Safeguarding Mississippi soybean production from interactive heat and drought stress-induced yield and quality losses (Project#: 43-2022)
FINAL REPORT

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BACKGROUND AND OBJECTIVES

Soybean yields have been inversely related to maximum summer air temperatures, with yield suppression of up to 6%, when the temperatures are above 85 °F during critical developmental stages under rainfed conditions. In addition to the various impacts observed, the detailed analysis highlights that combined drought and heat stress will amplify a negative effect on seed number, seed weight, and seed composition in current and future production systems. Not surprisingly, the highest yield and quality penalty are expected to occur when the heat and drought stresses coincide with flowering and pod filling in soybean (R1 to R6). Heat stress at flowering induces significant damage to male reproductive organ viability and causes a linear decline in seed set. Long-duration stress during pod filling impairs physiological responses and affects source-sink relations. In general, soybean yield is dependent on the amount of photo-assimilate produced in the leaves (source) to supply to the seed (sink) and the potential of the seed to utilize resources during seed filling. In most soybean growing regions, including Mississippi, maximum temperatures during reproductive and seed-filling stages are already >85 °F highlighting the crop's vulnerability to heat stress. For every 0.8 °C increase above 26.7 °C (80 °F), the current mean temperature of the southern United States, soybean yields are expected to decline by 2.4%. Under field conditions, soybean exposed to simultaneous high temperature (97 °F) and drought stress suppresses the plant health, quality, and nutritional value of beans by reducing leaf photosynthetic rate, stomatal conductance, and increasing canopy temperatures during R1 to R6. To date, soybean breeding programs are successful in increasing yield potential in a favorable environment. Breeding for interactive stress tolerance hasn't progressed much because of the complex interaction between stressors and the limited knowledge of how traits respond to stressors. To address these knowledge gaps, we quantified soybean plant health, yield, and seed composition under individual and interactive heat and drought conditions.

The objectives of the project were:

Objective 1: Determine soybean reproductive and physiological responses to interactive heat and drought stress.

Objective 2: Quantify the impact of heat and drought stress on soybean seed yield and quality dynamics.

REPORT OF PROGRESS/ACTIVITY

A two-year study was conducted during the soybean growing season of 2021 and 2022 (from May to September) at the R. R. Foil Plant Science Research Center Greenhouse Facility, Mississippi State University, to quantify the impacts of the individual and combined heat and drought stress on the reproductive success and yields of soybean. The graduate student-led experiment consisted of ten soybean cultivars/lines including eight commercially cultivated cultivars from different industries (Armor, AgriGold, Local Seed, Dyna-Gro, Progeny Ag, Donmario, and Delta Grow) and two breeding lines specifically chosen for their high-yield potential and rich protein content. The ten soybean cultivars were planted in pots (10 cultivars x 4 treatments x 8 or 10 replications) filled with farm soil and grown under non-stress conditions until flowering. At flowering, plants were subjected to four different treatments; control (32 °C daytime temperature + 100% irrigation, characterized as a present growing condition), drought (50% irrigation + 32 °C daytime temperature), heat stress (38 °C daytime + 100% irrigation), and drought and heat (38 °C daytime temperature + 50% irrigation, future predicted soybean growing conditions) for a duration of 30 days. The study quantified the stress-induced changes by individual and combined heat and drought treatments in photosynthetic pigments like chlorophyll content, and physiological traits such as stomatal conductance, transpiration, canopy/leaf temperature, and other relevant physiological parameters. Further, we examined yield traits (pod and seed weight and number) and seed quality traits (protein and oil) responses to stressors. Stress tolerance index rankings were used to identify drought or heat or combined stress-sensitive and tolerant soybean cultivars.

Obj. 1: Determine soybean reproductive and physiological responses to interactive heat and drought stress

Year 1: Cultivar, treatment, and cultivar-treatment interaction significantly influenced all physiological parameters (p <0.05 to p <0.001), except chlorophyll content. Compared to the control, the chlorophyll content was significantly decreased by 24% under combined stress followed by drought (9%) and heat stress (3%) (Figure 1A). Across the stress treatments, the cultivar R01-416F had the least reduction in chlorophyll content (Figure 2A). Under combined and drought stress, a substantial reduction (98% and 95% respectively) in stomatal conductance was observed compared to the control (Figure 1B). The transpiration rate followed a similar trend as the stomatal conductance with a reduction of 91% and 82% under combined and drought stress respectively. Whereas the stomatal conductance decreased by 11% and transpiration increased by 5% under heat stress as compared to the control (Figure 1C). The cultivar R01-416F lowest showed an increase in stomatal conduction under heat stress (Figure 3A). On average, the plants under combined stress had higher canopy temperature (+9 °C), followed by heat (+4.6 °C) and drought stress (+1.8 °C) than the optimum temperature (33.7 °C). Cultivars R01-416F and DM 45X61 maintained cooler canopy across treatments compared to the other cultivars (Figure 3B).

Year 2: A significant cultivar × treatment interaction (p < 0.05) was observed for pigments and physiological parameters except for the chlorophyll content, and canopy temperature (Table 1). The chlorophyll content did not vary significantly under control and drought stress, whereas it was reduced under heat (16%) and combined stress (10%) as compared to control (Figure 1E). The cultivar DM45X61 (23% decrease) and LS5009XS (18% decrease) displayed a maximum reduction in chlorophyll content under heat and combined stress compared to the control (Figure 2B). The cultivar P46A86X performed consistently better under heat, drought, and combined stress, with the lowest reduction in chlorophyll content. The plants subjected to combined stress had a lower stomatal conductance (93% decrease) and transpiration (68% decrease) (Figure 1F-G). On average, when compared with the control, the plants exposed to combined stress had a higher canopy temperature (+8 °C ± 1.5) followed by heat (+5 °C ± 1.3) and drought stress (+2 °C ± 1.5) (Figure 1H). Under drought, the cultivar G4620RX displayed a maximum reduction in stomatal conductance (75%) compared to the control. The cultivar S48XT90 had a maximum reduction in stomatal conductance (95%) under combined stress compared to the control. The cultivars 44-D49 and R15-2422 displayed a minimum reduction in stomatal conductance and a cooler canopy under combined stress (Figure 3C, D).

Overall, the physiological traits of cultivars responded differently to individual and combined stressors. For instance, drought stress led to a significant decline in stomatal conductance across all cultivars, leading to reduced transpiration to prevent loss of water. Conversely, under heat stress, transpiration was increased in all the cultivars to maintain the cooler canopy temperature. However, when these stresses were combined, both stomatal conductance and transpiration were significantly reduced resulting in higher canopy temperature in all cultivars. Cultivars R01-416F and R15-2422 were found to have better adaptability and resilience in terms of physiological performance across the stress treatments in both years.

Obj. 2: Quantify the impact of heat and drought stress on soybean yield and quality dynamics

Year 1 (2021): Significant differences were observed between cultivar, treatment, and their interactions for yield and quality parameters (Table 1). The reduction in seed number was significantly *at par* under drought stress (46%) and combined stress (43%) as compared to control (Figure 4). Whereas seed number was reduced by 19% under heat stress compared to the control (Figure 4A). The seed weight was severely impacted by combined stress (46% decline) followed by drought stress (33% decline) and heat stress (23% decline) compared to control (Figure 4B). Across the treatments, the cultivar P46A86X displayed a maximum reduction in seed number and weight, with a reduction of more than 50% under combined stress (Figure 5A, B). In addition, the hundred seed weight was also measured as a proxy of the seed size. Under drought stress, the seed size increased, whereas under heat and combined stress, the seeds were small and wrinkled in comparison to the control. The seed size increased by 20% under drought stress and reduced by 5% and 8% under heat and combined stress (Figure 4C). The cultivar R15-2422 had the smallest seed size across the stress treatments among the ten cultivars (Figure 5C). The seed protein was decreased by 6% under combined stress followed by heat stress (Figure 6A). Whereas increased protein (4%) under drought stress decreased oil by 2% compared to control. The oil content increased by 11% under combined stress conditions (Figure 6B). The protein and oil content were negatively correlated across treatments. The cultivar P46A86X showed the lowest reduction in protein and the highest increase in oil content under heat and combines stress (Figure 6A, B).

Year 2 (2022): Yield components showed significant variation between treatments ($p < 0.001$, Table 1). Significant reductions in seed number and weight were observed under drought (49%

and 36%) and heat (27% and 34%) stress compared to control (Figure 4D, E). This reduction was more than 50% under the combined stress as compared to the control. Under drought, the cultivar 44-D49 displayed a maximum reduction in seed number (55%), and the cultivar P46A86X displayed a maximum reduction in seed weight (42%) as compared to the control (Figure 5D-E). Cultivar LS5009X had the lowest reduction in seed weight (24%) under drought compared to the control (Figure 5E). Under heat and combined stresses, the cultivar G4620RX displayed a maximum reduction in the yield parameters compared to the control (Figure 5D-E). The hundred seed weight was increased significantly by 28% under drought stress compared to control, whereas it was decreased by 8% under heat and 6% under the combined stress (Figure 5F). Significant treatment ($p < 0.001$), cultivar ($p < 0.001$), and treatment × cultivar interaction ($p < 0.05$) were observed for protein and oil (Table 1). Compared to the control, protein content increased by 7%, while oil content decreased by 11% under drought. The cultivar 4775E3S had a maximum increase (13%) in protein content and a maximum reduction (16%) in oil content under drought as compared to the control (Figure 6C-D). The protein content under heat and combined stress were similar to that of the control. The cultivar R01-416F showing a maximum reduction in protein content had a maximum increase in oil (7%) under heat stress compared to the control. Under combined stress, the cultivar P46A86X recorded a maximum increase in protein content, and cultivar 44-D49 had a maximum decrease in oil content (Figure 6C-D).

In summary, the two-year study revealed that the combination of drought and heat stress during flowering and early-seed filling detrimental to yield and quality-determining traits followed by single stress such as drought and heat. All the cultivars displayed considerable variability in their response to individual and combined heat and drought stress. The stress tolerance index value revealed that G4620RX was the most stress-tolerant cultivar for yield traits (Figure 7), followed by R15-2422 and 44-D49, consistently performing better in both years across all stress treatments. In addition, R15-2422 and S48XT90 exhibited better performance for protein and oil content under stress among the ten cultivars, respectively. These findings suggest that the use of stress-tolerant cultivars, such as G4620RX, R15-2422, and 44-D49, might be beneficial for ensuring sustainable soybean production under hot and dry conditions.

IMPACTS AND BENEFITS TO MISSISSIPPI SOYBEAN PRODUCERS

Soybean farming is a vital part of Mississippi's agricultural economy, second to poultry and forestry. It is a major row crop, covering a total of 2,280 thousand acres and producing 128 million bushels with an average yield of 56 bushels per acre. Around 3,080 soybean farms in Mississippi could benefit from the findings of a two-year study that revealed the negative impact of heat, drought, and combined stress on soybean yield. The study found that yield reductions due to these factors were 28%, 34%, and 52%, respectively. These numbers suggest that, per acre, farmers could lose up to $414, $503, and $754 annually due to heat, drought, and interactive stress, respectively ($14.8 cost per bushel). Given these significant losses, it is important to find novel solutions that can mitigate the impact of drought and heat stress on soybean farms. Our research identified promising high-yield soybean cultivars that are better adapted to heat and drought stress.

By utilizing these cultivars, Mississippi soybean growers can reduce the risks associated with drought and heat, which could lead to a more sustainable and profitable future.

END PRODUCTS–COMPLETED OR FORTHCOMING

Publications

- Poudel S, Adhikari B, Dhillon J, Reddy KR, Stetina SR, Bheemanahalli R. 2023. Potential impacts of heat stress on soybean physiology, yield components, and quality. *Plant Stress* (Under review).
- Poudel S. 2023. On the cover: Quantifying impact of heat and drought stress on soybeans. *Crop Sciences*, 63-3. https://acsess.onlinelibrary.wiley.com/doi/pdf/10.1002/csc2.20785
- Poudel S. 2023. Physiological and yield responses of soybean cultivars to heat and drought stresses. Master's Thesis (Agronomy). Department of Plant and Soil Sciences, College of Mississippi State University. (Approved).
- Poudel S, Vennam, RR, Shrestha A, Reddy KR, Wijewardane N, Reddy KN, Bheemanahalli R. 2023. Resilience of soybean flowering and early-seed setting stages to drought stress. *Scientific Reports*, 13, 1277. https://doi.org/10.1038/s41598-023-28354-0
- Bheemanahalli R, Poudel S, Alsajri FA, Reddy KR. 2022. Phenotyping of southern united states soybean cultivars for potential seed weight and seed quality compositions. *Agronomy*, *12*(4), 839 https://doi.org/10.3390/agronomy12040839

Abstracts published or presentations at conferences

1. **Poudel S**, Reddy KR, Bheemanahalli R. 2023. Characterization of Southern United States soybean cultivars for heat and drought stress tolerance Feb 25, Spring Graduate Research symposium, Mississippi State University, Mississippi. (Poster presentation).
2. **Poudel S**, Reddy KR, Bheemanahalli R. 2023. Elucidating the impacts of drought stress during pod development in soybeans. Feb 25, Spring Graduate Research symposium, Mississippi State University, Mississippi. (Oral presentation).
3. **Poudel S**, Reddy KR, Bheemanahalli R. 2023. Characterization of Southern United States soybean cultivars for heat and drought stress tolerance. Feb 23-24, Annual MAS Meeting, Biloxi, Mississippi. (Poster presentation).
4. **Poudel S**, Reddy KR, Bheemanahalli R. 2023. Elucidating the impacts of drought stress during pod development in soybeans. Feb 23-24, Annual MAS Meeting, Biloxi, Mississippi. (Oral presentation).
5. **Poudel S**, Reddy KR, Bheemanahalli R. 2022. Heat stress impact on soybean physiology, yield, and quality during pod development. ASA, CSSA & SSSA Virtual International Annual Meeting, November 6-9, Baltimore, MD, USA. (Oral presentation).

6. **Poudel S**, Reddy KR, Bheemanahalli R. 2022. Phenotyping of soybean cultivars for interactive drought and heat stress tolerance. ASA, CSSA & SSSA Virtual International Annual Meeting, November 6-9, Baltimore, MD, USA. (Poster presentation).
7. **Poudel, S.,** Vennam R.R., Reddy, K.R, and Bheemanahalli, R. (2022). Impact of Elevated Temperature and Low Rainfall in Soybean Yield and Quality. 4th Annual MAS Summer Science and Engineering Symposium*,* June 8 Mississippi State University, MS. (3-Minute Oral Presentation)
8. **Poudel, S**., Vennam R.R., Reddy, K. R, and Bheemanahalli, R. (2022). Phenotypic characterization of soybean cultivars for heat and drought stress tolerance. 4th Annual MAS Summer Science and Engineering Symposium, June 8, Mississippi State University, MS. (Poster presentation)
9. **Poudel, S**., Bheemanahalli, R., Vennam R.R., and Reddy, K. R. (2022). Independent and Combined Effects of Heat and Drought Stress During Pod Filling on Soybean. 3rd Biennial International Scientific Conference of Association of Nepalese Agricultural Professionals of America, May 28-29, Atlanta, Georgia. (Oral presentation)
10. **Poudel, S**., Bheemanahalli, R., Vennam R.R., and Reddy, K. R. (2022). Impact of Heat Stress on Pollen Germination, Physiology, Yield, and Quality in Soybean. Mississippi Academy of Sciences, *86th* Annual Meeting, March 31- April 01, Biloxi, MS. (Poster presentation)
11. **Poudel, S**., Bheemanahalli, R., Vennam R.R. and Reddy, K. R. (2022). Independent and Combined Effects of Heat and Drought Stress During Pod Filling on Soybean. Mississippi Academy of Sciences, *86th* Annual Meeting, March 31- April 01, Biloxi, MS. (Oral presentation)
12. **Poudel, S**., Bheemanahalli, R., Vennam R.R., and Reddy, K. R. (2022). Interactive Effects of Heat and Drought Stress on Soybean Yield and Quality. Graduate Research Symposium, February 26, Mississippi State University, MS. (Oral presentation)
13. **Poudel, S**., Bheemanahalli, R., Vennam R.R., and Reddy, K. R. (2022). Drought Stress Impacts on Soybean Pollen Germination, Physiology, and Yield. Southern Branch of American Society of Agronomy, February 12-14, New Orleans, LA. (Poster presentation)
14. **Poudel, S**., Rangappa, R. B., Vennam R.R., and Reddy, K. R. (2022). Interactive Effects of Heat and Drought Stress on Soybean Physiology, Pollen Germination, Yield, and Quality. Southern Branch of American Society of Agronomy, February 12-14, New Orleans, LA. (Oral presentation)
15. **Poudel, S**., Bheemanahalli, R., and Reddy, K. R. (2021). Interactive Heat and Drought Stress Impacts on Pollen Germination and Physiological Attributes in Soybeans. ASA, CSSA & SSSA Virtual International Annual Meeting, November 9-11, Salt Lake City, UT, USA.
16. **Poudel, S**., Bheemanahalli, R., Sehgal, A., Brand, D., Walne, C. H., & Reddy, K. R. (2021). Impact of Salt Stress on the Growth and Development of C 3 and C 4 Crop Species.

ASA, CSSA & SSSA Virtual International Annual Meeting, November 9-11, Salt Lake City, UT, USA.

Awards and Honors

1. **2023 Graduate Research Assistant of the Year** (Master's Category) by The Graduate School, April 21, Mississippi State University, Mississippi.
2. **Awarded Second Position** in Poster Presentation at Spring Graduate Research symposium, Feb 25, Mississippi State University, Mississippi.
3. **Awarded Third Position** in Oral Presentation at Spring Graduate Research symposium, Feb 25, Mississippi State University, Mississippi.
4. **Awarded Second Position** in Poster Presentation at Annual MAS Meeting, Feb 23-24, Biloxi, Mississippi.
5. **Awarded Third Position** in Oral Presentation at Annual MAS Meeting, Feb 23-24, Biloxi, Mississippi.
6. **Awarded Second Position** in Poster Presentation at the Graduate Research Symposium, October 22, 2022, Mississippi State University, MS.
7. **Awarded Third Position** in Oral Presentation at the Graduate Research Symposium, October 22, 2022, Mississippi State University, MS.
8. **Awarded Second Position** in 3- Minutes Oral Presentation at the 4th Annual MAS Summer Science and Engineering Symposium. On June 8, 2022, Mississippi State University.
9. **Honored** by Mississippi INBRE for being selected as one of the top 20 graduate students in the Mississippi INBRE Graduate Scholars Symposium at the Mississippi Academy of Sciences Honoring Excellence in Science, 86th Annual Meeting, 2022, Biloxi, MS.
10. **Awarded Second Position** in Oral Presentation at 3rd Biennial International Scientific Conference of the Association of Nepalese Agricultural Professionals of America. On May 28-29, 2022, Atlanta, Georgia.
11. **Awarded First Position** in Poster Presentation at Mississippi Academy of Sciences, 86th Annual Meeting. On March 31- April 01, Biloxi, MS.
12. **Awarded Third Position** in Poster Presentation at the Southern Branch of the American Society of Agronomy. On February 12-14, New Orleans, LA.
13. **Honored** by Mississippi INBRE for being selected as one of the top 20 graduate students in the Mississippi INBRE Graduate Scholars Symposium at the Mississippi Academy of Sciences Honoring Excellence in Science, 85th Annual Meeting, 2021, Biloxi, MS.

TABLE AND GRAPHICS

Table 1. Summary of analysis of variance across the cultivar (C), treatment (T), and their interaction (T x C) for some of the key physiology, yield, and quality traits.

	2021			2022		
Traits	**T**	**C**	**T x C**	**T**	**C**	**T × C**
Chlorophyll content (μg cm^{-2})	***	ns	***	***	***	ns
Stomatal conductance (mol m^{-2} s^{-1})	***	***	***	***	***	***
Transpiration (mmol m^{-2} s^{-1})	***	*	***	***	***	***
Canopy temperature (°C)	***	***	**	***	*	ns
Seed number ($plant^{-1}$)	***	***	***	***	***	***
Seed weight (g $plant^{-1}$)	***	***	***	***	***	***
Hundred seed weight (g)	***	***	***	***	***	ns
Protein (%)	***	***	***	***	***	**
Oil (%)	***	***	***	***	***	***

*, **, and ***, indicate significance levels at $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively. 'ns' indicates nonsignificant.

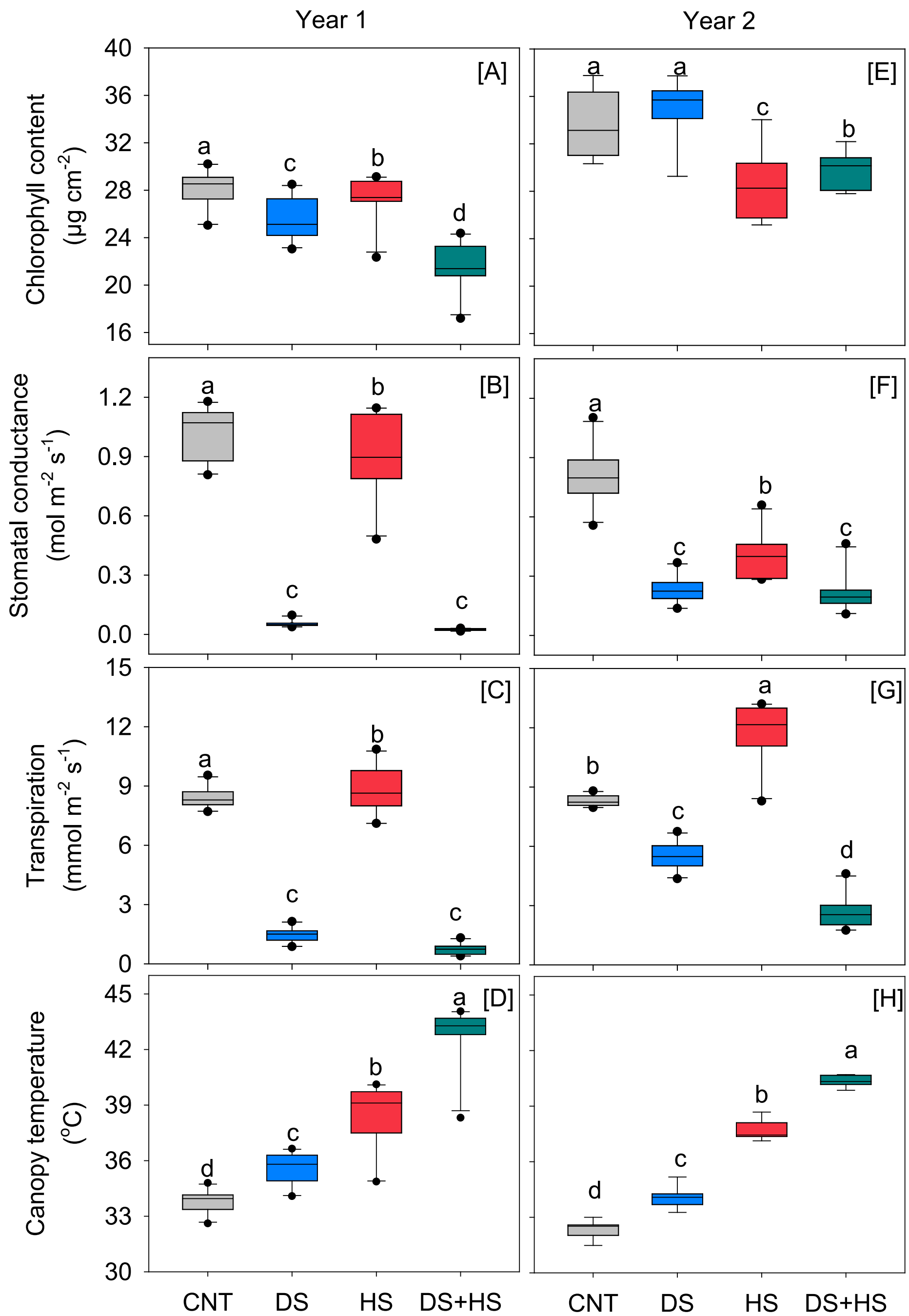


Figure 1. Box plots showing soybean physiological traits response to treatments (CNT- Control, DS- Drought Stress, HS- Heat Stress, and DS+HS- Heat and Drought) during the flowering seed setting stage in Years 1 (2021) and 2 (2022). Means followed by a common letter are not significantly different by the least significant difference (LSD) test at $p < 0.05$.

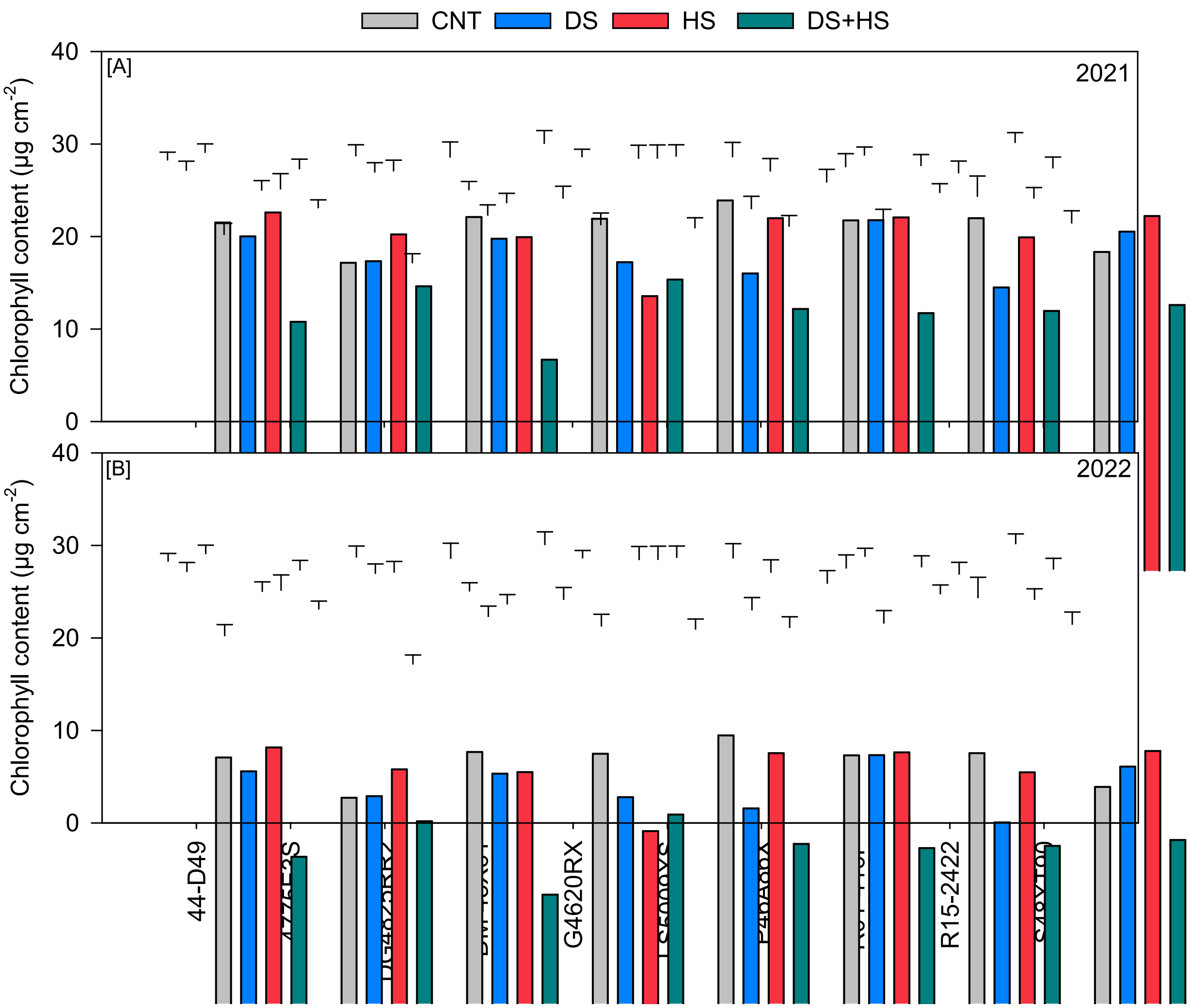


Figure 2. Effects of drought, heat, and their combination during the flowering seed setting stage of soybean cultivars on chlorophyll content (µg cm^{-2}) in Year 1 (A), and Year 2 (B). Vertical bars denote mean ± SE. CNT - control, DS - drought stress, HS - heat stress, and DS+HS - combined heat and drought stress.

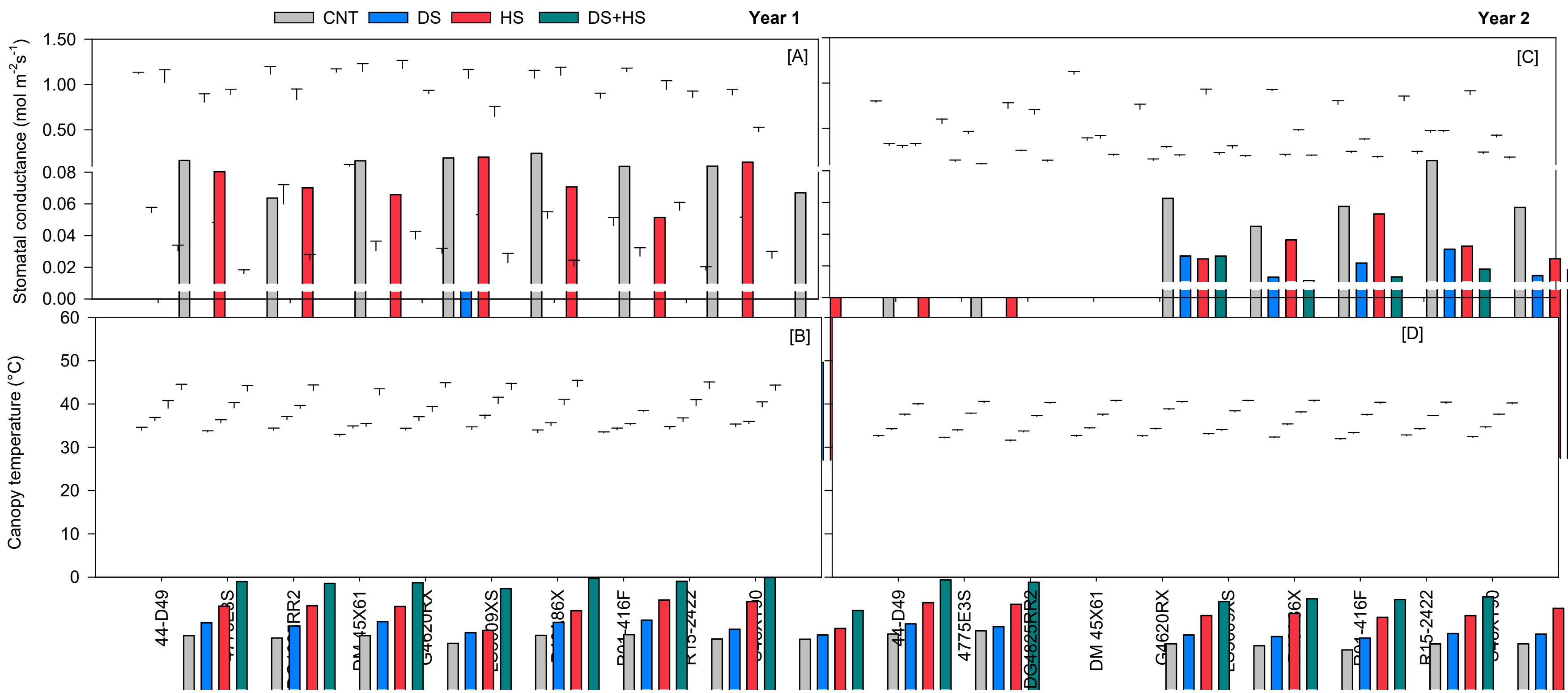


Figure 3. Effects of drought, heat, and their combination during the flowering seed setting stage of soybean cultivars on stomatal conductance (mol m^{-2} s^{-1}) and canopy temperature (°C) in the years 2021 (A and B), and 2022 (C and D). Vertical bars denote mean ± SE. CNT - control, DS - drought stress, HS - heat stress, and DS+HS - combined heat and drought stress.

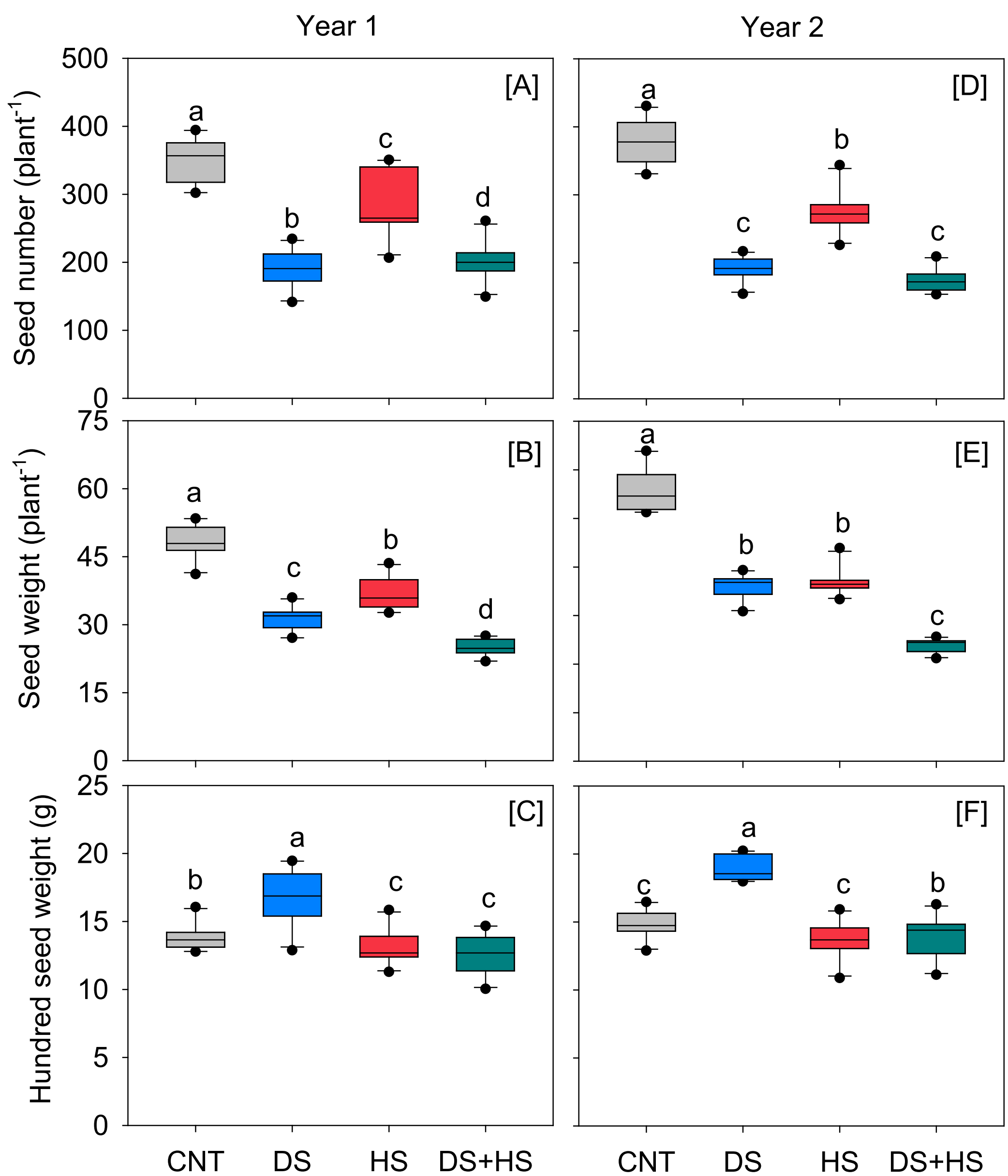


Figure 4. Box plots showing the yield components responses of soybean cultivars to different treatments (CNT- Control, DS- Drought stress, HS- heat stress, and DS+HS- combined heat and drought stress) during the flowering seed setting stage in Year 1 (2021) and Year 2 (2022). Means followed by a common letter are not significantly different by the least significant difference (LSD) test at $p < 0.05$.

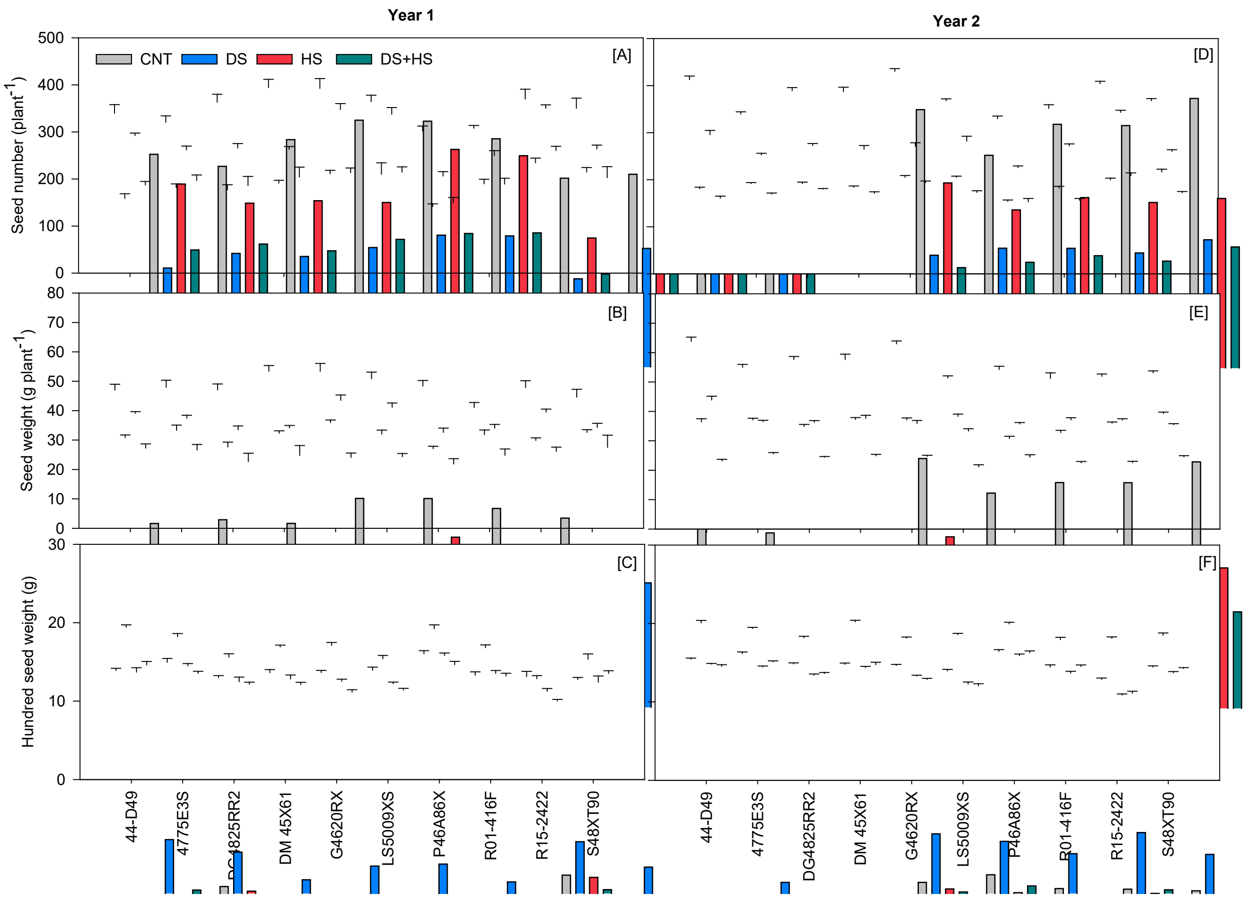


Figure 5. Effects of drought, heat, and their combination during the flowering, and seed setting stage of soybean cultivars on seed number, seed weight (g), and hundred seed weight (g) in the year 2021 (A, B, and C) and 2022 (D, E, and F). Vertical bars denote mean ± SE. CNT - control, DS - drought stress, HS - heat stress, and DS+HS - combined heat and drought stress.

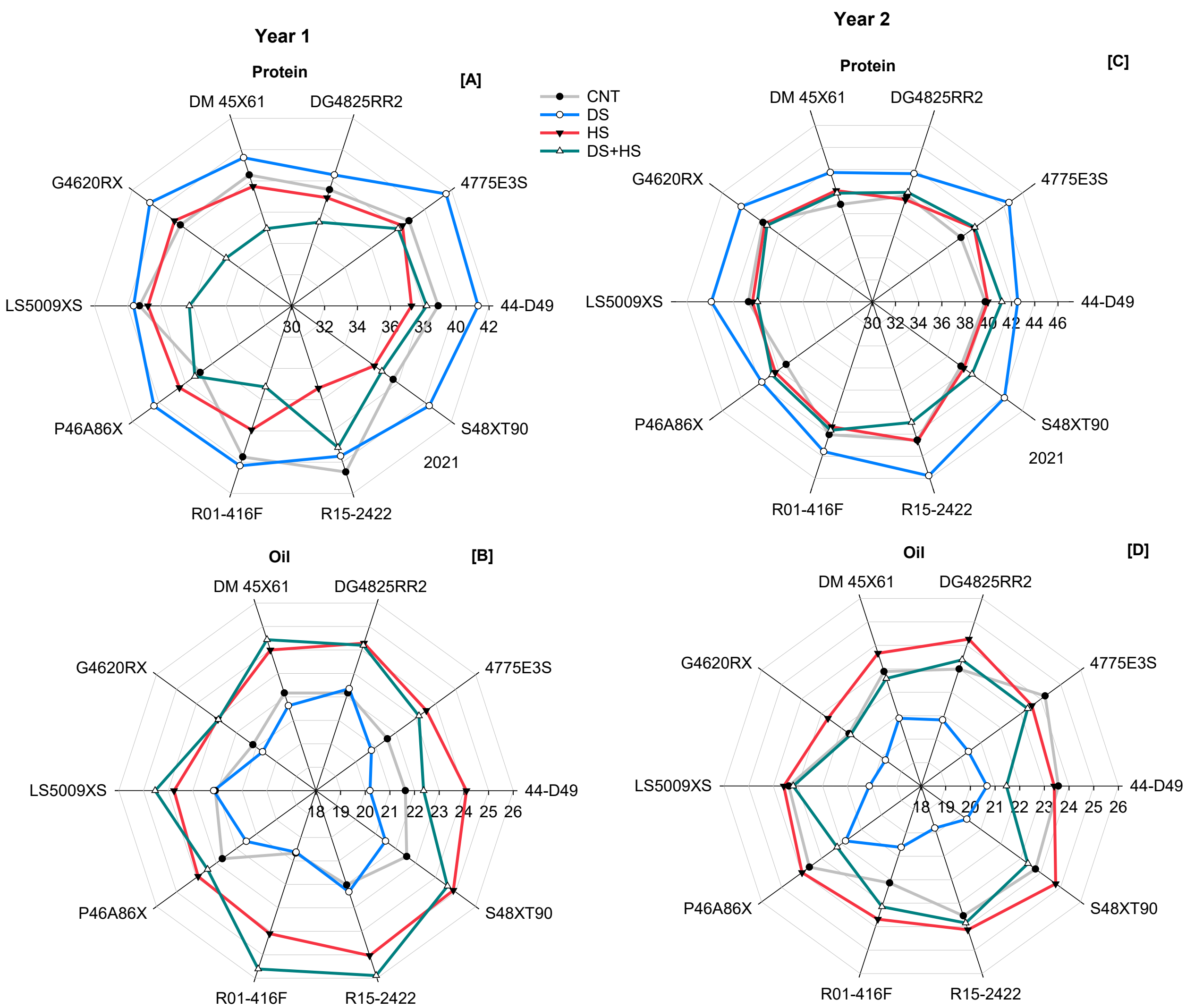


Figure 6. Seed protein and oil composition of ten soybean cultivars under control (CNT), drought stress (DS), heat stress (HS) and combined drought and heat stress (DS+HS) during the flowering seed setting stage in the Year 1 (A and B) and Year 2 (C and D).

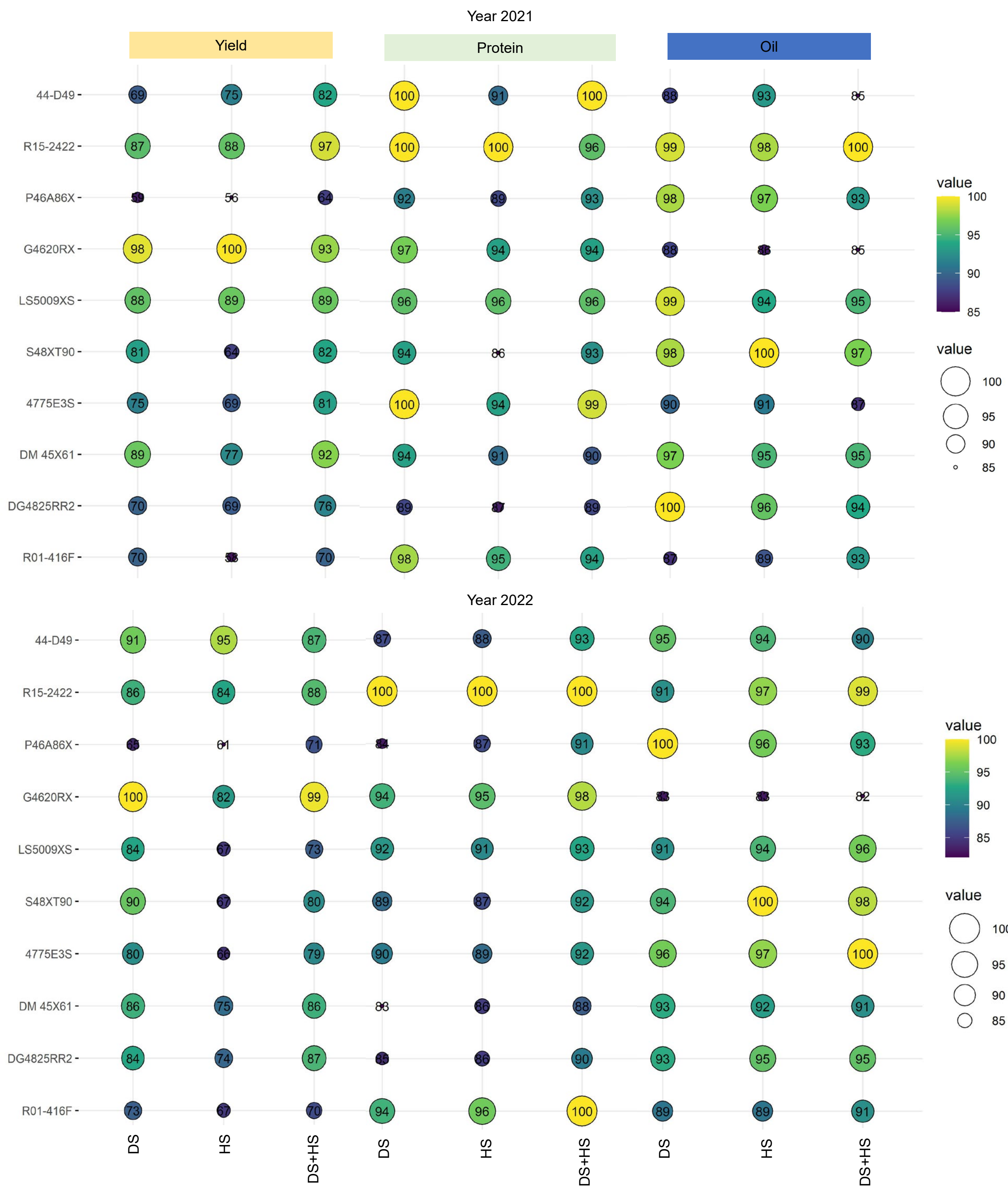
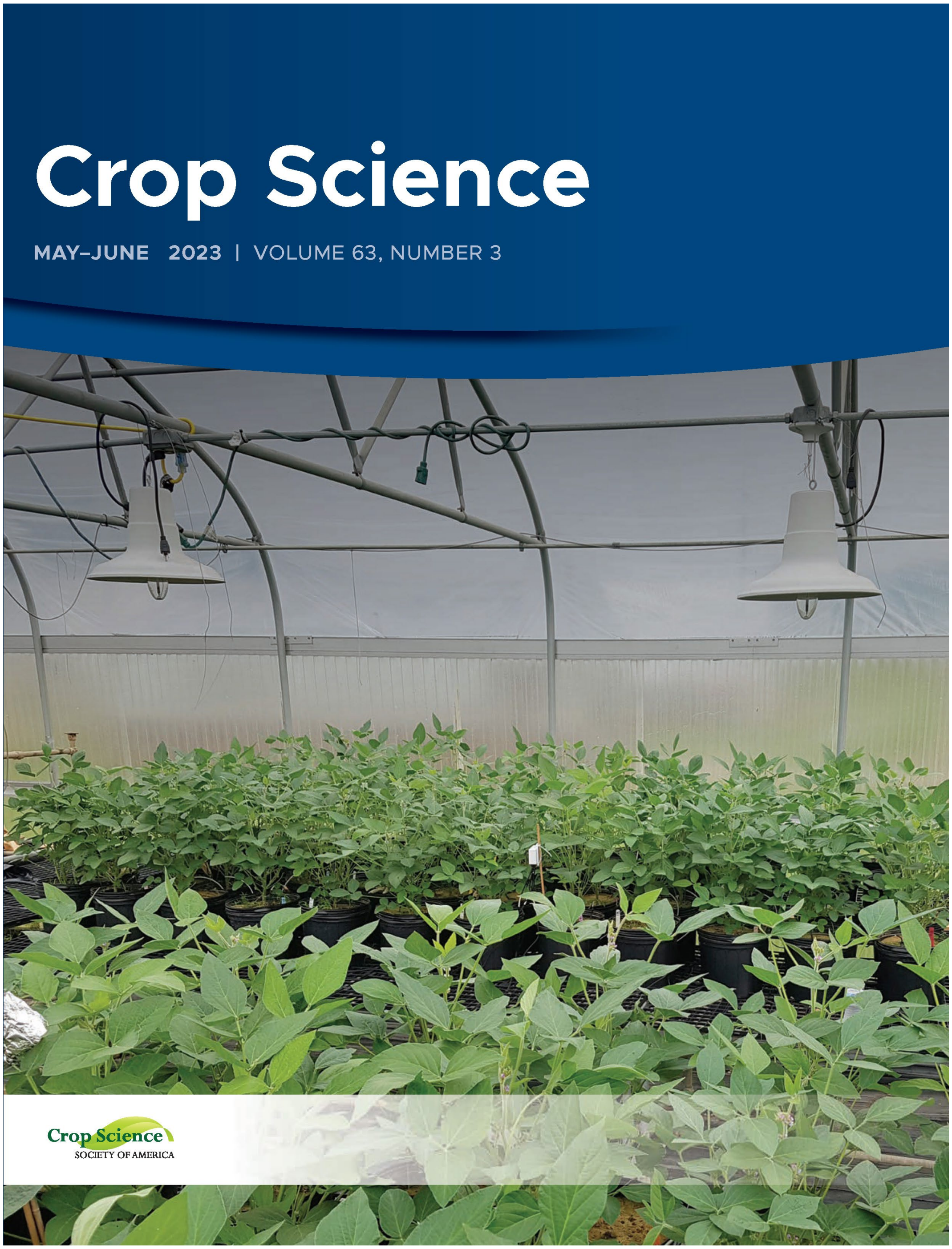


Figure 7. Balloon plot showing the stress tolerance index values (%) for seed yield (seed number and weight), protein and oil parameters in Year 1 and Year 2. The balloon plot's bubble size reflects the corresponding cultivar's stress tolerance indices under different stress treatments. Smaller bubble size or score represents a less tolerance under the given stress treatment. DS - drought stress, HS - heat stress, and DS+HS - combined heat and drought stress.



Featured on the cover of the crop science journal. Quantifying impact of heat and drought stress on soybeans. *Crop Sciences*, 63-3. https://acsess.onlinelibrary.wiley.com/doi/pdf/10.1002/csc2.20785