; Tu et al., 2023). These techniques also provide high-throughput means of assessing seed nutritional quality, a key factor influencing heat tolerance.

Beyond biochemical and structural traits, seed germination is a pivotal phenotype for evaluating thermotolerance. Tools like ScreenSeed facilitate high-throughput tracking of germination metrics such as time to 50 % germination (t50), germination uniformity (U80–20), and maximum germination percentage (Gmax). This platform is particularly valuable for screening large genomic libraries under controlled heat stress conditions (Merieux et al., 2021). In parallel, machine learning platforms such as AIseed extract up to 54 phenotypic features, integrating morphological, color, and texture data—to predict seed vigor, viability, and purity, which are key indicators of a seed's capacity to withstand high temperatures (Tu et al., 2023) (Table2). SmartGrain, another automated tool, focuses on precise morphological measurements such as length, width, and area, enabling the subtle detection of stress-induced changes in seed shape (Tanabata et al., 2012), (Table 2, Fig. 3).

While seed phenotyping has traditionally focused on physical and chemical traits, recent advances allow researchers to link these traits with molecular data, including transcriptomics and proteomics, offering a systems biology perspective on thermotolerance. For instance, gene expression profiling of heat-stressed seeds can uncover pathways involved in antioxidant activity, membrane stabilization, and heat shock protein regulation, all of which are crucial for maintaining cellular integrity during thermal stress. Furthermore, integrating phenotypic data with environmental sensors enables the simulation of real-world heat stress scenarios, enhancing the ecological relevance and applicability of research findings.

Advances in cellular phenotyping have enabled high-resolution analysis of cellular features across various focal planes, allowing researchers to study Arabidopsis roots beyond surface tissues (Ovečka et al., 2022). When combined with fluorescent protein markers targeting subcellular structures, live imaging offers valuable insights into dynamic processes such as cell division and expansion (Ovečka et al., 2022). These methods have significantly contributed to our understanding of shoot meristem development and anisotropic cell expansion. However, their application to thicker tissues, such as leaves and crop roots, remains challenging due to tissue density. For deeper structures like the mesophyll and vascular layers, effective imaging using confocal or multiphoton microscopy requires rigorous tissue fixation, clearing, and staining protocol (Wuyts et al., 2010), (Table 2, Fig. 2). These techniques allow researchers to observe tissue-specific responses to heat stress.

Cellular-level phenotyping also enables the investigation of intracellular Ca^{2+} signaling, which plays a crucial role in regulating plant responses to environmental stimuli and developmental signals. Genetically encoded calcium indicators (GECIs) have made it possible to visualize these dynamics at high resolution (Grenzi et al., 2021; Resentini et al., 2021). In Arabidopsis, recent studies have shown that heat stress rapidly (within seconds) alters cytosolic Ca^{2+} levels in leaves (Weigand et al., 2021) and disrupts chloroplast Ca^{2+} homeostasis (Lenzoni and Knight, 2019) potentially linking stress perception to changes in photosynthetic activity. These findings suggest that disruptions in Ca^{2+} homeostasis may represent one of the earliest detectable responses to high temperature stress in plants.

However, applying this type of phenotyping requires the generation of transgenic plants expressing Ca^{2+} indicators and the development of custom imaging systems. As a result, it is currently not suitable for field deployment. Nonetheless, it remains highly compatible with analyses conducted in controlled environments, including medium-scale phenotyping facilities. These technological advances are reshaping plant breeding strategies by providing a multi-dimensional toolkit for identifying and propagating thermotolerant genotypes. By integrating high-throughput, non-destructive phenotyping with molecular data, researchers can accelerate the development of climate-resilient crops. This comprehensive approach not only addresses the immediate challenges of rising global temperatures but also supports long-term food security and the sustainability of agricultural systems (Liu et al., 2024; Merieux et al., 2021; Tanabata et al., 2012; Tu et al., 2023).

Table 2
Heat stress phenotyping experiments.

Article	Species	Traits	Technology	Field / Control Conditions	References
Robot-as-a-Service as a New Paradigm in Precision Farming	grapevine	NDVI, vegetation indexes, plant morphological traits	Polibot	field	(Milella et al., 2024)
Prediction of biomass accumulation and tolerance of wheat seedlings to drought and elevated temperatures using hyperspectral imaging	Wheat	ChlF parameters (PAM imaging method), Morphometric: length, fresh and dry weight of the roots, shoots and whole plant. Hyperspectral images.	Open FluorCam FC 800-O/ 1010-S, Photon Systems Instruments,Specim IQ hyperspectral camera	control conditions	(Sherstneva et al., 2024)
From landraces to haplotypes, exploiting a genomic and phenomic approach to identify heat tolerant genotypes within durum wheat landraces	Wheat	Morphometric: plant height, leaf area and canopy %. Physiological: stomatal resistance, Infrared Leaf Temperature and canopy temperature depression (CTD), relative water content (RWC). Biochemical: MDA and H_2O_2	AP4 porometer, FLIR E75 and Lab systems	control conditions	(Palermo et al., 2024)
Drone-based imaging sensors, techniques, and applications in plant phenotyping for crop breeding: A comprehensive review	crop	Leaf surface temperature	UAV-based thermal imagery	review	(Gano et al., 2024)
Phenotyping for heat stress tolerance in wheat population using physiological traits, multispectral imagery, and machine learning approaches	Wheat	Yield related traits, NDVI, (B) NDRE, (C) CCCI, (D) EVI, (E) CVI, and (F) crop volume.	UAV	field	(Sharma et al., 2024)
High Throughput Image-Based Phenotyping for Determining Morphological and Physiological Responses to Single and Combined Stresses in Potato	Potato	Chlorophyll fluorescence, Thermal Infrared (IR), RGB, hyperspectral imaging	HTP	control conditions	(Abdelhakim et al., 2024)
Drought and heat stress: insights into tolerance mechanisms and breeding strategies for pigeonpea improvement	Pigeonpea	Antioxidant, Plant growth hormones	Lab systems	review	(Bakala et al., 2024)
Enhancing Cowpea Tolerance to Elevated Temperature: Achievements, Challenges and Future Directions	Cowpea	Yield traits, HSP, ROX, Floral development	Visual, biochemical and molecular	review	(Mohammed et al., 2024)
Testing the evolutionary potential of an alpine plant: phenotypic plasticity in response to growth temperature outweighs parental environmental effects and other genetic causes of variation	Alpine herbs	Flower characterization, photosynthesis related traits	PAM chlorophyll fluorescence imaging system, Peltier plate	control conditions	(Arnold et al., 2024)
Unlocking genetic insights: Evaluating wheat RILs for physiobiochemical traits under terminal heat stress conditions	Wheat RILs	Antioxidant activity (TAA), canopy temperature depression (CTD water-use efficiency (WUE), grain yield (GY), chlorophyll content (SPAD)	Lab systems	field	(Redhu et al., 2024)
Functional phenomics and genomics: Unraveling heat stress responses in wheat	Wheat	Review on Functional phenomics and genomics	NA	field/control	(Kayess et al., 2024)
Exotic alleles contribute to heat tolerance in wheat under field conditions	Wheat	NDVI, yield related traits	UAV, agronomic measurements	Field	(Molero et al., 2023)
Morphological, physio-biochemical, and molecular indications of heat stress tolerance in cucumber	Cucumber	Chlorophyll, Hormones, Proline, Antioxidant	Laboratory test, Manual score	control conditions	(El-Remaly, 2023)
Four-Dimensional Plant Phenotyping Model Integrating Low-Density LiDAR Data and Multispectral Images	Limonaria (Cymbopogoncitratus), Guaiacum bonsai	Vegetative Indices	Velodyne's VLP−16 LiDAR	semi control conditions	(Rincón et al., 2022)
Study of High-Temperature-Induced Morphological and Physiological Changes in Potato Using Nondestructive Plant Phenotyping	Potato	Gas exchange, chlorophyll fluorescence	LI−180 spectrometer, WET Sensor and HH2 Moisture Meter, PlantEye F500 multispectral 3D scanner, HortControl software	control conditions	(Lazarević et al., 2022)
LiDAR Platform for Acquisition of 3D Plant Phenotyping Database	maize	Seedling height h, Total Volume v, Classification of organs	LiDAR	semi control conditions	(Forero et al., 2022)
Using UAV-based hyperspectral imaging and functional regression to assist in predicting grain yield and related traits in wheat under	Wheat	grain yield components, thousand kernel weight (TKW), grain number per meter squared and cellular membrane	Hyperspectral sensing system, Pika L 2.4 (Resonon, Bozeman, MT, USA)	Field	(Costa et al., 2022)

(continued on next page)

Table 2 (*continued*)

Article	Species	Traits	Technology	Field / Control Conditions	References
heat-related stress environments for the purpose of stable yielding genotypes		thermostability, spike number and grains per spike traits, spectral data			
High-throughput sequencing data revealed genotype-specific changes evoked by heat stress in crown tissue of barley sdw1 near-isogenic lines	Barley *sdw1*.a (BW827) and *sdw1*.d (BW828) mutants	Photosynthetic parameters, index of chlorophyll, flavonols, and anthocyanins, RWC (%), relative water content	Lab systems	control conditions	(Mikołajczak et al., 2022)
Phenotyping methods to assess heat stress resilience in grapevine	Grapes	Photosynthetic parameter, chlorophyll content, RWC, electrolyte leakage, Chemical markers, markers for oxidative stress (MDA, H2O2, Antioxidant, Ascorbic acid, GSH, SOD, POD, CAT)	NA	NA	(Pettenuzzo et al., 2022)
Phenotyping diverse wheat genotypes under terminal heat stress reveal canopy temperature as critical determinant of grain yield	Wheat	Canopy temperature, chlorophyll content (SPAD), morphological traits and yield components	Custom system, visual scoring	field	(Singh et al., 2022)
A Review of High-Throughput Field Phenotyping Systems: Focusing on Ground Robots	Wheat, Cotton, Soybean, Triticale, Grape, Brassica, Tobacco and Maize	Canopy cover, canopy height, canopy temperature, NDVI, Crop water stress index, Biomass, Leaf area index, Photochemical reflectance index, Plant morphology, Spectral index, Green cover fractions, Green area index, Average Leaf Angle, Meris terrestrial chlorophyll index, canopy reflectance, NDRE, Grenn pixel fraction, Fraction of intercepted radion, Projected leaf area, In row width, Cross-row width, Seedling count.	Tractor based system, Breed Vision, Phenoliner, GPheno Vision, ProTractor, Pushcart, Phenocart, Proximal sensing cart, Motorized pushcart Professor, Gantry system, Field Scanalyzer, PhenoField, Cable system, Field phenotyping platform, NU-Spidercam.	NA	(Xu and Li, 2022)
High-Throughput Phenotyping Approach for the Evaluation of Heat Stress in Korean Ginseng (Panax ginseng Meyer) Using a Hyperspectral Reflectance Image	Korean Ginseng	Hyperspectral imaging (hyperspectral Vis/NIR and SWIR image)	Image based score	control conditions	(Park et al., 2021)
A phenotyping strategy for evaluating the high-temperature tolerance of wheat	Wheat	Yield traits (harvest index)	Visual and manual	field	(Thistlethwaite et al., 2020)
Heat stress in maize: characterization and phenotypic plasticity	Maize	Grain Yield, Yield components, Morphological and physiological indices, SPAD value	fPARi (LI-COR), SPAD, visual, manual score	field	(Parrado et al., 2020)
The Use of High-Throughput Phenotyping for Assessment of Heat Stress-Induced Changes in Arabidopsis	Arabidopsis	Imaging-Based phenotypic measurements: plant size, morphology, and chlorophyll fluorescence	PlantScreen™ system, Plant Screen analyser software, RGB camera, Thermal camera(A615) FLIR	control conditions	(Gao et al., 2020)
Phenotyping and characterization of heat stress tolerance at reproductive stage in rice (*Oryza sativa L.*)	Rice	Grain yield components, spikelet fertility and in vitro pollen viability, grain hulling (%) and milling (%) and quality attributes, net photosynthesis rate and gas exchange, Infra-Red (IR) thermal imaging of leaf and spikelets and tis- sue surface temperature, oxidative stress (H2O2) and membrane damage (TBARS),SOD, catalase, APX, GPX and estimation of endogenous free polyamines	Lab system	control conditions	(Karwa et al., 2020)
A Low-Cost Method for Phenotyping Wilting and Recovery of Wheat Leaves under Heat Stress Using Semi-Automated Image Analysis	Wheat	Leaves wilting	Smartphone equipped with video recording 1920 × 180 pixel camera	control conditions	(Rascio et al., 2020)

Articles are listed according to the date of publication

11. Discussion

Global agricultural production is increasingly challenged by climate change and the rising frequency of extreme weather events. Among abiotic stresses, heat stress remains one of the most complex to simulate and analyze due to its intricate interactions with various environmental factors. Standardized protocols for heat stress phenotyping are often disrupted by fluctuating conditions, complicating both data standardization and timely data sharing.

A central question in heat stress phenotyping is whether experiments should be conducted in controlled environments or field conditions. Field environments exhibit dynamic fluctuations in light intensity, air and soil temperatures, wind exposure, water and nutrient availability, and soil compaction (Poorter et al., 2016). These factors introduce significant variability, complicating the interpretation of phenotypic data from field trials (Langstroff et al., 2021). While multi-site and multi-year trials are a conventional approach, environmental variability can substantially weaken expected year-to-year correlations.

In contrast, controlled environments offer standardized conditions, ensuring repeatability and enabling comparisons across different geographical locations. They also allow precise measurement of traits that are difficult to assess under field conditions, thereby improving heritability estimation, a key factor in breeding programs (Langstroff et al., 2021). Consequently, investment in advanced infrastructure for high-throughput heat stress phenotyping is essential for facilitating accurate and reproducible research.

Despite recent advancements in plant phenotyping, heat stress research continues to rely heavily on traditional, often destructive methodologies, including molecular, biochemical, and physiological measurements (Fig. 3). The most recorded traits under heat stress remain yield-related, with fewer studies incorporating real-time monitoring or image-based indices (Table 2).

Traditional phenotyping approaches are labor-intensive, subjective, and low throughput, making it difficult to capture the dynamic nature of plant responses across different growth stages and environmental conditions. Additionally, these conventional methods predominantly focus on macroscopic traits, often neglecting critical subcellular processes that play a crucial role in plant responses to heat stress (Colaço et al., 2018; Li et al., 2021; Cembrowska-Lech et al., 2023). Consequently, there is a pressing need to transition towards high-throughput, objective, and precision-driven phenotyping techniques.

Emerging technologies aim at enhancing phenotyping approaches across multiple scales, from cellular mechanisms to whole-plant responses, under diverse environmental conditions (Hemming et al., 2019; Falcioni et al., 2023). These advancements enable researchers to identify novel genetic traits that confer heat resilience, aiding the development of climate-adaptive crop varieties.

However, access to high-throughput phenotyping infrastructure is often limited by funding constraints and project-based application models, such as those facilitated through European initiatives like AgroServ (https://agroserv.eu/). Large-scale phenotyping remains restricted due to these barriers, prompting the adoption of cost-effective and customized methodologies.

Recent studies highlight the efficacy of low-cost approaches in detecting heat stress phenotypes (Bar-Sella et al., 2023, Preprint). Technological innovations, such as Raspberry Pi-based imaging systems, allow conventional greenhouses to be transformed into high-throughput phenotyping platforms at an affordable cost (Minervini et al., 2017). Such solutions generate high-quality data suitable for deep neural networks and advanced data analysis (Samiei et al., 2020).

Moreover, real-time phenotyping methods have demonstrated their ability to track plant response dynamics under drought stress, and their application in heat stress studies is anticipated (Halperin et al., 2017; Janni et al., 2019).

The rapid advancement of high-throughput phenotyping, coupled with the development of novel sensors and robotic platforms, is increasingly driven by the integration of artificial intelligence (AI). These technologies enable efficient analysis of large-scale datasets and significantly enhance our understanding of plant responses to heat stress (Cembrowska-Lech et al., 2023). As phenotyping techniques gain broader adoption, the need for robust and scalable data management systems becomes more urgent. One of the key challenges in modern phenomics is ensuring long-term data stewardship in alignment with the FAIR principles.

Implementing FAIR-compliant data infrastructures requires considerable investment in time, expertise, and coordination. This includes the involvement of professionals specialized in standards development, domain-specific data curation, and the creation of sustainable data repositories. Equally important is the standardization of experimental protocols, particularly in heat stress research, where environmental variability poses a major obstacle to reproducibility across both laboratory and field conditions (Morisse et al., 2022).

Significant progress in multi-scale phenotypic characterization has deepened our understanding of plant defense mechanisms against heat stress. Furthermore, emerging technologies now allow in vivo monitoring of plant ionomic fluxes and offer unprecedented capabilities to track Ca^{2+} signaling dynamics during thermal stress and related physiological responses (Janni et al., 2019; Resentini et al., 2021; Zhang et al., 2023).

To address the urgent challenges posed by heat stress, plant phenotyping must continue to evolve through the adoption of innovative technologies and the expansion of access to high-throughput infrastructure. The integration of AI-driven phenotyping, real-time imaging and sensing, and cost-effective tools will significantly improve our ability to characterize plant responses to thermal stress and accelerate the breeding of heat-resilient crop varieties (Sheikh et al., 2024). As research in this area progresses, fostering interdisciplinary collaboration and investing in advanced, scalable phenotyping platforms will be critical to ensuring agricultural productivity and resilience in the context of a changing climate.

12. Conclusion

Addressing the challenges posed by heat stress in agriculture demands continuous advancements in plant phenotyping. The integration of high-throughput technologies, AI-driven analytics, and real-time sensing methods offers unprecedented opportunities to deepen our understanding of plant resilience mechanisms. However, ensuring broad accessibility to advanced phenotyping infrastructure and adopting standardized data-sharing frameworks remain critical priorities. Moving forward, interdisciplinary collaborations, increased investment in scalable phenotyping platforms, and the adoption of cost-effective methodologies will be essential to accelerate the development of heat-resilient crop varieties. By bridging cutting-edge technological innovation with practical applications, the scientific community can contribute to sustainable agricultural systems capable of withstanding the growing impacts of climate change.

CRediT authorship contribution statement

Nadia Palermo: Visualization, Formal analysis, Data curation. **Michela Janni:** Writing – original draft, Conceptualization. **Silvana Moscatelli:** Writing – original draft, Data curation. **Francesca Resentini:** Writing – original draft, Data curation. **Alex Costa:** Supervision. **Marta Adelina Mendes:** Data curation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

No data was used for the research described in the article.

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