Indian Journal of Agricultural Sciences **94** (5): 489–494, May 2024/Article
https://doi.org/10.56093/ijas.v94i5.142783

Unraveling the effect of drought and heat stresses on grain quality of wheat (*Triticum aestivum*)

NANDINI G A[1], ABHISHEK CHITRANSHI[1], MALLESH GAMPA[1], SUNEHA GOSWAMI[1], MONIKA DALAL[1], SUDHIR KUMAR[2], ARUNA TYAGI[1] and RANJEET R KUMAR[1]*

ICAR-Indian Agricultural Research Institute, New Delhi 110 012, India

Received: 22 September 2023; Accepted: 06 February 2024

ABSTRACT

Drought stress (DS) and heat stress (HS) have adverse effects on wheat (*Triticum aestivum* L.) growth, serving as the primary constraints that significantly limit grain yield. Present study was carried out during 2020–21 and 2021–22 at the research farm of ICAR-Indian Agricultural Research Institute, New Delhi to evaluate the effects of drought and heat stress on grain quality parameters of 4 contrasting wheat genotypes. Several physiological and biochemical parameters were analyzed in wheat cv. C306 (thermotolerant), HD3271 (thermosusceptible), H11500 (drought tolerant) and HD3226 (drought susceptible) under DS and HS. Our study found that disintegration of chlorophyll pigments was higher in wheat cv. HD3226 (43%) under combined stress. Grain weight per spike was reduced by 30% in C306 and 47.3% in HD 3271 under combined drought and heat stress treatment. All cultivars showed reduction in starch, amylose, amylopectin, AGPase and Soluble starch synthase activity under DS and HS. Additionally, a significant increase in total soluble protein and free amino acid content were observed in all genotypes under combined stress. Chlorophyll content and grain weight per spike were positively correlated with the starch, amylose, amylopectin, soluble starch synthase and AGPase activity, while negatively related to the total soluble protein and free amino acid content. The tolerant genotypes maintained higher chlorophyll content, grain weight per spike, starch, amylose, amylopectin, soluble starch synthase and AGPase activity could be used for breeding, for the adaptation to drought and heat under climate change.

Keywords: Combined stress, Drought stress, Heat stress, Starch, Starch synthase, Wheat

Wheat (*Triticum aestivum* L.) stands as the second most extensively grown crop globally, serving as a primary food source for 40% of the global population. It contains carbohydrates (78%), protein (14%), fat (2%), minerals (2.5%), as well as vitamins like thiamine and vitamin B, alongside trace amounts of minerals (Iqbal 2022). Abiotic stresses, such as drought and high temperatures, play a crucial role in diminishing global grain yields and their combined influence is increasingly evident in semi-arid regions that cultivate wheat globally (Fahad *et al*. 2017). Due to increase in global temperatures and shifts in precipitation patterns within dryland agriculture, the combined impact of both drought and heat stresses has a more pronounced effect on morphological, biochemical, and physiological processes compared to other environmental stressors (Zandalinas *et al.* 2018). Drought and heat stress have multiple consequences on grain filling processes, such as a decrease in photosynthesis, various types of oxidative damage to cell organelles, premature senescence, a shortened plant life cycle, and significant reductions in grain yield (Tricker *et al*. 2018).

Wheat endosperm primarily consists of starch, comprising amylose and amylopectin. They contribute to 65–75% of the grain's weight and play a pivotal role in determining the grain yield. ADP-Glc pyrophosphorylase (AGPase) and granule-bound starch synthase (GBSS) are responsible for the synthesis of amylose. AGPase, along with starch synthases (SSs), primarily contributes to the synthesis of amylopectin (Lu *et al*. 2019). Elevated temperatures experienced during the grain filling stage can lead to diminished yields, decreased grain weight and size, and impairment to the internal grain structure. This ultimately causes a decline in starch content and changes in protein composition (Kumar *et al*. 2022). Drought stress hastens the initiation and duration of grain development while diminishing the synthesis of starch, amylose, fat, and overall grain size (Golfam *et al.* 2021). There is a scarcity of information available regarding the combined impact of drought and heat stresses on the quality of wheat grains. In this study, we have examined the influence of drought and heat stresses on the grain quality attributes of distinct wheat cultivars. The following study was carried out to insight

[1]ICAR-Indian Agricultural Research Institute, New Delhi.
*Corresponding author email: ranjeetranjaniari@gmail.com

biochemical changes occurring in selected genotypes under different stress.

MATERIALS AND METHODS

Plant material and stress treatment: The research experiments were conducted during 2020–21 and 2021–22 at research farm of ICAR-Indian Agricultural Research Institute, New Delhi. Four contrasting wheat cultivars, C306 (thermotolerant), HD3271 (thermosusceptible), H11500 (drought tolerant) and HD3226 (drought susceptible) were selected for the present investigation. The seeds underwent pre-sowing treatment with 0.25% Bavistin before being planted in 48 pots (12 pots per cultivar), with an equal mixture of perlite, sand, and farmyard manure (FYM). The plants subjected to drought stress, heat stress, and a combination of drought and heat stress during the pollination and grain-filling stages, determined according to the Feeke's scale (Miller 1992). Drought stress (DS) was induced by withholding water for 7 days until the water potential reading in the tensiometer reduced to 50 centibars, while heat stress (HS) was given by maintaining a temperature of 38°C for 2 h inside heat chambers. The HS treatment was carried out using microprocessor-regulated chambers, with a gradual temperature increment of 1°C every 10 sec in a sinusoidal mode. Control plants were maintained at a day temperature of (22 ± 2°C) and a night time temperature of (18 ± 2°C). The relative humidity (RH) was maintained at 80%, and the photosynthetically active radiation (PAR) was set at 250 µmol/m^2/s, with 8 h light and dark cycle. Samples (leaf, spike and grain) were collected and stored for further biochemical analyses.

Estimation of physiological and biochemical traits linked with grain quality

Chlorophyll content: The chlorophyll content during the grain-filling stages was assessed using the flag leaf of different wheat cultivars. Arnon's method was used to determine the total chlorophyll content, expressed in mg/g. The average value was obtained from three observations taken from a one flag leaf.

Grain weight per spike (GWPS): GWPS was determined by weighing the threshed grains from single spike.

Total soluble protein and free amino acids: Using liquid nitrogen, the spike samples were ground into a fine powder and added to the extraction buffer (100 mM Tris-HCl, *p*H 6.8). After homogenization, the resulting mixture underwent centrifugation for 20 min at 4°C and the supernatant was employed for protein quantification based on the methodology outlined by Bradford *et al.* (1976). The measurement of free amino acids was estimated by method given by Moore and Stein (1954), with slight modification.

Starch, amylose and amylopectin content: Starch content was estimated through the Anthrone method. The fully dried mature wheat grains were extracted using 70% heated ethanol, followed by centrifugation at 12,000 rpm for 15 min. After centrifugation, the resulting residue was resuspended in 7.5 ml of perchloric acid and 5.0 ml of water, and then subjected to further centrifugation to recover the supernatant. An additional extraction was carried out using 5 ml of perchloric acid, and its supernatant was pooled. The volume was adjusted to 100 ml with water. A 100 µl aliquot was taken and mixed with anthrone reagent. The colour intensity generated was measured at 620 nm.

The amylose content was quantified by following the method outlined by Kumari *et al*. (2020). Initially, a 20 mg sample of starch was dispersed in 10 ml of 0.5 N potassium hydroxide. After vortexing for 5 min, the volume was adjusted to 100 ml using distilled water. A 10 ml aliquot was taken and mixed with 5 ml of 0.1 N hydrochloric acid (HCl) and 0.5 ml of iodine reagent. The resulting solution was brought to a total volume of 50 ml. At 625 nm, the absorbance was measured, and the amylose content (%) was calculated using a standard curve made from amylose and amylopectin blends. Subtracting the amylose content from the total starch content yielded the amylopectin content.

Activity assay of ADP-glucose pyrophosphorylase (AGPase) and starch synthase: AGPase activity was estimated by using a method, Nivelle *et al.* (2019). The spectrophotometric evaluation of AGPase's pyrophosphorolytic activity involved monitoring the rise in absorbance at 340 nm, corresponding to the conversion of NADP to NADPH.

The assay for starch synthase (SS) activity was conducted following the procedure outlined in the study by Kumar *et al*. (2013). The activity was measured by monitoring the rise in absorbance at 340 nm following the introduction of 1.4 I U of hexokinase and 0.35 I U of glucose 6-phosphate dehydrogenase. To determine the SS activity in nmol/min/mg protein, a standard curve of sodium pyruvate was used for calculation.

Statistical analysis: The analysis of variance of different biochemical parameters was analyzed by MINITAB statistical package using a linear model of variance. The variances in the impact of DS and HS on physio-biochemical parameters associated with carbon assimilation under various treatments were assessed through a two-way analysis of variance (ANOVA), and the least significant difference (LSD) was employed to determine significant differences at a significance level of $P \leq 0.05$.

RESULTS AND DISCUSSION

Wheat is highly sensitive to both drought and heat stress. The simultaneous occurrence of heat stress (HS) and drought stress (DS) can amplify the detrimental effects on wheat yield, resulting in a combined impact that significantly surpasses the simple additive effects of these individual factors. This study presents an analysis of pooled 2-year data on a range of physiological and biochemical parameters, along with their impact on the quality of wheat grains.

Chlorophyll content: A significant decrease in chlorophyll content was observed under DS and HS in all the cultivars during both the years (Fig. 1a). The chlorophyll content was observed maximum in wheat cv. C306 (6.3 mg/g) and HI1500 (6.09 mg/g) and minimum in

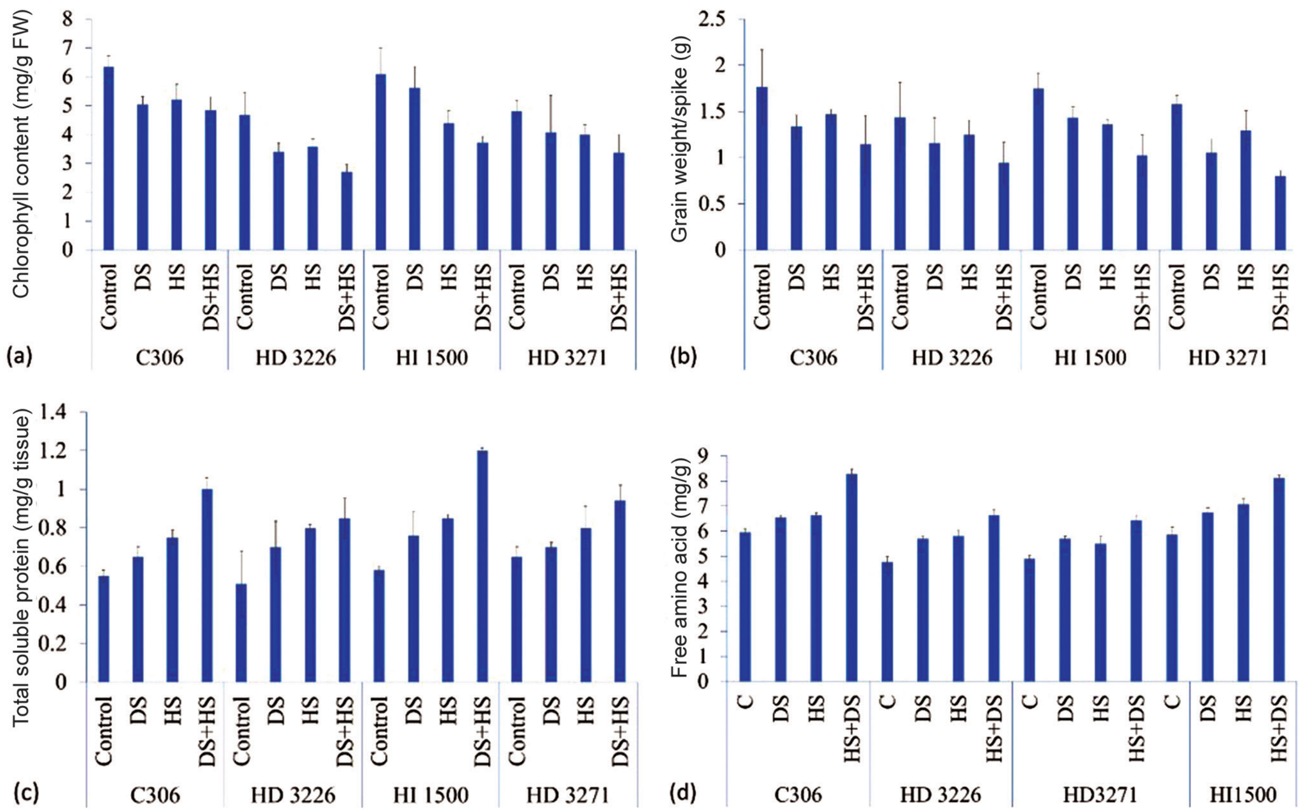

Fig. 1 Effect of drought stress (DS) and heat stress (HS) on (A) Chlorophyll content; (B) Grain weight/spike; (C) Total soluble protein; (D) Free amino acid (FAA) content in wheat.
Means for varieties, treatments and interaction are significant at $P<0.05$; vertical bars indicate s.e. (n=3).

HD 3271(4.8 mg/g) and HD3226 (4.6 mg/g) under control condition. The combination of drought and heat stress led to a more pronounced decrease in chlorophyll content with HD3226 (2.6 mg/g) and HD3271 (3.3 mg/g). Exposure to drought and heat stress led to a substantial reduction in chlorophyll levels, showing a significant decline under the combined conditions (Kumar *et al*. 2018). Heat stress leads to a decrease in chlorophyll content by damaging thylakoid membranes. Similarly, the reduction in chlorophyll content during drought stress is often associated with the deactivation of various enzymes involved in chlorophyll synthesis, along with an elevation in the activity of enzymes responsible for chlorophyll degradation. Therefore, minimal accumulation of chlorophyll in plants may result from increased chlorophyll breakdown, decreased chlorophyll production, or a combined effect of both (Meena *et al.* 2023).

Grain weight per spike (GWPS): GWPS provides a clear indication of the plant's yield and is influenced by various factors. The highest values were noted in wheat cultivars C306 (1.76 g) and HI1500 (1.74 g) under control conditions, while the lowest values were recorded in wheat cultivars HD3271 (0.79 g) and HD3226 (0.94 g) under simultaneous drought and heat stress conditions (Fig. 1b). The wheat cultivar HD3271 exhibited the highest percentage decrease in GWPS in both the years. Our results are in conformity with the findings of Shaukat *et al*. (2018) who reported that HS deteriorate the development of grain per spike in wheat, which has adverse impact on the yield and quality of the grains. In their study 108 different wheat genotypes were exposed to combined stress from heading to maturity, and average yields decreased by 44.66%. The combined stress treatment impacts grain yield by diminishing the metabolism and mobilization of reserves to developing grains and leaves (Qaseem *et al.* 2019).

Total soluble protein (TSP) and free amino acid (FAA): We observed a notable rise in TSP in all genotypes subjected to stress across both years. Maximum accumulation was occured in C306 (1.0 mg/g FW) and HI1500 (1.2 mg/g FW) under combined stress treatment while minimum observed in HD3226 (0.51 mg/g FW) under control treatment (Fig. 1c). Increased level of TSP was noted in the presence of combined stresses. TSP levels typically rise in response to drought and heat stress because plants accumulate small molecular mass proteins as a result of increased *de novo* synthesis or inhibition of amino acid degradation, and higher protein content may confer better tolerance by aiding in osmotic balance. Our results are aligned with the findings of Wang and Liu *et al*. (2021) who observed a rise in the TSP content in the grains of various wheat genotypes subjected to high temperature stress.

FAA content was significantly increased in grains in all the selected genotypes under DS and HS treatment. Maximum FAA accumulation was observed in C306 (8.28 mg/g FW) under combined drought and heat stress treatment while minimum accumulation was observed in HD3226 under control condition (4.7 mg/g FW) (Fig. 1d). The cause of this could be the interruption of protein synthesis or the incomplete hydrolysis of proteins under stress. Our results align with the findings of Kumar *et al.* (2017).

Starch, amylose and amylopectin content: A reduction in starch accumulation was observed under both DS and HS conditions in all the cultivars during both the years (Fig. 2a). Under the control condition, starch accumulation was maximum in C306 (70.14%) and HI1500 (71.10%) as compared to HD 3271(63.64%) and HD3226 (63.29%). Per cent decrease in C306 was observed to be 14.25% while in HD3226, 21.94% decrease was observed under combined DS and HS. Wheat exposure to HS, DS, and the combination of HS and DS led to reduction in starch content. The storage of starch is linked to a cascade of enzymes such as sucrose synthase, sucrose transporter, ADP-gluco-pyrophosphorylase (AGPase), and soluble starch synthase (SSS). These enzymes are sensitive to high temperatures and can be easily inactivated by heat. A significant reduction in starch content, with decreases of up to 35%, has been documented in various wheat cultivars when exposed to high temperature stress (HS) (Lu *et al*. 2019).

A reduction in amylose content was noted across all cultivars under stress conditions when compared to the control (Fig. 2b). The highest amylose content was recorded in C306 (17%) under normal conditions, whereas the lowest was observed in HD3226 (12.28%) under combined drought stress (DS) and heat stress (HS). Although there were no significant variations in amylose content, a noteworthy decline in amylopectin content occurred during DS and HS conditions. HI1500 exhibited the maximum amylopectin content (54.2%) under control conditions, while HD3226 showed the minimum (37.41%) under the combined DS and HS treatment (Fig. 2c). Lu *et al.* (2019) reported that HS, DS, and the combination of HS and DS led to reductions in amylopectin and amylose contents and the ratio of amylose to amylopectin increased due to decrease in the amylopectin content (Lu *et al*. 2019).

AGPase and starch synthase (SS) activity: We observed significant difference in AGPase activity in all selected genotypes in response to drought and heat stress treatment across both the years. The specific activity of AGPase was found maximum in C 306 (1.64 U/mg) and HI1500 (1.58 U/mg) under control treatment while while minimum activity was found in HD3271 (1.03 U/mg) and HD3226 (1.08 U/mg) under combined stress (Fig. 2d). Due to the heat sensitivity of the enzymes responsible for starch biosynthesis, an increase in temperature adversely affects the activity of AGPase (Kumar *et al.* 2017). Lu *et al.* 2019 reported that activity of AGPase rapidly declines, particularly in response to a severe drought stress, resulting in an early halt to starch accumulation.

We observed a notable reduction in SSS activity in response to DS and HS. In the control conditions, the highest SSS activity was observed in C306 and HI1500, whereas HD3226 and HD3271 exhibited lower activity. C306 showed

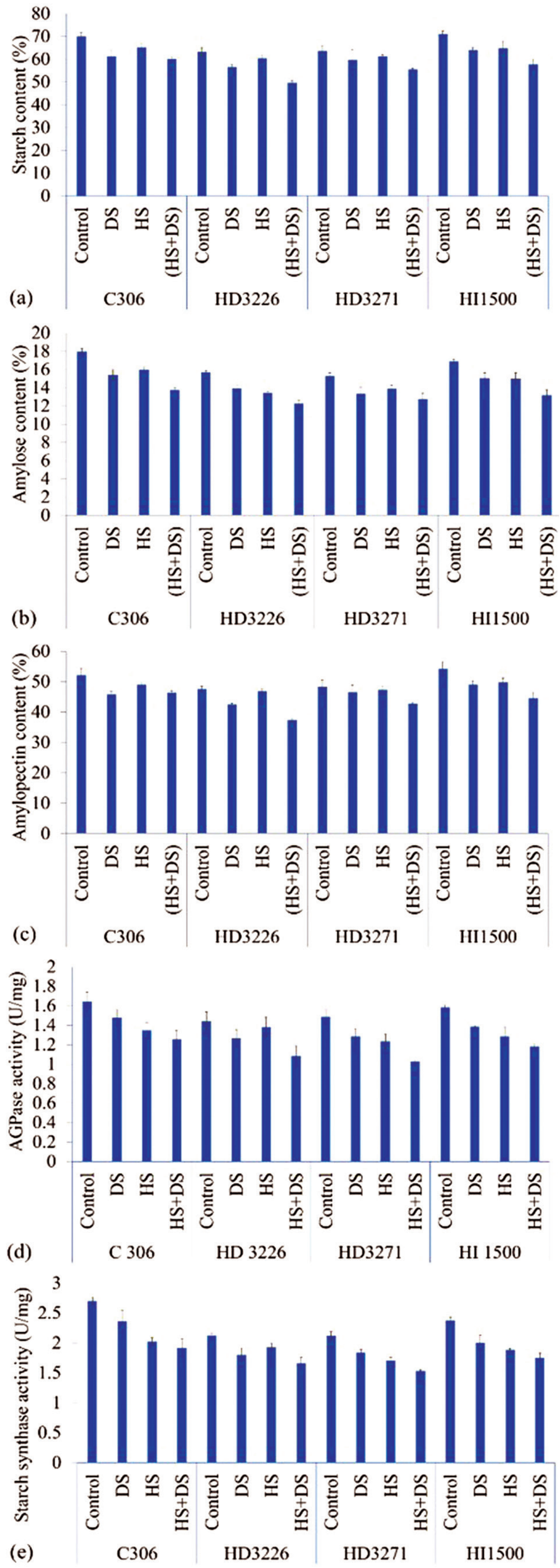

Fig. 2 Effect of drought stress (DS) and heat stress (HS) on grain quality related parameters (A) Starch content; (B) Amylose content; (C) Amylopectin content; (D) AGPase activity; (E) Soluble starch synthase activity in wheat.
Means for varieties, treatments and interaction are significant at *P*<0.05; vertical bars indicate s.e. (n=3).

Table 1 Correlation analysis for physio-biochemical parameters of wheat under drought and heat stress

	Chlorophyll	GWPS	Starch	Amylose	Amylopectin	SSS	FAA	TSP
Chlorophyll	1							
GWPS	0.864251	1						
Starch	0.690148	0.747187	1					
Amylose	0.687776	0.766651	0.908381	1				
Amylopectin	0.656632	0.702199	0.986267	0.826845	1			
AGPase activity	0.703877	0.79557	0.852882	0.893801	0.794142	1		
SSS	0.700701	0.726649	0.791701	0.909826	0.70552	0.948146	1	
FAA	-0.601088	-0.664764	-0.92943	-0.94620	-0.87641	-0.87416	-0.8899	1
TSP	-0.52053	-0.68965	-0.57878	-0.72596	-0.49175	-0.83848	-0.7525	-0.649

GWPS, Grain weight per spike; SSS, soluble starch synthase; FAA, Free amino acid; TSP, Total soluble protein.

the highest SSS activity (2.69 U/mg), whereas HD3236 showed the lowest (2.12 U/mg) activity under DS and HS conditions (Fig. 2e). Starch synthase and ADP-Glucose Pyrophosphorylase (AGPase) are crucial enzymes that play a significant role in starch biosynthesis. Elevated temperatures have been linked to a reduction in grain starch content, sometimes resulting in reductions of up to one-third of the total endosperm starch. This decrease is primarily attributed to a decline in the efficiency of the enzymes involved in starch biosynthesis. Furthermore, the reduced activity of SSS at higher temperatures, typically around 40°C, contributes to both smaller grain size and decreased starch deposition (Poudel *et al.* 2020). Under conditions of drought and heat stress, it was observed that soluble starch synthase (SS) exhibited the highest degree of sensitivity among the enzymes involved in starch biosynthesis. Moreover, the activity of SSS tends to decrease at elevated temperatures, usually around 40°C.

Correlation analysis: Pearson's correlations among the physiological and biochemical traits of wheat genotypes subjected to drought, heat and combined stresses (Table 1) revealed that chlorophyll content and GWPS exhibit a positive correlation with starch, amylose, amylopectin, AGPase and SSS. Conversely, a negative correlation was found between TSP and FAA content. TSP and FAA accumulation displayed a negative correlation with starch, amylose, amylopectin, AGPase, SSS content in wheat.

To conclude, drought and heat stress significantly influence the grain quality in wheat. We evaluated the biochemical changes in wheat cultivars C306, HD3271, HI1500 and HD3226 under both drought stress (DS) and heat stress (HS), to understand its impact on grain quality. Our study found that tolerant genotypes maintain higher chlorophyll content, starch, amylose, amylopectin, SSS, AGPase, TSP and FAA content. All these parameters can be used as physio-biochemical markers as tools for identifying wheat genotypes that exhibit tolerance to drought, heat, or the combination of both stresses. This study offers valuable insights to breeders for development of wheat varieties with improved tolerance mechanisms to combat challenging environmental conditions. Overall, the findings suggest that the combined effect of drought and heat stress has more detrimental effects on grain quality than the individual stresses.

ACKNOWLEDGEMENT

The authors are grateful to the NICRA Project and ICAR-Indian Agricultural Research Institute New Delhi for providing financial support to carry out this research work.

REFERENCES

Bradford M M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry* **72**(1-2): 248–54.

Fahad S, Bajwa A A, Nazir U, Anjum S A, Farooq A, Zohaib A, Sadia S, Nasim W, Adkins S, Saud S and Ihsan M Z. 2017. Crop production under drought and heat stress: Plant responses and management options. *Frontiers in Plant Science* **8**: 1147.

Golfam R, Kiarostami K, Lohrasebi T, Hasrak S and Razavi K. 2021. A review of drought stress on wheat (*Triticum aestivum* L.) starch. *Farming and Management* **6**(1): 47–57.

Iqbal M J, Shams N and Fatima K. 2022. Nutritional quality of wheat. *Wheat-Recent Advances*. IntechOpen.

Kumar R R, Sharma S K, Goswami S, Singh G P, Singh R, Singh K, Pathak H and Rai R D. 2013. Characterization of differentially expressed stress associated proteins in starch granule development under heat stress in wheat (*Triticum aestivum* L.). *Indian journal of Biochemistry and Biophysics* **50**(2):126–38.

Kumar A S, Sharma S K, Lata C, Devi R, Kulshrestha N, Krishnamurthy S L, Singh K and Yadav R K. 2018. Impact of water deficit (salt and drought) stress on physiological, biochemical and yield attributes on wheat (*Triticum aestivum*) varieties. *The Indian Journal of Agricultural Sciences* **88**(10):1624–632.

Kumar R R, Goswami S, Shamim M, Mishra U, Jain M, Singh K, Singh J P, Dubey K, Singh S, Rai G K and Singh G P. 2017. Biochemical defense response: Characterizing the plasticity of source and sink in spring wheat under terminal heat stress. *Frontiers in Plant Science* **8**: 1603.

Kumar R R, Praveen S and Rai G K. 2022. *Thermotolerance in Crop Plants*, 1st edn, pp. 1–321. Springer Nature, Verlag, Singapore.

Kumari A, Kumar R R, Singh J P, Verma P, Singh G P, Chinnusamy V, Praveen S and Goswami S. 2020. Characterization of the starch synthase under terminal heat stress and its effect on grain quality of wheat. *3 Biotech* **10**: 1–10.

Lu H, Hu Y, Wang C, Liu W, Ma G, Han Q and Ma D. 2019. Effects of high temperature and drought stress on the expression of gene encoding enzymes and the activity of key enzymes involved in starch biosynthesis in wheat grains. *Frontiers in Plant Science* **10**: 1414.

Meena S, Arora A, Taria S, Yadav S, Kumar S, Pushkar S, Chinnusamy V, Kumar R R and Padaria J C. 2023. Unraveling the physiological and molecular mechanisms regulating grain yield under combined drought and heat stress in wheat (*Triticum aestivum*). *The Indian Journal of Agricultural Sciences* **93**(8): 821–826.

Miller T D. 1992. Growth stages of wheat. Better crops with plant food. *Potash and Phosphate Institute* **76**: 12–19.

Moore S and Stein W H. 1954. A modified ninhydrin reagent for the photometric determination of amino acids and related compounds. *Journal of Biological Chemistry* **211**: 907–13.

Nivelle M A, Remmerie E, Bosmans G M, Vrinten P, Nakamura T and Delcour J A. 2019. Amylose and amylopectin functionality during baking and cooling of bread prepared from flour of wheat containing unusual starches: A temperature-controlled time domain 1H NMR study. *Food chemistry* **295**: 110–19.

Poudel P B and Poudel M R. 2020. Heat stress effects and tolerance in wheat: A review. *Journal of Biology and Today's World* **9**(3): 1–6.

Qaseem M F, Qureshi R and Shaheen H. 2019. Effects of pre-anthesis drought, heat and their combination on the growth, yield and physiology of diverse wheat (*Triticum aestivum* L.) genotypes varying in sensitivity to heat and drought stress. *Scientific Reports* **9**(1): 6955.

Shaukat S, Khan A S, Hussain M, Kashif M and Ahmed N. 2018. Selection of superior parents and cross combinations for quality traits in bread wheat (*Triticum aestivum* L.) under normal and heat stressed conditions. *Pakistan Journal of Agricultural Sciences* **55**(4): 801–07.

Tricker P J, ElHabti A, Schmidt J and Fleury D. 2018. The physiological and genetic basis of combined drought and heat tolerance in wheat. *Journal of Experimental Botany* **69**(13): 3195–210.

Wang X and Liu F. 2021. Effects of elevated CO_2 and heat on wheat grain quality. *Plants* **10**(5): 1027.

Zandalinas S I, Mittler R, Balfagón D, Arbona V and Gomez-Cadenas A. 2018. Plant adaptations to the combination of drought and high temperatures. *Physiologia Plantarum* **162**(1): 2–12.