Journal of Experimental Botany, Vol. 76, No. 22 pp. 6589–6603, 2025
https://doi.org/10.1093/jxb/eraf368 Advance Access Publication 14 August 2025

DARWIN REVIEW

Enhancing plant resilience under combined stress: the role of reflectance spectroscopy

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Received 15 May 2025; Accepted 13 August 2025

Editor: Alistair Rogers, Lawrence Berkeley National Laboratory, USA

Abstract

Plants in natural environments often face unpredictable, co-occurring stresses, such as heatwaves and droughts, a trend that is intensifying with climate change. Reflectance spectroscopy, a valuable tool for monitoring plant health, has been widely used to detect single stress, but its potential for assessing combined stresses remains underexplored. While several reviews have explored plant molecular and physiological responses to combined stress, none has discussed the role of spectroscopy in this context. This review addresses this gap by synthesizing existing findings on plant spectral responses to two common stress combinations: drought + nitrogen deficiency and drought + heat stress. Although a limited number of studies exist, they reveal that plant spectral responses to combined stresses are often unique compared with individual stresses. These results point to three potential pathways by which spectroscopy can enhance plant resilience under combined stress: generating new hypotheses, facilitating the selection of broad-spectrum stress-tolerant genotypes, and improving stress detection for precision management. This review also suggests that spectral responses to combined stresses differ from individual stresses across spectral regions, plant species, scale of spectral sensing, and possibly other factors not yet considered here. To advance reflectance spectroscopy as a tool for studying combined stress, future research should prioritize enhanced experimental designs, standardized data presentation, integrated modeling, and sensor synergies.

Keywords: Concurrent stress, drought, heat stress, hyperspectral data, nitrogen deficiency, reflectance spectroscopy.

Introduction

Plants experience various stressors both from the physical environment (abiotic stress) and from biological interactions with microorganisms and pests (biotic stress). As global climate change intensifies, the frequency, intensity, and duration of many environmental stressors, such as heatwaves and rainfall, have increased, which may further accelerate the spread of pests and diseases (Chávez-Arias *et al.*, 2021; Chaudhry and Sidhu, 2022). Meanwhile, plants are more likely to encounter multiple stresses simultaneously or sequentially. While individual stresses often share overlapping signaling pathways, their combination can lead to unique responses. As a result, combined stress conditions need to be investigated as distinct scenarios rather than being inferred from single-stress studies (Kissoudis *et al.*, 2014; Pandey *et al.*, 2015; Zandalinas and Mittler, 2022). Although much research has focused on individual stresses, the study of combined stress conditions remains largely unexplored and has become a critical topic in plant stress research.

Stress alters how light interacts with plants by changing their physiological, biochemical, and structural properties. Stressed plants exhibit changes in the intensity and direction of the light they reflect or emit (Jackson, 1986). Reflectance spectroscopy measures the light reflected from plants in contiguous, narrow spectral bands using instruments such as spectroradiometers or hyperspectral imagers (Peñuelas and Filella, 1998; Berger *et al.*, 2020), providing a window into assessing individual and combined stresses in plants. This technique normally divides the spectrum into three regions: visible (VIS, 400–700 nm), near infrared (NIR, 700–1300 nm), and shortwave infrared (SWIR, 1300–2500 nm) (Berger *et al.*, 2020; Skendžić *et al.*, 2023). The VIS region is dominated by the absorption of foliar photosynthetic pigments, such as chlorophylls, carotenoids, and anthocyanins; the NIR region is mainly related to light scattering within the leaf or canopy; and the SWIR region is dominated by the absorption of water, lignin, cellulose, and proteins (Berger *et al.*, 2020). Numerous studies have used reflectance spectroscopy to evaluate individual stresses, such as drought (Asaari *et al.*, 2019; Watt *et al.*, 2021; Zhou *et al.*, 2021; Sapes *et al.*, 2024), nutrient deficiency (Zhu *et al.*, 2022; Wang *et al.*, 2024), salinity stress (El-Hendawy *et al.*, 2021; Vennam *et al.*, 2024), and other abiotic and biotic stresses (Sanaeifar *et al.*, 2023; Zhang *et al.*, 2024). These successful applications imply the great potential of reflectance spectroscopy in studying the effects of combined stresses. Indeed, this technique has been reported in several studies of stress combinations, such as drought and nutrient deficiency (Ihuoma and Madramootoo, 2020; Wasonga *et al.*, 2021), drought and pathogen infection (Sapes *et al.*, 2024), as well as drought and heat (Couture *et al.*, 2015; Bheemanahalli *et al.*, 2022). These studies generally focused on distinguishing stress and detecting the effect of combined stress using reflectance spectroscopy.

While recent reviews have focused attention on combined stresses, they predominantly address molecular and physiological aspects (Pandey *et al.*, 2015; Zhang and Sonnewald, 2017; Zandalinas and Mittler, 2022; Nadeem *et al.*, 2023; Nawaz *et al.*, 2023). There is a notable gap in reviews focusing on how reflectance spectroscopy has been used to understand plant responses to combined stresses. This review aims to bridge this gap by synthesizing existing studies conducted under two common stress combinations—drought + nitrogen deficiency and drought + heat stress. These combinations were selected since they are more frequently studied by using reflectance spectroscopy and are representative of challenges under a changing climate.

The number of studies available on these two stress combinations are limited; thus, rather than providing comprehensive conclusions, this review serves to: (i) highlight current applications of reflectance spectroscopy in combined stress research; (ii) demonstrate the potential of the technique for enhancing plant resilience in changing environments; and (iii) stimulate further investigation in this emerging field. Accordingly, this review begins with an overview of plant spectral responses to individual and combined stresses. It then highlights the potential of reflectance spectroscopy for deciphering stress combinations and enhancing plant resilience in the challenging climate. The review concludes with a call for future actions and improvements.

Review methodology and summary

In this review, we focused on two common stress combinations: drought + nitrogen deficiency and drought + heat stress, which represent the majority of the published studies involving reflectance spectroscopy. Other abiotic and biotic stresses were not included primarily due to the lack of relevant publications. Although we found a few papers addressing combined drought + biotic stress, they were not included because the types of biotic stress (e.g. fungus, pathogen, and pest) varied among these studies, which further complicates the interpretation of results.

A search for peer-reviewed publications from 1995 to 2025 was conducted using Web of Science, Science Direct, Scopus, and Google Scholar. For drought + nitrogen deficiency combination, keywords included (spectr* OR reflectance) AND (‘water stress’ OR drought) AND (‘nitrogen stress’ OR ‘nitrogen defici*). For studies on combined drought and heat stress, the search keywords were (spectr* OR reflectance) AND (‘water stress’ OR drought) AND (heat OR ‘temperature stress’ OR ‘elevated temperature’). Studies addressing each stress separately without combined treatments were excluded, as were studies that did not report spectral reflectance measurements in the VIS–NIR–SWIR region (400–2500 nm), such as those focusing only on plant growth or using other sensing technologies such as thermal sensors. This search resulted in 37 papers, with 28 on drought + nitrogen deficiency and 9 on drought + heat stress combination (Supplementary Table S1). A summary of these papers is given in Supplementary Fig. S1. Maize and wheat are the most frequently studied crops under both stress combinations. Under drought and nitrogen stress combination, more than half (56%) of the studies utilized spectral reflectance measurements from sensors on ground-based platforms (‘canopy_proximal’). On the other hand, studies on combined drought and heat stress primarily relied on spectral reflectance at the leaf level. Canopy reflectance derived from remote sensing platforms (‘canopy_remote’), such as unmanned aerial vehicles (UAVs), aircraft, and satellites, has not been reported under combined drought and heat stress conditions. For drought and nitrogen stress combination, 20 out of 28 (71%) studies were conducted in open-field environments, whereas most studies (78%) under the drought and heat stress combination were conducted indoors, probably due to the difficulty of controlling temperature in field conditions.

Quantitative synthesis analysis

We emphasize 'synthesis analysis' rather than 'meta-analysis' because the latter requires sufficient data to calculate effect size metrics, such as standardized mean difference (Gurevitch *et al.*, 2018). These metrics are typically calculated using the mean and variance of the data. However, most of the papers identified in this review only provided average spectral reflectance values without including the associated variance or range, making it impossible to calculate effect size metrics. Therefore, instead of a formal meta-analysis, we conducted a quantitative synthesis analysis focused on summarizing the plant spectral reflectance responses under combined stress conditions. Readers are advised to interpret these synthesis results as descriptive summaries that suggest the potential for this method to resolve plant responses to multiple stresses rather than statistically derived conclusions.

We first contacted the corresponding authors of the collected papers to request access to the raw spectral reflectance data. When no response was received, PlotDigitizer (https://plotdigitizer.com/) was used to extract data from published figures, if available. To gather additional data, we searched for open-source data from the EcoSIS (Ecosystem Spectral Information System) website (https://ecosis.org/). This synthesis analysis focused solely on continuous spectral reflectance data and excluded vegetation indices. This was due to the variation in the type of reported vegetation indices across studies, making it difficult to compile a consistent set of indices for further analysis. For consistency, a fixed step size of 50 nm was maintained when sampling spectral reflectance data from the raw data and published figures. When multiple severity levels or treatment rates for a stress factor were present, the lowest and highest level/rate were consistently selected. In cases where reflectance data from multiple dates were available, the final date was used, ensuring that the stress conditions had been well established. For studies involving multiple genotypes, species, locations, or years, we treated each unique combination of these factors as a single sample. Overall, 21 samples were collected for the drought and nitrogen stress combination, and 18 samples for the drought and heat stress combination.

To assess plant spectral responses to stress, we calculated the relative response (RR) for each spectral waveband, referencing to the control treatment (i.e. the group of plants receiving no stress treatment). As shown in Equation (1), for a stress factor (drought, nitrogen stress, heat stress, or combined stress):

$$\mathrm{RR}_{\lambda_{\mathrm{i}},\mathrm{stress}} = \frac{(\lambda_{\mathrm{i,stress}} - \lambda_{\mathrm{i,control}})}{\lambda_{\mathrm{i,control}}} \times 100\ (\%) \qquad (1)$$

where $\mathrm{RR}_{\lambda_{\mathrm{i}},\mathrm{stress}}$ represents the RR at the ith waveband, $\lambda_{\mathrm{i,stress}}$ is the reflectance value at the ith waveband under stress conditions, and $\lambda_{\mathrm{i,control}}$ is the reflectance value at the same waveband under control conditions. A positive RR indicates that stress increased reflectance at the ith waveband; in contrast, a negative RR indicates reduced reflectance as compared with control treatment.

Drought + nitrogen deficiency

Visible spectral region

In general, plants under stressed conditions had VIS reflectance higher than the controls, especially for nitrogen deficiency and the combined stress (Fig. 1). Among these samples, the wheat crops grown in the field had the largest RRs in this region (Fig. 2A). Eight of these wheat samples obtained from Raya-Sereno *et al.* (2024) are depicted in Fig. 2B with their reflectance under each treatment. Previous work has shown that increased VIS reflectance is a common response to drought in maize (Schepers *et al.*, 1996 ; Schlemmer *et al.*, 2005), wheat (Bandyopadhyay *et al.*, 2014), and other plant species (Lassalle, 2021). In this spectral region, reflectance is dominated by the absorption of various leaf pigments, including Chl *a* and *b*, carotenoids, and anthocyanins (Knipling, 1970 ; Blackburn, 2007; Ollinger, 2011), with higher pigment content leading to lower reflectance. Among these, chlorophyll is the major light-harvesting compound in plants and has strong absorption in the red (~650–700 nm) and blue (~400–500 nm) regions (Curran, 1989; Ollinger, 2011). A reduction in chlorophyll under drought conditions has been widely reported (Fig. 1), which may help explain the observed increase in VIS reflectance. Our review also confirms that nitrogen deficiency leads to increased VIS reflectance, consistent with prior studies (Schepers *et al.*, 1996 ; Clay *et al.*, 2006; Ranjan *et al.*, 2012; Corti *et al.*, 2017). For example, Zhao *et al.* (2003, 2005) reported that nitrogen stress increased leaf reflectance at ~550 nm and 710 nm in corn and sorghum. Similarly, Blackmer *et al.* (1996) found that the spectral responses of corn to different nitrogen treatments were centered around 550 nm and 710 nm. The rise in VIS reflectance under nitrogen deficiency is primarily attributed to the stress-induced decrease in chlorophyll synthesis (Fig. 1). Under combined drought and nitrogen deficiency, chlorophyll also tends to be reduced, leading to increased VIS reflectance (Fig. 1). Additionally, the magnitude of the RR of each species under combined stress was greater than that under either stress alone (Fig. 2A). This evidence suggests potential additive or synergistic effects of drought and nitrogen stress on VIS reflectance.

Near infrared spectral region

The average NIR spectral reflectance was reduced under all three stress conditions as compared with the control (Fig. 1). The overall reduced reflectance under water deficit is consistent with findings from field studies in wheat, where spectroscopy measurements were taken ~1 m above the canopy (Bandyopadhyay *et al.*, 2014; EI-Hendawy *et al.*, 2017). In

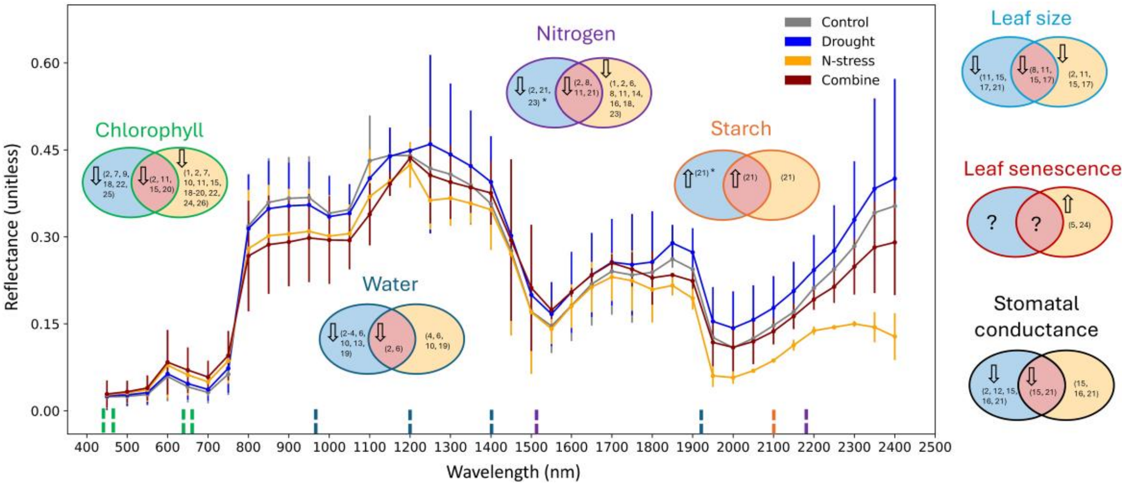

Fig. 1. Average spectral reflectance curves and physiological responses under combined drought and nitrogen (N) deficiency stress. Spectral data were averaged across seven species–celery, maize, sugar beet, wheat, and three tree species (sycamore, sweetgum, and loblolly pine) ($n = 21$). Vertical bars indicate 1 SD. Overlapping circles summarize common trends in physiological traits: a downward arrow indicates a decrease, an upward arrow an increase, no arrow (with cited studies) indicates no change, and a question mark denotes a lack of available studies. This summary is based on 26 reviewed papers, most of which examined combined drought and N deficiency stress. The trends shown in the figure reflect the majority consensus among studies. An asterisk (*) indicates an equal number of studies reporting opposite trends. Circles shaded in different colors represent stress types, i.e. drought stress, nitrogen stress, and combined stress. Studies cited in those circles are as follows: [1](Boussadia *et al.*, 2010), [2](Caine *et al.*, 2024), [3](Colovic *et al.*, 2022), [4](Corti *et al.*, 2017), [5](Ding *et al.*, 2005), [6](Dodig *et al.*, 2019), [7](Elmetwalli and Tyler, 2020), [8](El-Shikha *et al.*, 2007), [9](Fahad *et al.*, 2017), [10](Ihuoma and Madramootoo, 2020), [11](Kang *et al.*, 2023), [12](Klem *et al.*, 2018), [13](Kusnierek and Korsaeth, 2015), [14](Lebourgeois *et al.*, 2012), [15](Li and Wang, 2023), [16](Pancorbo *et al.*, 2021), [17](Saravia *et al.*, 2016), [18](Schepers *et al.*, 1996), [19](Schlemmer *et al.*, 2005), [20](Shangguan *et al.*, 2000), [21](Shi *et al.*, 2017), [22](Siłuch *et al.*, 2023), [23](Wang *et al.*, 2011), [24](Wen *et al.*, 2020), [25](Yang and Qin, 2023), [26](Zhao *et al.*, 2005).

contrast, a greenhouse study by Schepers *et al.* (1996) reported an increase in NIR reflectance under drought. Notably, their measurements were taken at the leaf level, unlike the canopy-level measurements in the previous studies—a distinction that may account for the discrepancy. At the leaf level, drought-induced changes in plant water status (Fig. 1) can alter the intercellular air spaces in leaf tissues, causing more microcavities between cell walls. This creates more interfaces, leading to enhanced NIR reflectance (Knipling, 1970; Schepers *et al.*, 1996). In contrast, at the canopy level, the NIR spectral response is strongly influenced by the leaf area index (Jacquemoud *et al.*, 2009). The decline in NIR reflectance observed in our review, as well as in the studies by Bandyopadhyay *et al.* (2014) and El-Hendawy *et al.* (2017), is likely to be due to reduced leaf area and canopy cover under drought stress (Fig. 1). Nevertheless, spectral reflectance can be affected by confounding factors such as weather and soil conditions. As shown in Fig. 2B, three out of four samples in 2020 demonstrated increased or unchanged reflectance under drought compared with the control. The combined effect of annual weather patterns, preceding crops, and genotypes could alter the reflectance. To accurately isolate the impact of these factors, well-controlled and carefully designed experiments are required in future research.

Similarly, nitrogen deficiency also led to decreased NIR reflectance, probably due to reduced plant growth and smaller leaf size under nutrient-limited conditions (Fig. 1). Additionally, nitrogen deficiency is often associated with accelerated leaf senescence (Fig. 1), which can further reduce NIR reflectance due to degradation of cell walls (Knipling, 1970). These findings align with previous observations in corn (Clay *et al.*, 2006; Wang *et al.*, 2011) and wheat (Ranjan *et al.*, 2012; Devadas *et al.*, 2015). Under combined drought and nitrogen stress, the average reduction in NIR reflectance appeared to be the sum of reductions observed under each individual stress (Fig. 1), suggesting a potential additive effect.

Shortwave infrared spectral region

A clear pattern in the SWIR region could not be established due to the high variability and limited sample size (i.e. only four samples across three species). However, previous studies have reported that SWIR reflectance generally increases under individual drought (Bandyopadhyay *et al.*, 2014; El-Hendawy *et al.*, 2017) and nitrogen deficiency conditions (Ranjan *et al.*, 2012), which is consistent with our findings for the two wheat samples (Fig. 2A). The other two samples also exhibited a slight increase in SWIR reflectance under drought stress (Fig. 2A). This region is known for its water absorption bands at wavelengths such as 1200, 1450, 1930, and 2500 nm (Knipling, 1970; Curran, 1989). Drought-induced reductions in plant water status (Fig. 1) are likely to be a primary factor contributing to the increased SWIR reflectance. In addition to water absorption bands, the SWIR region also includes absorption

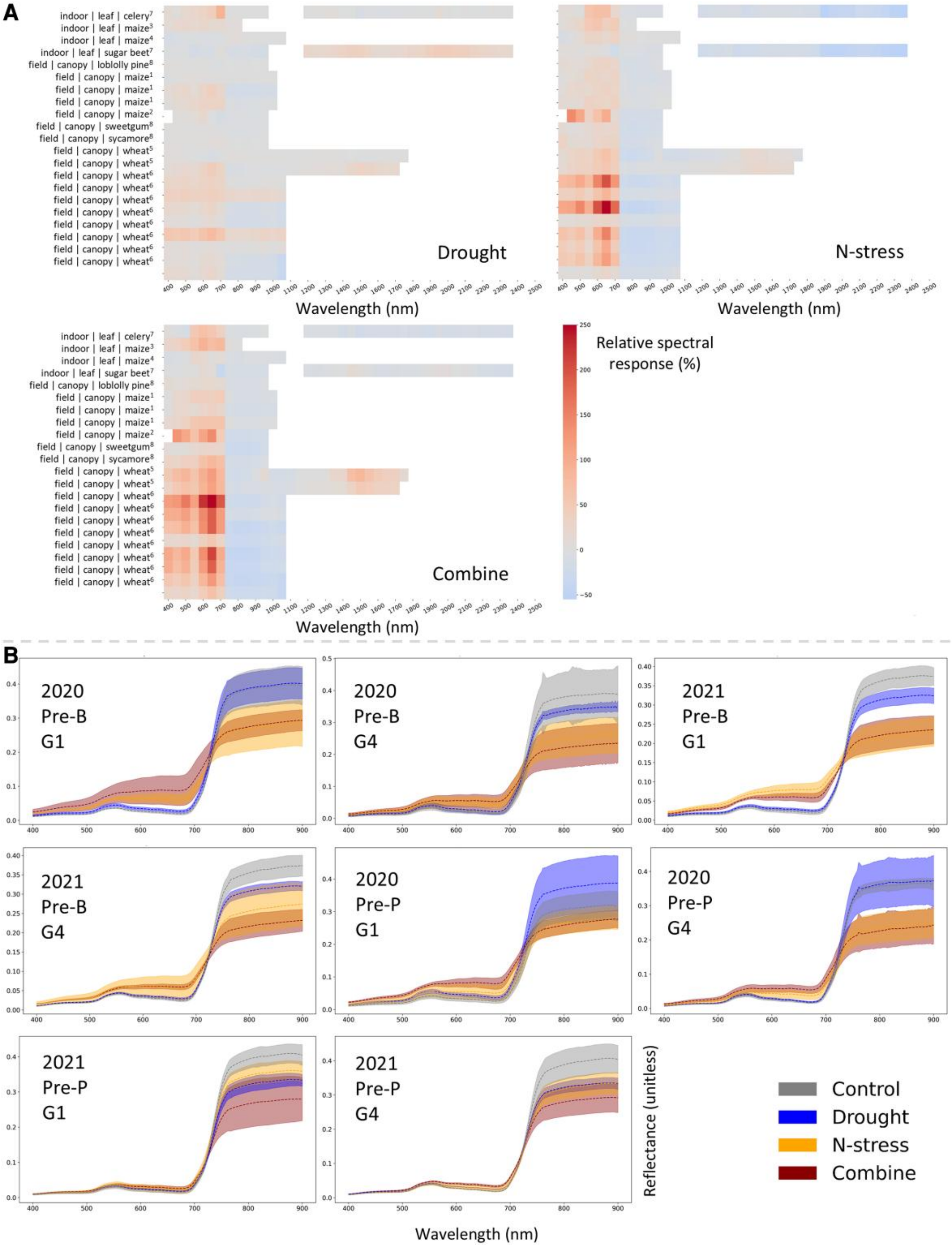

Fig. 2. Relative spectral response (Equation 1) for all samples (A) and spectral reflectance of wheat samples (B) from a selected case study (Raya-Sereno *et al*. (2024), bottom) under drought + nitrogen deficiency combination. In the top heatmap, the *y*-axis lists each sample along with details on experimental setting (indoor versus field), spectral sensing scale (leaf versus canopy), and species. Among the 21 samples: six maize samples were derived from figures published in [1](Li *et al*., 2025), [2](Ramachandiran and Pazhanivelan, 2015), [3](Schlemmer *et al*., 2005), and [4](Wang *et al*., 2011); two wheat samples were derived from figures published in [5](Pancorbo *et al*., 2021); eight wheat samples were derived from the raw data published in [6](Raya-Sereno *et al*., 2024); one celery and one sugar beet sample were derived from figures published in [7](Siłuch *et al*., 2023); three tree species were derived from figures published in [8](Gong *et al*., 2012). In the bottom spectral curves, each subplot indicates one wheat genotype (G1, G4) from 1 year of the experiment (2020, 2021) and with different preceding crops (B for barley and P for pea). The dashed curve shows the mean value of replicates, and the shaded area indicates 1 SD.

features associated with various chemical compounds, such as proteins and lignin, which contain nitrogen. Key absorption bands occur at 1510, 1690, 1940, 2060, 2180, 2300, and 2350 nm (Curran, 1989), reflecting the presence of organic molecules with C–H, N–H, and O–H bands. Since nitrogen deficiency often leads to reduced leaf nitrogen content (Fig. 1), the increased SWIR reflectance under such conditions may also result from reduced nitrogen-related absorption. Under combined drought and nitrogen deficiency, the two wheat samples in our review showed a greater RR in the 1350–1700 nm range than under either stress alone (Fig. 2A). This suggests that co-occurring drought and nitrogen stresses may act additively or synergistically on wheat water and/or nitrogen status, leading to a greater reduction in the SWIR spectral reflectance.

Drought + heat

Visible spectral region

While both individual and combined drought and nitrogen deficiency led to increased VIS reflectance, this pattern did not hold for the drought + heat combination. In the latter case, some samples showed a slight increase in VIS reflectance, but most exhibited a reduction under individual drought or heat stress (Fig. 4A). These contrasting patterns may be attributed to the use of stress-tolerant genotypes in studies involving combined drought and heat stress. Most drought + nitrogen deficiency studies were field based and involved a single genotype with unreported stress tolerance, whereas the drought + heat studies were predominantly conducted indoors and intentionally compared genotypes with varying stress tolerance. For example, Couture *et al.* (2015) used milkweed seeds from northern and southern populations and observed distinct trait responses to experimental treatments. The spectral reflectances of these populations are summarized in Fig. 4B, where the zoomed-in regions highlight subtle yet noticeable differences between northern and southern groups. Lobos *et al.* (2019) also reported genotypic differences in response to drought and heat. These inherent variations in stress tolerance may help explain the diverse VIS reflectance patterns observed in our synthesis. Additionally, environmental factors may also contribute to these contrasting patterns. In particular, when comparing maize samples from greenhouse studies under drought + nitrogen deficiency versus drought + heat stress, the day/night temperatures maintained in each greenhouse were different. These temperature variations could interactively influence crop spectral responses to drought conditions.

Heat stress is known to impair PSII activity and reduce photosynthetic pigments (Fig. 3), typically resulting in increased VIS reflectance. However, this pattern may not apply to heat-tolerant genotypes. As shown in Lobos *et al.* (2019), two groups of blueberry cultivars showed contrasting spectral responses in the VIS region: one exhibited higher reflectance under heat stress compared with the control, while the other displayed slightly lower reflectance (Fig. 4A). In Bheemanahalli *et al.* (2022), two maize genotypes exhibited opposite RRs in the VIS region under heat stress—one positive and the other negative (Fig. 4A). Similarly, Park *et al.* (2021) observed that heat-resistant ginseng varieties maintained similar VIS reflectance after heat exposure, whereas susceptible varieties displayed increased reflectance. Zhou *et al.* (2015) further found that heat stress increased chlorophyll content in the heat-tolerant tomato groups but reduced it in the heat-sensitive groups. One proposed mechanism for heat tolerance is the accumulation of anthocyanins (Fig. 3), which are water-soluble vacuolar pigments that strongly absorb light at ~550±15 nm (Gitelson *et al.*, 2001). In our synthesis, blueberry plants showed a slight positive response near 550 nm, whereas milkweed exhibited a significantly negative response, potentially reflecting differences in anthocyanin content and heat tolerance (Fig. 4A).

Overall, our synthesis suggests that genotypic variation in stress tolerance can be detected through spectral reflectance. Under combined drought and heat stress, plant responses became more complex. Carob trees and maize showed positive RRs, while blueberry and milkweed exhibited negative responses (Fig. 4A). Notably, maize and carob trees displayed opposite response directions under combined stress compared with individual drought or heat. This suggests that these species may have unique responsive mechanisms under combined stress that are not simply additive from the responses to each stress alone.

Near infrared spectral region

Although less pronounced than the pattern observed under the drought + nitrogen deficiency combination, the NIR spectral reflectance was generally reduced under stress conditions (Fig. 3). Moreover, varying levels of stress tolerance can lead to differences in spectral responses. For example, a maize sample in Bheemanahalli *et al.* (2022) showed a significant negative response under drought (Fig. 4A). This particular sample belonged to a genotype that appeared more drought tolerant than the other genotype studied in the same research. Although the exact mechanisms responsible for the reduction in NIR reflectance remain unclear, the spectral evidence suggests possible drought tolerance mechanisms related to leaf structure. In our synthesis, heat stress slightly increased NIR reflectance in blueberry, potentially due to dehydration-induced microcavities forming between cell walls (Knipling, 1970). In contrast, the other three species showed decreased NIR reflectance, possibly resulting from cell wall degradation as leaves aged—a response consistent with the acceleration of leaf senescence under heat stress (Fig. 3). Although NIR reflectance under heat stress varies across species, these differences highlight the potential of spectroscopy as a tool for evaluating stress resistance. When drought and heat were combined, the average NIR reflectance was nearly identical to that under control

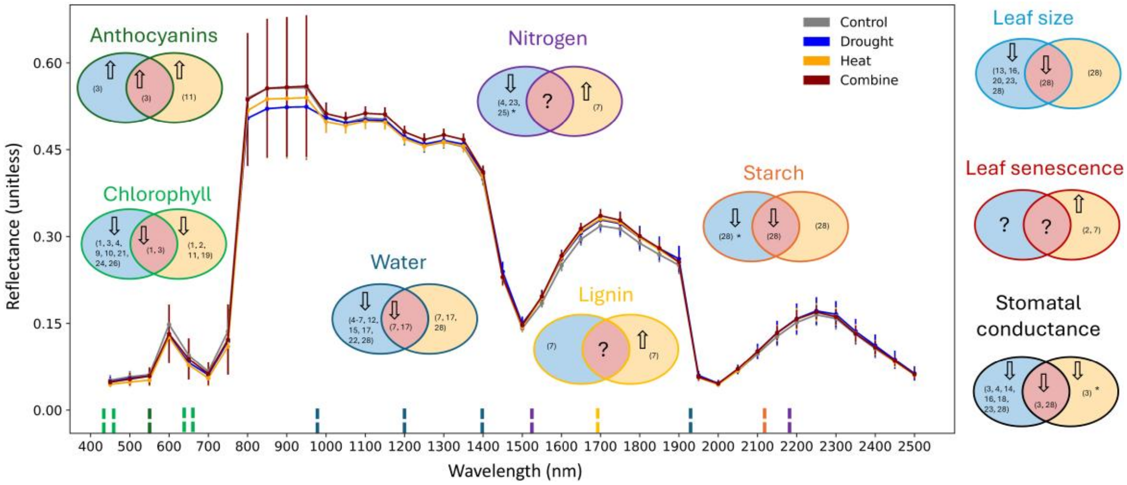

Fig. 3. Average spectral reflectance curves and physiological responses under combined drought and heat stress. Spectral data were averaged across four plant species—blueberry, carob tree, maize, and milkweed (*n*=18). Vertical bars indicate 1 SD. Overlapping circles summarize common trends in physiological traits: a downward arrow indicates a decrease, an upward arrow an increase, no arrow (with cited studies) indicates no change, and a question mark denotes a lack of available studies. This summary is based on 28 reviewed papers, most of which examined combined drought and heat stress. The trends present in the figure reflect the majority consensus among studies. An asterisk (*) indicates an equal number of studies reporting opposite trends. Circles shaded in different colors represent stress types, i.e. drought stress, heat stress, and combined stress. Studies cited in those circles are: [1](Abdelhakim *et al*., 2021), [2](Akter and Islam, 2017), [3](Bheemanahalli *et al*., 2022), [4](Caine *et al*., 2024), [5](Colovic *et al*., 2022), [6](Corti *et al*., 2017), [7](Couture *et al*., 2015), [8](Dodig *et al*., 2019), [9](Elmetwalli and Tyler, 2020), [10](Fahad *et al*., 2017), [11](Hassan *et al*., 2021), [12](Ihuoma and Madramootoo, 2020), [13](Kang *et al*., 2023), [14](Klem *et al*., 2018), [15](Kusnierek and Korsaeth, 2015), [16](Li and Wang, 2023), [17](Osório *et al*., 2012), [18](Pancorbo *et al*., 2021), [19](Rezaei *et al*., 2015), [20](Saravia *et al*., 2016), [21](Schepers *et al*., 1996), [22](Schlemmer *et al*., 2005), [23](Shi *et al*., 2017), [24](Siłuch *et al*., 2023), [25](Wang *et al*., 2011), [26](Yang and Qin, 2023), [27](Zhou *et al*., 2015), [28](Zhou *et al*., 2017).

conditions (Fig. 3), suggesting a non-additive effect of the two stresses on the NIR region. For example, in maize, both drought and heat stress individually led to negative RRs in the NIR region (Fig. 4A). If their effects were additive, a more pronounced negative response would be expected under combined stress. However, the observed response was close to zero (Fig. 4A), supporting the idea of a non-additive interaction between drought and heat in influencing NIR reflectance.

Shortwave infrared spectral region

Although only two plant species—blueberry and milkweed—had available SWIR region measurements for analysis, distinct spectral patterns were observed across the three stress conditions (Fig. 4A). In the blueberry samples, SWIR reflectance under drought remained similar to the control, but it increased under both heat stress and the combined drought + heat stress. Notably, one blueberry sample exhibited a reflectance 'hotspot' near 1900–1950 nm under heat and combined stress (Fig. 4A)—an area primarily associated with water absorption (Fig. 3). This probably indicates dehydration in these blueberry samples under those stress conditions. In contrast, milkweed displayed the opposite trend. The samples showed positive RRs in the SWIR region under drought but exhibited minimal changes under heat or combined stress. When examining individual milkweed populations, increased SWIR reflectance under drought was mainly observed in southern populations, suggesting greater drought sensitivity (Fig. 4B). This aligns well with findings from Couture *et al.* (2015), which indicate that northern populations may perform better under future climate conditions with more drought events. Interestingly, at ~1950 nm, milkweed reflectance tended to remain unchanged or slightly decrease, in contrast to the positive responses observed in blueberry under heat and combined stress (Fig. 4A). These contrasting patterns suggest that blueberry and milkweed employ different physiological mechanisms in response to stress. They highlight the potential of spectral data to generate and support hypotheses about plant stress responses.

Standardized protocols for reporting data

Although not evident in the synthesis analysis, variations in experimental regimes may introduce differences in the intensity and duration of the stress, which may further affect spectral responses. For example, nitrogen fertilization has been shown to enhance plant growth under short-term drought but may exacerbate stress effects under prolonged drought conditions (Araus *et al.*, 2020). To minimize such variability, we collected spectral data from the lowest and highest treatment levels at the latest sampling date or growth stage reported in each study. Nevertheless, this approach cannot entirely eliminate potential sources of error. Identical treatment levels may not induce the

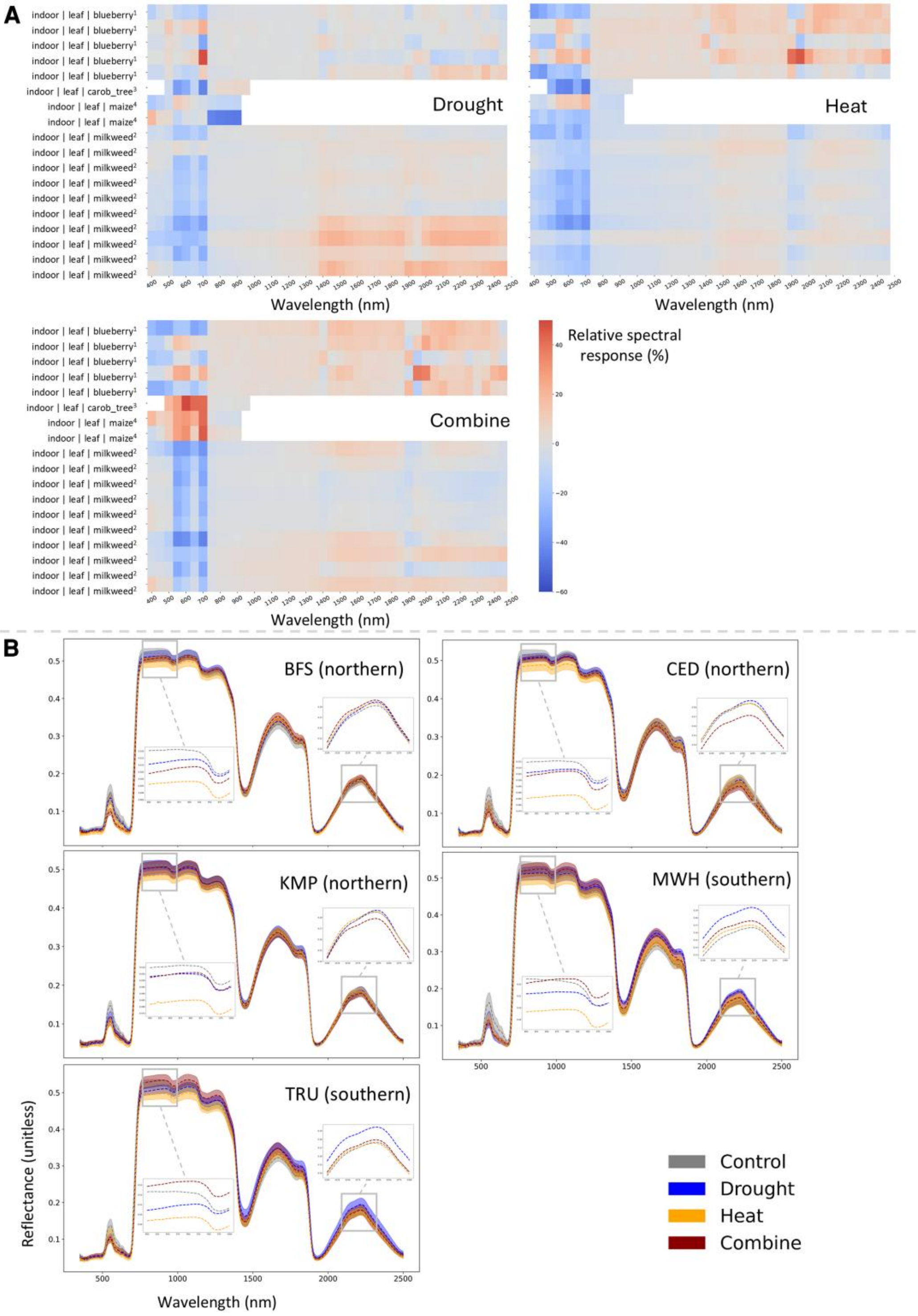

Fig. 4. Relative spectral response (Equation 1) for all samples (A) and spectral reflectance of milkweed samples (B) from a selected case study (Couture *et al.*, 2015, bottom) under drought + heat combination. In the top heatmap, the *y*-axis lists each sample along with details on experimental setting (indoor versus field), spectral sensing scale (leaf versus canopy), and species. Among the 18 samples: five blueberry samples were derived from figures published in [1](Lobos *et al.*, 2019); 10 milkweed samples were derived from the open-source platform [2]EcoSIS. (https://ecosis.org/package/common-milkweed-leaf-responses-to-water-stress-and-elevated-temperature); one tree sample was derived from the figure published in [3](Osório *et al.*, 2012); two maize samples were derived from figures published in [4](Bheemanahalli *et al.*, 2022). In the bottom spectral curves, each subplot indicates one milkweed population (BFS, CED, KMP, MWH, and TRU) from either northern or southern regions. The dashed curve indicates the mean value of replicates, and the shaded area indicates 1 SD. For clarity, the zoomed-in plots only show the mean value of the reflectance.

same degree of physiological stress across different studies due to varying experimental conditions. Environmental factors such as local climate and soil conditions may also confound spectral responses (Kissoudis *et al.*, 2014; Akter and Islam, 2017). For example, if a drought treatment is applied under lower ambient temperatures in one study compared with another, differences in spectral reflectance may result from temperature effects rather than drought alone.

These uncertainty factors underscore the need for improved protocols in experimental design and standardized data presentation in scientific publications. Publications should include detailed ancillary data along with spectral measurements, such as plant growth stages, ambient weather conditions, sensor specifications, and the scale of sensing (leaf or canopy). Furthermore, the reporting of spectral data must be standardized. Our review showed that <50% of the identified studies provided average spectral curves or raw spectral data for each treatment condition. Some studies only reported a few vegetation indices, despite having continuous spectral measurements, while others focused primarily on estimating plant traits under stress conditions without discussing spectral behaviors or disclosing the spectral data. To advance this field, we encourage researchers to share raw spectral data through open repositories such as EcoSIS. If necessary, a new data platform can be developed specifically to host plant spectral data under individual and combined stresses.

The role of spectroscopy in enhancing plant resilience to complex stress conditions

While the spectral responses observed are often case and species specific, this review nonetheless provides valuable insights into how plant stress responses may be reflected in different spectral regions. Most importantly, our synthesis underscores the potential of spectroscopy as a tool for studying plant stress resilience, as discussed in the following sections.

Driving hypothesis

In this era of rapid global climate change, developing broad-spectrum stress-tolerant plant varieties is vital for sustainable development. Advancements in transcriptomic, proteomic, and metabolic technologies have improved the understanding of the mechanisms underlying plant responses to combined stresses (Atkinson and Urwin, 2012; Pandey *et al.*, 2017; Zhang and Sonnewald, 2017; Nawaz *et al.*, 2023). These advancements have also facilitated the identification of breeding targets for developing broad-spectrum stress tolerance. Despite the growing understanding of the unique and shared plant responses to individual and combined stresses, many remain unknown due to the complexity of combined stresses. Although spectroscopy alone cannot directly uncover these mechanisms—since it captures the integrative impacts of stress—it can be useful in hypothesis generation and validation. For example, leaf chlorophyll content, which is closely linked to photosynthesis, is strongly associated with the VIS spectral region at ~430, 460, 640, and 660 nm (Curran, 1989). Observing plant VIS spectral features under individual and combined stresses allows us to formulate hypotheses about photosynthetic responses to different stress conditions. As observed in the synthesis analysis, wheat subjected to combined drought and nitrogen deficiency exhibited a greater magnitude of RR in the SWIR region than those experiencing either stress alone (Fig. 2). Given the strong association between the SWIR region and water absorption, this observation suggests a hypothesis that combined drought and nitrogen deficiency may synergistically impact wheat leaf water content. Additionally, the speed and efficiency of spectroscopy allow for the tracking of diurnal and seasonal dynamics in plant spectral responses. This capability is particularly valuable because the effects of combined stress on plants vary across different growth stages (Pandey *et al.*, 2015). Continuous monitoring of spectral reflectance under combined stress conditions allows us to hypothesize about stage-specific stress effects and identify the most vulnerable growth stages for each plant species.

Genotype selection

When selecting genotypes for broad-spectrum stress tolerance, it is not enough to simply identify those that can tolerate or survive stress conditions—it is also important to select genotypes that can maintain high yields (Atkinson and Urwin, 2012). Yield and its component traits are integrative in nature since they integrate plant performance over time (Araus *et al.*, 2023). Conventional methods to measure these traits are often destructive and time-consuming. Spectroscopy offers a valuable, non-destructive alternative for estimating yield and yield components, helping to accelerate the selection of elite genotypes with broad-spectrum stress tolerance and high yield potential. Another direction is to use spectroscopy directly by treating plant spectral signatures as a unique trait or breeding target. Since spectroscopy provides an integrative measurement of plant performance under stress, using the full spectrum may be more effective than estimating plant traits for selecting elite genotypes (Kothari and Schweiger, 2022). In ecology, the concept of 'optical types' has been proposed to optically distinguish functional plant types based on their spectral properties (Ustin and Gamon, 2010). This concept could be adapted for selecting genotypes with broad-spectrum stress tolerance. Positioning genotypes with different stress tolerances in the multidimensional spectral space may open up a new perspective on genotype selection. Advanced knowledge on how plant spectral signatures relate to stress tolerance is critical for this pursuit.

Stress detection

Increasing plant resistance to combined stress conditions can be achieved through breeding and engineering resilient plants, as well as through adaptive management practices. From the management perspective, the ability to distinguish between combined and individual stress events is crucial for precision management. For example, accurately differentiating between water stress, nutrient deficiency, and their combined effects could optimize fertigation systems that apply water and nutrient together. Several studies have explored the use of spectroscopy technology to distinguish between drought, nitrogen deficiency, and their combination. Karimi *et al.* (2005) identified a set of narrow spectral wavebands for the discrimination of nitrogen and water stress in corn. Kusnierek and Korsaeth (2015) used principal component analysis (PCA) to separate nitrogen and water treatments in spring wheat, with results showing that the first PCA component was more related to water treatment, while the second component correlated more with nitrogen fertilizer. Similarly, Elmetwalli and Tyler (2020) used penalized linear discriminant analysis to distinguish water and nitrogen deficiency stress from hyperspectral data (350–1050 nm), achieving promising results, with a misclassification rate of 0.24. Additionally, Siłuch *et al.* (2023) reported high accuracy using a random forest model to distinguish between different nitrogen supply levels under varying water availability in sugar beet (85% overall accuracy) and in celery (78% overall accuracy).

Other studies have focused on developing unique vegetation indices that can isolate individual drought or nitrogen deficiency stress. For example, in El-Shikha *et al.* (2007) and Pancorbo *et al.* (2021), the canopy chlorophyll content index (CCCI) was found to be highly sensitive to nitrogen stress but insensitive to water stress. On the other hand, the water deficit index (WDI) has shown potential in detecting crop water stress by effectively differentiating between varying levels of water treatments. Masseroni *et al.* (2017) found that the crop water stress index (CWSI) could detect plant water status without dependence on plant nitrogen conditions. Ihuoma and Madramootoo (2020) identified several indices—PRI550 (photochemical reflectance index centered at 550 nm), PRInorm (normalized PRI), and WI (water index)—as being the most sensitive to water stress. Conversely, the RDVI (renormalized difference vegetation index), PRInorm, and TCARI (transformed chlorophyll absorption in reflectance index) correlated well with nitrogen stress indicators. Recently, Li *et al.* (2025) optimized a new nitrogen stress indicator, the ratio between the normalized red edge index (NDRE) and the normalized difference vegetation index (NDVI). This new indicator successfully minimized the confounding influence of soil water variability. Altogether, these findings demonstrated the potential of spectroscopy for distinguishing between individual and combined drought and nitrogen deficiency. Future studies should investigate how spectroscopy can be applied to detect or distinguish other common stress combinations, such as drought and heat stress, and drought and biotic stress.

Calls to action

Although there has been increasing attention on studying plant molecular and physiological responses to combined stress conditions, this review highlights a lack of research on how reflectance spectroscopy can be used to understand these responses, as evident by the limited number of identified papers. This is the primary limitation of our review and constrains the generalizability of the observed spectral patterns. Findings presented in this study should be interpreted with caution, as the spectral responses reported here may not universally apply across different plant species, genotypes, or environmental settings. Additionally, this review focused on two stress combinations occurring simultaneously, as these are the most studied in publications. However, in natural conditions, plants can experience three or more abiotic and/or biotic stressors simultaneously or sequentially—what Zandalinas *et al.* (2021) referred to as 'multifactorial stress combination'. Further, sequential stresses often cause priming effects that prepare plants to perform better under future stress (Zhang and Sonnewald, 2017; Fu *et al.*, 2022), leading to more complex and dynamic variations in plant spectral responses. Given these gaps in the literature, we call for more research into multiple stress combinations, both simultaneous and sequential, with a particular focus on the role of reflectance spectroscopy.

Experimental setting

Investigating combined stress conditions in natural settings or at least in conditions that closely mimic the natural environment is crucial yet challenging. As shown in this review, most studies on combined drought and heat stress were conducted indoors, possibly due to the difficulty of regulating the temperature in open-field conditions. Controlled environments are advantageous for studying plant responses to particular stresses because they offer better control of stress intensity, timing, and duration, as well as other environmental factors. However, these settings often limit plants to certain growth conditions, such as confining plants to a limited soil volume (Araus *et al.*, 2023), which can confound the effects of combined stresses. If experiments must be conducted in controlled environments, they should be carefully designed to closely replicate field conditions, particularly in terms of growth media, stress duration and severity, timing of stress, and nutrient availability (Atkinson and Urwin, 2012). Alternatively, free-air carbon dioxide enrichment (FACE) facilities offer a promising solution for in-field control of certain stress factors. For example, the SoyFACE facility at the University of Illinois at Urbana–Champaign (IL, USA) allows altering of climate conditions including atmospheric CO_2 and O_3 levels, air temperature, and soil water availability (Ainsworth *et al.*, 2004; Bernacchi *et al.*, 2006). This facility has been well documented in the literature for investigating interactions between rising atmospheric CO_2 and O_3 levels (Eastburn *et al.*, 2010), rising CO_2 and warming temperature (Bagley *et al.*, 2015), as well as elevated CO_2 and intensified drought (Gray *et al.*, 2016). Future studies are encouraged to use these FACE facilities to explore more stress combinations. With the open-field settings, spectroscopy sensors mounted on remote sensing platforms, such as satellites, UAVs, and cable-suspended systems, can be used to collect spectral data more quickly and efficiently.

Integrated modeling

Implementing various stress combinations in controlled or field experiments can be impractical. An alternative approach is to use process-based models, which are mathematical representations of the physical world and grounded in decades of observations and experiments (Read *et al.*, 2019). For example, crop growth models (CGMs) simulate crop growth and development under pre-defined weather, soil, and management conditions (Hoogenboom *et al.*, 2004). By adjusting input parameters, CGMs can simulate plant phenology and growth patterns under different stresses such as drought and temperature stress (Rezaei *et al.*, 2015; Araus *et al.*, 2023; Leisner *et al.*, 2023). However, due to constraints in parameter settings and the complexity of crop–environment interactions, CGMs have limited capability to simulate the impacts of many stress conditions such as biotic stress factors. To better simulate crop growth under diverse stressors, a promising strategy is to couple CGM with other holistic models, such as climate models (Rötter *et al.*, 2011) and pest and disease models (Donatelli *et al.*, 2017). Skelsey *et al.* (2016) proved this strategy by coupling a CGM with an aerobiological model and an infection risk model to assess the impact of future climate conditions on potato late blight risks.

From a spectroscopy perspective, radiative transfer models (RTMs), which simulate light interactions with plants (Jacquemoud *et al.*, 2009), can be integrated with CGMs using their shared variables. This integration efficiently connects the plant spectral signature with its dynamic growth, as well as the environmental conditions. A successful example is demonstrated by Chen *et al.* (2022), where a CGM was integrated with an RTM for the estimation of crop traits. Another benefit of integrating RTMs, especially leaf-level models such as PROSPECT (Jacquemoud and Baret, 1990), is their ability to simulate not only reflectance, but also transmittance and absorptance. While this review has focused primarily on reflectance spectroscopy, other optical properties such as transmittance and absorption can offer complementary insights into plant responses to environmental stress. For example, Chen *et al.* (2023) proposed transmittance-based vegetation indices that were less influenced by confounding leaf traits compared with traditional reflectance-based indices when estimating carotenoids, leaf water content, and leaf mass per area. Although measuring transmittance and absorptance typically requires more complex instruments (e.g. spectrophotometers with integrating spheres), RTMs provide a promising alternative for simulating and exploring these properties. Future studies should consider incorporating transmittance and absorptance data to better isolate the physiological mechanisms underlying spectral responses to combined stress conditions.

While process-based models offer a robust framework for understanding underlying mechanisms, they often require complex and time-consuming calibration processes (Chang *et al.*, 2023). In contrast, statistical and empirical data-driven models, such as machine learning and deep learning, have become increasingly popular due to their efficiency and accuracy. These empirical models excel at learning from observational data to identify driving factors affecting crop growth that might not be captured by process-based models (Rezaei *et al.*, 2022). Integrating process-based models with these empirical models can enhance their complementary strengths by combining mechanistic insights with the capacity to uncover new patterns from data (Li *et al.*, 2023, 2024). Collectively, such an integrated approach may open up the way for an improved mechanistic understanding of multistress interactions, as well as enhanced modeling efficiency. Yet, achieving this comprehensive integration, from stress-driving factors to plant responses and from process-based to data-driven models, will require intensive collaboration among modelers, engineers, climatologists, agronomists, and plant pathologists in future research.

Sensor synergy

A significant limitation of reflectance spectroscopy is that spectral changes often reflect alterations in plant physiological states that are collectively influenced by multiple stress factors. In natural conditions, where the specific type of stress may not be known in advance or multiple stresses occur simultaneously, spectroscopy alone cannot provide sufficient information to identify or disentangle the stresses (Jackson, 1986). To address this limitation, a multisensor synergy might offer a more effective solution for deciphering complex responses under combined stress conditions. Although this review focuses on reflectance spectroscopy, we acknowledge the value of other sensing technologies, including thermal imaging, chlorophyll fluorescence, and light detection and ranging (LiDAR), which can complement spectral data to provide a more holistic view of plant stress responses.

Thermal sensing measures canopy temperature. Based on the leaf energy balance equation, leaf or canopy temperature is related to the plant transpiration rate and thus is a function of stomatal conductance (Gerhards *et al.*, 2019). This sensing technique has been found in assessing plant water stress (Gerhards *et al.*, 2019). Chl *a* fluorescence, which is closely related to photosynthetic function, is another powerful tool for stress detection (Stirbet *et al.*, 2018). It has proven useful for evaluating tomato growth performance under combined water deficit and salinity stress (Kautz *et al.*, 2014) and under combined waterlogging and salinity stress (Zhou *et al.*, 2022). LiDAR, by emitting laser pulses that can partially penetrate crop canopies through foliage gaps, provides valuable plant structural information beneath the canopy. This makes it especially valuable for detecting structural changes linked to stress such as drought (Su *et al.*, 2019; Mulugeta Aneley *et al.*, 2023) and salinity (Zhang *et al.*, 2023). Given their complementary benefits, integrating different sensors holds promise for providing a more detailed and accurate assessment of plant responses to complex stress conditions.

There are two common approaches to combine different sensors: data-level and feature-level fusion. At the data level, raw data from multiple sensors is directly combined before further analysis. For example, Gu *et al.* (2024) combined UAV-derived LiDAR point clouds and multispectral images to form a 3D multispectral point cloud, from which the photosynthetic traits of wheat were derived for stress monitoring. At the feature level, key variables such as vegetation indices are first derived from individual sensors and then integrated for further analysis. For example, Pancorbo *et al.* (2021) suggested that simultaneously measuring VNIR reflectance and thermal information can improve water and nitrogen management strategies. Their approach used CCCI derived from VNIR reflectance to distinguish nitrogen treatment levels while minimizing the confounding effects of soil water status. Conversely, WDI calculated using both VNIR and thermal data showed stronger sensitivity to water status than nitrogen status. Caine *et al.* (2024) enhanced abiotic stress monitoring by integrating thermal sensing with reflectance-based indices to improve transpiration modeling and water flux estimation. Finally, incorporating other data sources, such as soil properties, weather variables, and management history, could further enhance our ability to identify the driving factors of plant stress.

Conclusion

This review presents a new perspective on studying plant responses to combined stresses through reflectance spectroscopy. The synthesis results showed that plants had different spectral responses to individual and combined stresses, indicating the potential to distinguish between these stresses. It is also observed that plant spectral responses to combined stress differ from those to individual stresses, and these differences were affected by plant species, genotypes, spectral sensing scales, and potentially other experimental factors. These findings highlight the potential of reflectance spectroscopy as a tool to help improve plant resilience in a changing climate by driving new hypotheses, facilitating breeding programs, and refining precision stress management practices. Nevertheless, the limited number of studies in this area underscores the need for continued research. Future advancements will require comprehensive approaches, including improved experimental designs, standardized data presentation protocols, advanced modeling methods, and integrated sensing strategies, to fully unlock the potential of reflectance spectroscopy in deciphering plant responses to combined stresses.

Supplementary data

The following supplementary data are available at *JXB* online.

Table S1. Summary of the collected papers involving reflectance spectroscopy and combined stress.

Fig. S1. A summary of reviewed papers under drought + nitrogen deficiency and drought + heat stress combinations.

Acknowledgements

The authors wish to thank Dr Miguel Quemada from Universidad Politécnica de Madrid and Dr Yeyin Shi from the University of Nebraska-Lincoln for sharing their raw spectral data.

Author contributions

JL, PF, and CJB: conceptualization, methodology, and writing—review and editing; JL: data curation, formal analysis, writing—original draft, and visualization; CJB: project administration and funding acquisition; PF and CJB: supervision and resources. All authors have read and agreed to the published version of the manuscript.

Conflict of interest

The authors declare no conflict of interest.

Funding

This work was supported by Bill & Melinda Gates Agricultural Innovations grant investment ID 57248 and the Global Change and Photosynthesis Research Unit of the USDA Agricultural Research Service, Urbana, IL, USA.

Data availability

Data is available upon request to the corresponding authors.

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