Effects of drought and heat stresses during reproductive stage on pollen germination, yield, and leaf reflectance properties in maize (Zea mays L.)

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Funding information
Mississippi Agricultural and Forestry Experiment Station, Special Research Initiative (MAFES-SRI); National Institute of Food and Agriculture, Grant/Award Number: MIS 043050; USDA-Agricultural Research Service (USDA-ARS), Grant/Award Number: 58-6064-9-007

Abstract
Drought and heat stresses are the major abiotic stress factors detrimental to maize (Zea mays L.) production. Much attention has been directed toward plant responses to heat or drought stress. However, maize reproductive stage responses to combined heat and drought remain less explored. Therefore, this study aimed to quantify the impact of optimum daytime (30°C, control) and warmer daytime temperatures (35°C, heat stress) on pollen germination, morpho-physiology, and yield potential using two maize genotypes (“Mo17” and “B73”) under contrasting soil moisture content, that is, 100% and 40% irrigation during flowering. Pollen germination of both genotypes decreased under combined stresses (42%), followed by heat stress (30%) and drought stress (19%). Stomatal conductance and transpiration were comparable between control and heat stress but significantly decreased under combined stresses (83% and 72%) and drought stress (52% and 47%) compared with the control. Genotype “Mo17” reduced its green leaf area to minimize the water loss, which appears to be one of the adaptive strategies of “Mo17” under stress conditions. The leaf reflectance of both genotypes varied across treatments. Vegetation indices associated with pigments (chlorophyll index of green, chlorophyll index of red edge, and carotenoid index) and plant health (normalized difference red-edge index) were found to be highly sensitive to drought and combined stressors than heat stress. Combined drought and heat stresses caused a significant reduction in yield and yield components in both Mo17 (49%) and B73 (86%) genotypes. The harvest index of genotype “B73” was extremely low, indicating poor partitioning efficiency. At least when it comes to “B73,” the cause of yield reduction appears to be the result of reduced sink number rather than the pollen and source size. To the best of our awareness, this is the first study that showed how the leaf-level spectra, yield, and quality parameters respond to the short duration of independent and combined stresses during flowering in inbred maize. Further studies are required to validate the responses...
of potential traits involving diverse maize genotypes under field conditions. This study suggests the need to develop maize with improved tolerance to combined stresses to sustain production under increasing temperatures and low rainfall conditions.

**KEYWORDS**
drought stress, flowering, heat stress, hyperspectral reflectance, maize, pollen

## 1 | INTRODUCTION

Maize (*Zea mays* L.) has become one of the most important cereal crops globally for food, animal feed, and fuel (Ranum et al., 2014). It is the third most important cereal crop globally, following wheat and rice, with a total production of 1.16 billion metric tons from 201.98 million hectares cultivated (FAOSTAT, 2021). Among the major maize-producing countries, the United States is the largest producer of maize, with a production of 360.3 million metric tons corresponding to over one-third of the global corn production. Access to irrigation water is becoming an increasingly limiting factor in growing maize under well-managed conditions (Scanlon et al., 2012). In addition, low rainfall events amplify the likelihood of heat stress in the corn-growing regions (Lobell et al., 2014). These climate extremes have caused significant damage to crop production in the United States and other countries in the last three decades (Li et al., 2019; Zhao et al., 2017), contributing to a loss of up to $220 billion (Teresa, 2021). Maize yields are foreseen to decline up to 50% based on empirical and statistical methods (Lobell et al., 2014; Tigchelaar et al., 2018). Drought and heat stresses are the two major abiotic stress factors that often affect the growth and productivity of crop species, including maize. It is anticipated that the impacts of drought and heat stresses events are likely to be exacerbated in the future as these events become more frequent, intense, and erratic (Sanderson et al., 2011).

Among the various growth stages, flowering and grain filling are the two critical phases for yield determination (Ndlovu et al., 2021). Stresses during these sensitive stages impact reproductive success, photosynthesis and biomass-related (source) attributes, resulting in yield loss (Oury et al., 2016; Wang et al., 2021). A decline in seed set was reported under heat stress in wheat (Bheemanahalli et al., 2019), sorghum (Chiluwal et al., 2020), and rice (Jagadish et al., 2007) mainly due to increased pollen sterility. Though drought and heat stresses usually co-occur (Moffat, 2002; Shah & Paulsen, 2003), their effects on plant development are often studied separately. The combined effects of drought and heat stresses have been investigated in some field crops such as wheat (Qaseem et al., 2019), rice (Costa et al., 2021), barley (Zhanassova et al., 2021), peanut (Hamidou et al., 2013), canola (Elferjani & Soolanayakanahally, 2018), sorghum (Johnson et al., 2014), and soybean (Cohen et al., 2021) at different development stages. The above studies reported that exposure of plants to combined stresses could lead to more acute damage than individual stress. Combined stresses, particularly during the stress-sensitive reproductive stages, decreased pollen viability (Bheemanahalli et al., 2021; Rang et al., 2011), gas exchanges (Reddy et al., 2020), yield, and quality parameters (Assefa et al., 2018) in different crops. Maize is highly sensitive to drought and heat stresses compared with other cereals (Barnabás et al., 2008). The short duration of heat stress around anthesis affects the pollen shedding, pollen germination ability, and embryo development in maize (Wang et al., 2021). Further, plants stressed at flowering-early grain filling impair starch synthesis and result in a limited supply of assimilates to the grain during the seed development (Liu et al., 2022). Exposure of early grain filling events to combined stresses attributed to the low endosperm cells and low photosynthetic rates result in reduced seed size (Liu et al., 2022). Several studies reported heat or drought stress-induced physiological changes as stress adaptive strategies (Barnabás et al., 2008; Fahad et al., 2017). The stress tolerance strategies or traits response, including reproductive responses, vary with species (Cohen et al., 2021; Prasad et al., 2017). For example, plants under drought stress close the stomata partially or completely, reduce transpiration, and push plants closer or beyond the critical leaf temperature threshold (Liu et al., 2011). Under heat stress, plants depend on evaporative cooling via transpiration to prevent thermal damage, but little is known about stomatal conductance and transpiration responses to combined stresses in maize at the reproductive stage. In addition, maize pollen germination and physiological traits responses of maize to combined stresses have not been addressed.

Leaf spectral reflectance is a real-time and nondestructive remote sensing application that helps understand plant physiological and biochemical processes with the help of spectral vegetation indices (Yendrek et al., 2017). When exposed to stress, plants undergo various physiological and biochemical modifications, altering leaf reflectance properties. Stress-affected changes in the reflectance properties of leaf in visible wavelengths indicate metabolic disturbance due to changes in leaf chlorophyll concentration (Grzesiak et al., 2010). Recent studies exhibited that drought- or heat-stressed plants tend to have higher reflectance at red-edge and near-infrared regions than non-stressed plants in various crops (Park et al., 2021), indicating that the plant spectral reflectance response varies with the type of stressors it exposed. Literature reports that drought stress can increase leaf/canopy reflectance in the visible region while decreasing in the near-infrared region due to stress responses (Feng et al., 2013; Wijewardana et al., 2019). Similarly, heat stress could increase the leaf reflectance in the near-infrared region (Park et al., 2021).
Even though heat stress often coincides with low rainfall (drought) under field conditions in the United States and other crop-growing regions (Tijchelaar et al., 2018), limited studies investigated the impact of combined stress effects at flowering compared with individual stress due to the complexity and little knowledge on plant functional responses. On the other hand, most previous studies used maize hybrids to understand the drought and heat stresses impact on yield and quality (Liu et al., 2022; Wang et al., 2021). Hybrids often display superior phenotypes due to their vigorous nature (van Dijk et al., 2014), making it hard to compare with inbred maize or breeding line performances. Moreover, traits responses to the combination of drought and heat stresses could be unique from individual stress (Zhou et al., 2017). Thus, the acquired knowledge of plant responses to individual drought or heat stress may not help breed combined stress tolerance unless investigated. Limited information is available on the responses of maize inbred, mainly using the nested association mapping founder lines, to individual and combined drought and heat stresses during flowering. The two whole genomes sequenced inbred lines (“B73” and “Mo17”) with contrasting genome composition and plant phenotype that is extensively used in the maize genomic community were assessed in this study. The current study reports the responses of pollen germination, physiology, spectral properties, yield, and quality attributes of two inbred genotypes to individual and combined drought and heat stresses at the reproductive stage.

2 | RESULTS AND DISCUSSION

Maize genotypes, “B73” and “Mo17”, are the most commonly used parental lines for generating different genetic resources, such as recombinant inbred lines (Lee et al., 2002) and nested associated mapping populations (Gage et al., 2020). Reid yellow dent (represented by “B73” genotype) and Lancaster (represented by “Mo17” genotype) are the two best-known maize variety groups (Sun et al., 2018). Genetic resources created using these parents have been widely used for developing genetic maps and dissecting the genetic architecture of numerous traits (https://nam-genomes.org/). Hybrids created using these two parental lines were commonly grown in the United States and other countries (Troyer, 2004). With a high-quality reference genome availability, these two genotypes are the most routinely used pair of maize inbred donors in genetic and molecular studies (Sun et al., 2018). However, these parental lines’ reproductive, yield, and quality responses to heat, drought, and their combination remain unknown. The current study assessed the reproductive stage responses of these two genotypes to individual and combined drought and heat stresses.

2.1 | Combined drought and heat stresses had a significant effect on pollen germination

Fresh pollen grains collected from four treatments (control, drought, heat, and combined stresses) were incubated at 30°C for 45 min to study the interactive stress effect on pollen viability during flowering. Pollen germination in both genotypes decreased in individual and combined stresses (Figure 1). A short-duration of stress treatments during flowering (anthesis) had a maximum negative impact on pollen germination under combined stresses (42%), followed by heat (30%) and drought stress (19%). However, the responses of the two genotypes were different for heat or combined stresses. Compared with drought-stressed plants, pollen grains from the heat-stressed plants showed a higher reduction (11%) in pollen germination, suggesting the greater sensitivity of maize reproductive processes to heat stress. A similar impact of short duration heat stress on pollen germination was noted in other field crops such as wheat (Bheemanahalli et al., 2017), rice (Jagadish et al., 2010), and sorghum (Chiluwal et al., 2020), including maize hybrids (Wang et al., 2021). The current study shows that pollen grains of inbred maize lines are susceptible to combined drought and heat stresses during pollination, similar to rice (Rang et al., 2011). The observed decrease in pollen germination under heat stress could be due to the damage to pollen physical structure, pollen wall composition, and lower energy (sugar) status during anthesis (Djanaguiraman et al., 2018; Jagadish et al., 2010). The pollen response to drought and heat stresses is genotype-dependent (Schoper et al., 1987). However, considering the pollen germination potential, “Mo17” showed higher reproductive resilience to combined stresses among the two genotypes. Despite 40% pollen germination recorded under drought and combined stresses, significantly lower kernels were noted under stress conditions. Viable pollen of about 20% was sufficient for a successful seed set under stress in upland crops (Chiluwal et al., 2020). This finding suggests

![Figure 1](image1.png)

FIGURE 1 In vitro pollen germination of maize genotypes (B73 and Mo17) under individual and combined drought and heat stresses. Vertical bars denote mean ± SE. Means followed by a common letter are not significantly different by Duncan’s multiple range test at the 5% level of significance. CNT, control; DS, drought stress; HS, heat stress; and DS + HS, combined drought and heat stresses (DS + HS).
that successful reproduction is beyond pollen germination (Begcy et al., 2018; Oury et al., 2016). Investigating male and female reproductive organ or ovary development responses to drought and heat stresses would help understand sensitive events associated with poor grain set in maize. Thus, future research needs to explore the impact of combined stresses on male and female reproductive organs.

2.2 Responses of photosynthetic pigments and physiological attributes to combined drought and heat stresses

Treatments showed significant differences (p < 0.05) for all the physiological traits except chlorophyll and flavonoids (Figure 2). The nitrogen status of the plant significantly influences the chlorophyll or leaf greenness and has been considered the best indicator of crop health (Deng et al., 2019). The leaf pigment is considered a good stress indicator for evaluating tolerance levels under stress conditions (Arumyankar et al., 2008). Measurement of chlorophyll content 13 days after treatments showed the highest reduction under combined stresses (23%) followed by drought (18%) treatment. Both “B73” and “Mo17” had comparable chlorophyll content under heat stress (Figure 2a). However, the additive decrement in chlorophyll content was observed under combined stresses. Both genotypes showed a significant increase in the anthocyanin under combined stresses (14% for “B73” and 21% for “Mo17”), followed by drought stress (8% for “B73” and 14% for “Mo17”). “Mo17” responded to drought and heat stressors by accumulating higher anthocyanin such responses often correlated with stress tolerance (Qin et al., 2021). Unlike drought, heat stress did not affect plant pigments but deteriorates when the heat coincides with drought stress (Figure 2a,b). The reduction in chlorophyll pigments is attributed to the production of reactive oxygen species (ROS) (Chalanika De Silva & Asaeda, 2017), which affects the biosynthesis of the pigment. These results infer that the changes in maize pigment responses to combined stresses are not always similar to the reaction caused by heat or drought stress. Observed genotype-dependent responses might be due to differences in genome structure, sometimes even opposing signaling pathways induced by combined stresses (Zhou et al., 2017).

Stomatal conductance and transpiration are reduced under combined stresses (83% and 72%, respectively), followed by drought stress (52% and 47%, respectively). Heat stress reduced stomatal conductance (52%) but increased transpiration (2%) compared with the plants under control conditions (Figure 2c,d). The transpiration (“B73”) under heat stress was higher than the control and other stress treatments. A decreased stomatal conductance often results in less productivity under drought and heat stress conditions. However, the mechanisms limiting the photosynthesis process are different, requires further investigation. Heat stress decreases photosynthesis by restricting the activity of rubisco (Crafts-Brandner & Salvucci, 2000), whereas drought stress decreases photosynthesis by reducing the stomatal aperture (Griffin et al., 2004). Few studies have isolated the stomatal conductance and transpiration functioning to drought or heat stress and suggest that both stomatal conductance

![Figure 2](https://example.com/figure2.png)

**Figure 2** Effects of drought, heat, and their combination during the flowering stage of maize genotypes (B73 and Mo17) on (a) chlorophyll ($\mu g cm^{-2}$), (b) anthocyanin index, (c) stomatal conductance (mol m$^{-2}$ s$^{-1}$), (d) transpiration (mmol m$^{-2}$ s$^{-1}$), (e) PSII actual photochemical quantum yield (PhiPS2), and (f) photosynthetic electron transport rate (umol e$^{-}$ m$^{-2}$ s$^{-1}$). Vertical bars denote mean ± SE. CNT, control; DS, drought stress; HS, heat stress; and DS + HS, combined drought and heat stresses. Means followed by a common letter are not significantly different by Duncan’s multiple range test at the 5% level of significance.
and transpiration decreased under drought stress. However, transpiration increases in most species under heat stress (Cohen et al., 2021; Grossiord et al., 2020), as observed in the current study. This increased response can be relatable because high temperatures raise respiration and thus require extensive carbon fixation for sustained growth and survival (Crafts-Brandner & Salvucci, 2000). Higher transpiration caused by heat stress induces stomatal closure, which indirectly affects the fixation of CO2 in photosynthesis (Balla et al., 2019) and could have affected dry matter production in this study. Whereas under combined stresses, stomatal conductance and transpiration were decreased in both genotypes. Such responses subsequently increase canopy temperatures (Damour et al., 2010) and impact photosynthesis which could cause declines in growth and yield by creating a higher demand for carbon.

Traits such as quantum efficiencies of photosynthetic electron transport through photosystem II (PhiPS2) and electron transport rate (ETR) are found to co-optimize the photosynthetic capacity in plants (Koike, 1990), thereby improving the crop growth rate (Lusk & Del Pozo, 2002). In this study, PhiPS2 did not vary significantly across treatments in “B73”, and the reduction was significant only for the combined stresses (53%) in “Mo17” compared with other treatments (Figure 2e). The genotype “Mo17” maintained optimal PhiPS2, ETR, stomatal conductance, and transpiration under drought and heat stresses compared with “B73”. These findings clearly explain the ability of “Mo17” to carry out a superior physiological process than “B73” (Figure 2). In addition, reductions in photosynthetic ETR around PSII would cause a decrease in CO2 assimilation rates in other crops (Yamori et al., 2011). Moreover, reductions in ETR could be influenced by the decline in CO2 assimilation rates, as seen in other studies (Yamori et al., 2011).

2.3 Responses of vegetation indices (VIs) to combined drought and heat stresses

Leaf reflectance spectra were collected on the same leaves used for pigments and other physiological measurements to investigate how the leaf reflectance changes in response to stressors. Observed spectra compositions varied substantially across treatments and genotypes (Figure S2). A subset of bands within the 350–880 nm range, which are commonly used in field aerial phenotyping studies, were chosen to understand the discriminating ability of spectral reflectance across treatments. Precisely five sets of bands were chosen as follows: (i) centered at 475 nm/bandwidth of 32, (ii) centered at 560 nm/bandwidth of 28, (iii) centered at 668 nm/bandwidth of 16, (iv) centered at 717 nm/bandwidth of 12, and (v) centered at 842 nm/bandwidth of 58. These five regions were selected to simulate the response of a commercially available MicaSense RedEdge multispectral sensor (Figure S2).

The impact of stress on physiology and biochemical processes can be monitored using the leaf reflectance properties. Previous studies showed the use of spectral changes in determining crop responses/health under stress conditions (Feng et al., 2013; Wijewardana et al., 2019). In addition, studies have shown that VIs based on leaf reflectance can precisely estimate leaf chlorophyll and other biophysical properties (Chen et al., 2021; Gitelson et al., 1996).

To understand the effects of stressors on maize plant health, six commonly used plant health indicators such as green chlorophyll index (Clgreen), red-edge chlorophyll index (Clred-edge), chlorophyll vegetation index (CVI), carotenoid index (CARI), normalized difference red-edge index (NDRE), and normalized difference vegetation index (NDVI) were extracted across treatments using the five regions (Figures 3 and S2). The Clgreen, Clred-edge, and CVI (indicators associated with leaf pigments) showed a significant reduction under combined stresses compared with control (Figure 3a–c). These three VIs (Clgreen, Clred-edge, and CVI) displayed responses comparable with the measured chlorophyll pigment (Figure 2a). As anticipated, the calculated CARI was increased in response to stressors compared with control (Figure 3d). In addition, the NDRE values measured during flowering significantly decreased under drought and combined stresses in both genotypes (Figure 3e). Heat-stressed plants showed NDRE values comparable with control plants. However, lower NDRE values were associated with variations in chlorophyll content (Figure 2a) and green leaf area/shoot biomass (Figure 4). One of the widely used plant health indicators, that is, NDVI, remained relatively stable in response to stressors, except in genotype “Mo17” under drought stress (Figure 3f). A weaker response of NDVI to stressors could be due to the leaf-level measurement rather than canopy level. The present study did not explore leaf-level NDVI to canopy-level NDVI due to the size and height of maize plants. On a positive note, the current results showed that pigment (Clgreen, Clred-edge, and carotenoids) and plant health/vigor associated VIs (NDRE) could better distinguish the stress impacts than the NDVI at the reproductive stage. The present study showed that the variations in light absorption and reflection of the leaves could be used to determine the plant health-related physiological processes under drought and heat stress. However, the current results were achieved using leaf clip reflectance spectra under greenhouse conditions; further field-based validation studies are encouraged before using them for phenotyping studies.

2.4 Responses of aboveground biomass parameters to interactive drought and heat stresses at reproductive stage

Green leaf area and other biomass components were measured across treatments before releasing from the stress treatment. Treatments significantly affected all the measured shoot traits (Figure 4). On average, there was a three-fold increase in dead leaves under combined stresses compared with control treatment during flowering (Figure 4a). At the reproductive stage, the mean green leaf area decreased by 34% (29% for “B73” and 39% for “Mo17”), 22% (20% for “B73” and 24% for “Mo17”), and 18% (24% for “B73” and 13% “Mo17”) under combined, drought, and heat stresses, respectively (Figure 4b). The response of “Mo17” for chlorophyll content and
green leaf area was comparable across treatments. Genotype “Mo17” reduced its green leaf area to minimize the water loss through transpiration, which appears to be one of the adaptive strategies under drought stress conditions. Programmed cell death in response to stress is associated with efficiently recycling of nutrients from the senescing cells to the other parts of the plant, such as young leaves, reproductive organs, or other sink (Munné-Bosch & Alegre, 2004). The reduction in corn leaf dry weight and shoot biomass were highest under combined stresses (35% and 44%, respectively), followed by drought (27% and 34%, respectively) and heat stress (15% and 29%, respectively) compared with the control (Figure 4c,d). Although individual drought and heat stresses had the opposite effect on physiological traits, the green leaf area and shoot biomass parameters were less affected by single stress (Figure 4b,d). The current results suggest greater sensitivity of physiological characteristics to short-duration stressors than the biomass traits at the reproductive stage.

2.5 | Combined drought and heat stresses had the highest impacts on yield and yield-related traits

A short-term drought, heat and combined stresses during flowering reduced the kernel number by 63%, 72%, and 87% across genotypes compared with control, respectively (Figures 5a and S3). Treatment effects were significant for yield-related traits (Table S1). Grain yield, single kernel weight, and harvest index were declined under combined stresses (87%, 29%, and 74%, respectively) compared with individual stresses. Mean values for grain yield varied across the treatments, “B73” was more affected by all treatments (Figure 5b). Drought stress had a lower negative effect on “Mo17” compared with heat or drought and heat stresses. The reduction of single kernel weight and harvest index were higher under drought and combined stresses for “B73” (Figure 5c,d). The single kernel weight, an indirect measure of kernel size (Tiwari & Singh, 2012), was not affected in “Mo17” but was significantly affected in “B73” (Figure 5c). There were variations
between stress treatments for genotypes, with “Mo17” maintaining a higher grain yield than “B73.” A similar trend was noticed for kernel number, single kernel weight, and a harvest index (Figure 5a, c, d). Drought-stressed “Mo17” plants had a harvest index comparable with the control, indicating balanced source–sink or partitioning efficiency (Figures 4d and 5d). Conversely, the genotype “B73” partitioning efficiency is low and extremely sensitive to stressors. Short-term drought and heat stresses around flowering had a significant effect on yield components or sink size and number, as shown in maize (NeSmith & Ritchie, 1992) and other crops (Farooq et al., 2014; Talukder et al., 2014). It was demonstrated that stress-induced changes in starch synthesis and enzymatic activity are associated with
TABLE 1  Effects of drought, heat and their combination on maize seed quality parameters

| Genotype | Treatment | Protein | Oil   | Starch | Fiber  |
|----------|-----------|---------|-------|--------|--------|
| B73      | CNT       | 11.1 ± 0.3c | 3.2 ± 0.2b | 61.3 ± 0.5a | 2.1 ± 0.2c |
|          | DS        | 12.4 ± 0.3b | 3.5 ± 0.1b | 57.6 ± 0.3b | 2.0 ± 0.1bc |
|          | HS        | 13.7 ± 0.3a | 3.7 ± 0.3b | 56.4 ± 0.7b | 2.2 ± 0.2b |
|          | DS + HS   | 13.1 ± 0.2ab | 2.9 ± 0.3a | 60.2 ± 0.4a | 3.1 ± 0.3a |
| Mo17     | CNT       | 11.4 ± 0.1b | 3.6 ± 0.2a | 59.2 ± 0.4a | 1.8 ± 0.1a |
|          | DS        | 11.5 ± 0.1b | 3.9 ± 0.1a | 59.0 ± 0.3a | 1.5 ± 0.1a |
|          | HS        | 12.7 ± 0.2a | 3.5 ± 0.1a | 57.6 ± 0.7a | 1.6 ± 0.1a |
|          | DS + HS   | 12.7 ± 0.2a | 3.5 ± 0.2a | 57.1 ± 0.3a | 1.7 ± 0.1a |

Note: The values shown are mean ± SE. ns indicates nonsignificant. Means followed by a common letter are not significantly different by Duncan’s multiple range test at the 5% level of significance.

Abbreviations: CNT, control; DS, drought stress; HS, heat stress; DS + HS, combined drought and heat stresses.
*p < .05, **p < .01, ***p < .001.

2.6  Warmer growing temperature in combination with drought stress affects maize quality

Maize kernels are predominantly composed of carbohydrates (mainly starch), protein, lipids, minerals, and crude fiber (Baye et al., 2006). Significant variations in kernel quality parameters were recorded for genotype, treatment, and interaction (Table 1), but the treatment and interaction were not substantial for oil. Compared with control, seed protein was increased in response to stress treatments for both genotypes, and the differences were 6% for drought stress, 17% for heat stress, and 15% for combined stresses. Drought- and heat-stressed “B73” recorded a decline in starch. The most significant increase in fiber was recorded in “B73” (10%) under combined stresses compared with control. Considerable increase of protein (15%) in “Mo17” was associated with a decrease (7%) in starch under combined drought and heat stresses. Similar responses to stressors have been observed in wheat (Impa et al., 2020), maize (Yang et al., 2018), soybean (Assefa et al., 2018, 2019), and other crops (Farooq et al., 2014). These findings suggest that shortduration stress during flowering can affect maize’s seed quality. Moreover, breeding for high yielding varities often compromises seed quality and stress tolerance. Reduction in starch ranged from 3% to 5% (Table 1). The seed that accumulated more protein tends to have lesser starch content and vice versa, supporting the earlier observations in maize and other crops (Impa et al., 2020; Scrob et al., 2014; Stevanovic et al., 2012). The poor yielding genotype “B73” recorded higher protein and fiber under stress than the control treatment or “Mo17” (Table 1), indicating that few numbers of fertile ovaries or viable embryos under stress might have received adequate assimilates due to less competition. The current results suggest that breeding for higher seed quality in maize is necessary to attract higher market revenue while targeting higher yield, particularly in environments with high temperature and low rainfall during flowering and grain filling (Scrob et al., 2014; Stevanovic et al., 2012).

3  CONCLUSION

The genotypes “B73” and “Mo17” examined in this study exhibited differential responses to drought, heat, and combined stresses. The effect of drought stress was similar to combined stresses and more significant than heat stress. Stomatal conductance and transpiration responses were opposite for heat stress (high) and drought or combined stresses (low). Heat stress was found to have a highly detrimental effect on pollen germination than drought stress. We observed a decrease in physiology, yield components, and impaired kernel quality.
under combined drought and heat stresses. The impact of stressors on physiology, and yield followed the order HS < DS < DS + HS, except pollen germination. Though “Mo17” showed relatively higher tolerance to drought stress, it appears sensitive to heat and combined stresses in yield. Overall research results suggest that (a) pigments associated with VIs could be used to differentiate drought and heat stress effects at the reproductive stage, (b) a genotype capable of maintaining maximum viable pollen and a balanced source–sink during flowering could help minimize yield loss under stress conditions, and (c) traits responses to individual and combined drought and heat stresses are not necessarily summative.

4 | MATERIALS AND METHODS

4.1 | Crop husbandry

Two whole-genome sequenced genotypes, “B73” and “Mo17”, with contrasting plant types and genomic compositions, were used to evaluate the impact of drought stress, heat stress and their combinations at the reproductive stage. The experiment was conducted in greenhouses at the R Rodney Foil Plant Science Research Center, Mississippi State University, Mississippi State (33°28’ N, 88°47’ W), Mississippi, USA. Four seeds were sown at a depth of about 3 cm in a 23-L pot (top diameter = 35.5 cm, bottom diameter = 29.5 cm, and height = 29.2 cm), filled with farm soil, and grown under natural solar radiation with no supplement light. The seedling was thinned to one per pot at the two-leaf stage.

All pots were fertilized with a controlled-release Osmocote (4 g) fertilizer (14–14–14 of N-P-K; ICL Specialty Fertilizers, Dublin, Ohio, USA) before sowing and top dressed (4 g) once a week throughout the vegetative and reproductive periods. After the seedling emergence, a systemic insecticide Marathon 1% G (imidacloprid, OHP, Inc., Mainland, Pennsylvania, USA) was applied to each pot (4 g) to avoid sucking pest infestation. Plants were sprayed with Sanmite insecticide (Gowan®, Yuma, Arizona, USA) at a rate of 1.89 g gal⁻¹ to control mites before tasseling. All plants were grown under optimum temperature (30°C/22°C, maximum day/minimum night, control) and volumetric soil moisture content (VWC) above 0.15 m³ m⁻³ (100% irrigation) until the start of stress treatments. All pots were supplied with optimal irrigation (100%) through an automated time-based pre-programmed drip irrigation schedule until treatment impositions (62 days after sowing).

4.2 | Stress treatment

At tassel emergence, plants grown under control condition were subjected to four (control [30°C daytime temperature, characterized as irrigated, 100% irrigation], heat stress [HS, 35°C + 100% irrigation], drought stress [40% irrigation; 0.06 m³ m⁻³ VWC], and drought and heat stresses [35°C + 40% irrigation]) treatments for 13 days. A minimum of four replicates (each replication had three plants) were maintained for each genotype per treatment. The thermostat regulating the maximum temperature inside the greenhouses was set to 30°C (control) or 35°C (heat stress) in the daytime (08:00–14:00 h) and 22°C at nighttime (18:15–5:45 h) during the treatment period. The cooling pad and backside ventilation flaps of the greenhouse were programmed to automatically open when the temperature exceeds the set maximum daytime temperature inside the greenhouses.

Two greenhouse microclimatic (temperature and relative humidity) conditions were monitored at 15-min intervals using the HOBO data loggers (Onset Computer Corporation, Bourne, Massachusetts, USA). The soil moisture was monitored daily by placing a soil moisture sensor at 15-cm depth (Model EM55b Soil Moisture, Decagon Devices, Inc., Pullman, Washington, USA). The irrigation was adjusted with a semi-automated irrigation method based on evapotranspiration (Gajanayake et al., 2014). In drought stress treatment, irrigation was cut off until soil water content reached the desired soil moisture content t½ (0.06 m³ m⁻³ VWC). Then, all pots were supplied with the necessary volume of water to maintain 40% irrigation until the end of stress treatment. Air temperatures inside the greenhouses during the day/night were close to the set temperatures of 30°C/22°C (actual 31.4°C/21.8°C, SD ± 1.3/0.7) and 35°C/22°C (actual 35.9°C/25.3°C, SD ± 1.6/0.5) in control and heat stress treatments, respectively (Figure S1). All plants were open pollinated across treatments. After the treatment (13 days after stress), plants were transferred back to control conditions and maintained until physiological maturity.

4.3 | In vitro pollen germination

To determine the effects of stress on the maize male reproductive organ (pollen), fresh pollen grains were collected, from the middle portion of the tassel, on the seventh day of stress imposition across treatments between 8:30 and 9:30 a.m. and dusted onto a liquid pollen germination media containing H2BO3 (0.0005% [w/v]), CaCl2 (10 mM), KH2PO4 (0.05 mM), sucrose (10% [w/v]), and polyethylene glycol 6000 (6%) without agarose, with slight modification to Begcy et al. (2018) protocol. The chamber slides with pollen grains were incubated at 32°C for 45 min (Fisher Scientific, Inc., Suwanee, Georgia, USA) and visualized using a light compound microscope (AmScope with MU035 camera, California, USA) at 100× magnification. In vitro pollen germination was calculated as the ratio of germinated pollen to a total number of pollen grains. Approximately over 100 pollen grains were counted from each chamber slide. Pollen grains were considered grown if the length of the pollen tube was higher than the diameter of the pollen grain (Bheemanahalli et al., 2019).

4.4 | Pigments, physiological, and shoot traits at reproductive stage

Leaf chlorophyll content, flavonoids index, anthocyanin index, and nitrogen balance index (NBI, the ratio of chlorophyll content and
flavonoids) were measured on the leaf close to cob, across treatments using a handheld Dualex® Scientific instrument (Force A DX16641, Paris, France) at 13 days after stress (75 days after sowing). A portable handheld LI-600 porometer system integrated with a fluorometer (LI-COR Biosciences, Lincoln, USA) was used to measure stomatal conductance, transpiration, ETR, and photosynthetic efficiencies photosystem 2 (PhiPS2) across treatments between 10:00 and 12:00 h. At 13 days after stress, a subset of plants in each treatment were hand harvested, and plant height (cm) and dead and green leaf numbers were recorded. Total green leaf area (cm² plant⁻¹) was measured using LI-3100 leaf area meter (LI-COR Inc.). Plant shoot components such as leaf weight and stem weight were determined by drying the samples at 75°C for until a constant weight was reached.

4.5 Spectral vegetation indices

Leaf hyperspectral spectral (350 and 2500 nm) data were collected between 11:00 and 12:00 h using a PSR + 3500 spectroradiometer (Spectral Evolution, Massachusetts, USA) attached to a leaf clip assembly with fiber optical cable and internal light source. The spectral range of the instrument is 350–2500 nm (2150 bands) with a spectral resolution of 2.8 nm at 700 nm, 8 nm at 1500 nm, and 6 nm at 2100 nm full width at half maximum. A white reference panelboard was used to calibrate the instrument. Ten instantaneous spectral reflectance measurements were recorded from the adaxial surface in each treatment for each genotype and treatment by keeping the leaf vertical to the optical probe at 13 days after stress imposition. Each measurement is an average of 10 readings. At the beginning of each treatment, a white reference measurement was taken; each measurement was then radiometrically calibrated based on the white reference. To simulate the response similar to a commercially available MicaSense RedEdge multispectral sensor, five sets of bands ([a] centered at 475 nm/bandwidth of 32 [blue–B], [b] centered at 560/bandwidth of 28 [green–G], [c] centered at 668/bandwidth of 16 [red–R], [d] centered at 717/bandwidth of 12 [red edge–RE], and [e] centered at 842/bandwidth of 58 [near infrared–NIR]) were used to calculate the VIs. Further, principal components of 146 bands (~7% of total spectral bands) revealed distinguishing abilities like the full spectrum of bands (2150 bands) with an overall classification accuracy of 87%. Thus, using the five sets (Figure S2) of bands (blue–B, green–G, red–R, red edge–RE, and near infrared–NIR), six VIs such as CIgreen (Gitelson et al., 2003), Cred-edge (Steele et al., 2008), CVI (Vincini et al., 2008), CARI (Zhao et al., 2003), NDRE (Jorge et al., 2019), and NDVI (Carlson & Ripley, 1997) were calculated based on the following equations.

\[
\text{Chlorophyll index of green (CIgreen)} = \frac{(R_{\text{NIR}} - R_{\text{G}})}{R_{\text{G}}},
\]

\[
\text{Chlorophyll index of red edge (Cred-edge)} = \frac{(R_{\text{NIR}} - R_{\text{RE}})}{R_{\text{RE}}},
\]

\[
\text{Normalized difference red edge index (NDRE)} = \frac{(R_{\text{NIR}} - R_{\text{RE}})}{(R_{\text{NIR}} + R_{\text{RE}})},
\]

\[
\text{Normalized difference of vegetation index (NDVI)} = \frac{(R_{\text{NIR}} - R_{\text{G}})}{(R_{\text{NIR}} + R_{\text{G}})},
\]

\[
\text{Carotenoid index (CARI)} = \frac{R_{720}}{R_{810}},
\]

\[
\text{Chlorophyll vegetation index (CVI)} = \frac{(R_{\text{NIR}} + R_{\text{RE}})}{(R_{\text{G}} + R_{\text{RE}})}.
\]

where \(R_{\text{G}}, R_{\text{RE}}, \text{and } R_{\text{NIR}}\) represent leaf reflectance in green (547–574 nm), red (660–676 nm), red-edge (711–723), and near-infrared (814–871 nm) regions, respectively.

4.6 Yield and quality parameters

Plants were manually harvested at physiological maturity to obtain aboveground biomass, yield, and yield components. The cobs were separated from the plant. The shoot was oven-dried to estimate the harvest index. Cobs were air-dried in the greenhouse conditions for 7 days and hand threshed to obtain yield components such as kernel numbers, kernel weight, and single kernel weight. Harvest index was estimated as the ratio of economic yield per plant to the total aboveground biomass. A Perten DA7250 (Perten Instruments, Springfield, Illinois, USA) near-infrared spectrometer (NIRS) was used to scan the seed quality compositions (protein, oil, starch, and fiber) of maize samples. Seed samples were examined using a small white stationary cup. Each sample was scanned two times by repacking the sample cup after each scan.

4.7 Statistical analysis

All measured physiology, yield, quality, and spectral data across treatments were subjected to statistical analysis. The significance of means was estimated through the F value for each trait. Duncan’s multiple range test was performed to assess the significant difference between individual treatment in each genotype using GEN STAT (12th edn). The graphs were generated using SigmaPlot 14.5 software (Systat Software, Inc. SigmaPlot for Windows). Principal component analysis was performed to identify the unique spectral differences across treatments using the scikit-learn package (Pedregosa et al., 2011) in Python (Python Software Foundation, 2019).

SIGNIFICANCE STATEMENT

This study showed that short-term combined drought and heat stresses around flowering stage can significantly affect maize’s pollen germination, leaf spectral properties, yield- and quality-related traits. The impacts were more acute under drought and heat stress combination followed by drought and heat stresses on most
parameters. Leaf-level spectra can determine drought and heat stress-induced changes in plant health at the reproductive stage. Phenotypic responses of maize genotypes differed between individual and combined stresses. Genotypes capable of maintaining maximum pollen viability and a balanced source–sink potential during flowering could help minimize yield reduction under stress conditions.

ACKNOWLEDGMENTS
We thank Mr. David Brand for technical assistance and Plant Stress Physiology Laboratory undergraduate students for their support during data collection. We would also like to thank Dr. Peter Balint-Kurti (Department of Entomology and Plant Pathology) for donating the pure inbred seeds used for this study. This work was funded by Mississippi Agricultural and Forestry Experiment Station, Special Research Initiative (MAFES-SRI), the USDA-Agricultural Research Service (USDA-ARS) (58-6064-9-007), and National Institute of Food and Agriculture (MIS 043050).

AUTHOR CONTRIBUTIONS
R.B. conceptualized the research. R.B. and K.R.R. designed the experiments. R.B., P.R., S.S., and N.W. analyzed the data. R.B., S.P., and R.B. conceptualized the research. R.B. and K.R.R. designed the experiment. S.S. performed the data collection. R.B. and P.R. wrote the first draft of the article. All authors read and approved the manuscript.

CONFLICT OF INTEREST
The Authors did not report any conflict of interest.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Bheemanahalli, R., Ramamoorthy, P., Poudel, S., Samiappan, S., Wijewardane, N., & Reddy, K. R. (2022). Effects of drought and heat stresses during reproductive stage on pollen germination, yield, and leaf reflectance properties in maize (*Zea mays L.*). *Plant Direct*, 6(8), e434. https://doi.org/10.1002/pld3.434