



Combined effects of drought and heat stress on morphology, physiology and yield of quinoa (*Chenopodium quinoa*)

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ABSTRACT

Climate change threatens global crop production and food security, especially in marginal areas prone to drought, high temperatures, and resource limitations compounded by rapid population growth. In this context, quinoa (*Chenopodium quinoa* Willd.) is gaining attention as a climate-resilient crop due to its exceptional adaptability to harsh environments. Despite its tolerance, quinoa often encounters multiple abiotic stresses simultaneously primarily water scarcity and elevated temperatures which can significantly impair its physiological functions and yield. The study was carried out during the winter (*rabi*) seasons of 2022 and 2023 at the controlled environment facility (growth chamber) of ICAR-National Institute of Abiotic Stress Management, Baramati, Maharashtra to investigate the combined effects of drought and heat stress on quinoa. The experiment was laid out in a completely randomised design (CRD) with eight replications having four treatments, viz. Control [100% field capacity (FC)], 32/20°C (day/night), drought (50% FC, 32/20°C); heat (100% FC, 36/24°C) and combined drought + heat (50% FC, 36/24°C). Quinoa variety used for the study was 'Jaipur local'. The stresses were imposed at crop vegetative, flowering, and grain filling stages to evaluate their influence on overall growth, physiology and productivity. Findings indicated that the concurrent application of drought and heat stress had the most detrimental effect, surpassing the impact of each stress applied independently. Morphological parameters were most affected by stress imposed at vegetative stage while physio-chemical parameters were affected by stress imposed at flowering and grain filling stages. Drought + heat, heat and drought stress reduced quinoa seed yield by 82, 67 and 48%, respectively as compared to control (1106 kg/ha). These findings provide valuable insights for researchers and farmers, highlighting the need to optimize crop production techniques for quinoa in marginal environments.

Keywords: Climate change, Drought, Heat, Quinoa, Sustainability

The climate change has accelerated and intensified in recent years, posing one of the greatest threats to global food production (IPCC 2022). Securing food and ensuring nutritional stability for a growing population faces significant hurdles primarily due to occurrence of abiotic stresses, viz. moisture deficit, high temperature, waterlogging, soil salinity, alkalinity and nutrient deficiency. In this context, cropping system diversification is considered as a crucial strategy for mitigating the negative impacts of climate change on farmers and enhancing their economic resilience. This entails the exploration and adoption of alternative crops, tolerant varieties and strategic management practices. Alternative crops, while less widespread, confer economic, ecological, and societal benefits compared to conventional

crops. They possess characteristics including resilience to biotic and abiotic stresses and demonstrate higher nutritional profiles (Pradhan *et al.* 2021). Quinoa (*Chenopodium quinoa* Willd.) presents itself as a promising agricultural candidate with substantial worldwide potential owing to its versatility across diverse agro-climatic environments and its exceptional protein quality. It showcases a well-balanced amino acid composition, augmented mineral content, and a wide array of vitamins. Moreover, this crop demonstrates resilience to drought, salinity, frost, and high temperature stresses, rendering it a viable prospect for agricultural diversification in various regions worldwide, including India (Ain *et al.* 2023).

Though quinoa is well known for its remarkable adaptation to the harsh climatic conditions, still there exists a high degree of variability in the tolerance level among the ecotypes as well as their growth stages. Despite quinoa's array of drought-resistant mechanisms, including attributes such as deep-rooted systems, adaptability in ontogenic processes, quick stomatal closure, antioxidant defence

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mechanisms, osmotic adjustments, cell membrane stability, elevated ABA concentration for hormonal regulation, and the activation and accumulation of osmoprotectants and aquaporins, it is still common to observe reductions in yield attributable to drought stress (Hinojosa *et al.* 2018, Pradhan *et al.* 2022). Though quinoa exhibits broader temperature tolerance than numerous crops, with optimal growth occurring around 25°C but elevated temperatures, especially during flowering, detrimentally affect yield by decreasing pollen viability (up to 50% loss) (Hinojosa *et al.* 2019). Therefore, quinoa responses to drought (moisture deficit) and heat (high temperature) stress under marginal conditions are a research priority since these regions are characterised by a harsh climate, erratic rainfall and often accompanied by high temperature. In this context, the current experiment was conducted with the main objective to assess the impact of drought and heat stress subjected at the vegetative, flowering, grain filling stages of quinoa on its morphology, physiology, yield, different yield attributes and water productivity.

MATERIALS AND METHODS

The study was carried out during the winter (*rabi*) seasons of 2022 and 2023 at the controlled environment facility (growth chamber) of ICAR-National Institute of Abiotic Stress Management, Baramati (18°09'30.62" N, 74°30'03.08" E; at an elevation of 570 m amsl), Maharashtra. The experiment was laid out in a completely randomised design (CRD) with eight replication. The quinoa variety 'Jaipur Local' was grown in plastic pots filled with 14 kg of clay loam soil (72% clay, 24.4% silt, and 4% sand). The soil had a pH of 8.2, electrical conductivity of 0.22 dS/m, bulk density of 1.33 Mg/m³, porosity of 54.2%, organic carbon content of 6.3 g/kg, and was supplied with 160 kg nitrogen (N), 15 kg phosphorus (P), and 130 kg potassium (K)/ha. Prior to sowing, the recommended fertiliser dose of 100: 50: 50 N, P₂O₅, K₂O kg/ha was applied, calculated based on soil weight and thoroughly mixed with the soil. Ten seeds were sown per pot at 3–4 cm depth. However, after one week, only two seedlings/pot were maintained under ambient atmospheric conditions and later transferred to the growth chamber for stress imposition at specific growth stages i.e. vegetative, flowering, and grain filling. At each targeted growth stage, drought stress (D) was imposed by maintaining soil moisture at 50% field capacity under regular day/night temperatures of 32/20°C. Heat stress (H) was induced by transferring plants to a growth chamber set at 36/24°C (day/night) while providing regular irrigation (100% field capacity). For the combined drought and heat stress treatment (D+H), plants were kept in the same heat-stress chamber but with soil moisture maintained at 50% field capacity. Control plants (C) were grown under optimal conditions, with regular irrigation and temperature of 32/20°C (day/night). The stress treatments were applied for one week, with eight replicate pots/treatment. To minimise positional bias, pots were repositioned randomly every other day within the growth chamber, set at 65–70% relative humidity and

450–750 μmol/m²/s PAR. During post-stress period, all pots were returned to ambient conditions (32/20°C day/night) and irrigated regularly to facilitate recovery. Throughout the experiment, all pots were maintained free of weeds, and no incidence of pests or diseases was observed, negating the need for any protective measures.

Each treatment at each growth stage comprised eight pots, of which four were used for destructive sampling immediately after stress application to measure various morphological and physiological parameters. The remaining four pots were retained until harvest to evaluate the effects of the treatments on yield attributes and yield. Morphological parameters such as plant height, stem girth, leaf area, and shoot biomass were recorded at harvest. Physiological and biochemical measurements, viz. relative water content (RWC), normalized difference vegetation index (NDVI), membrane stability index (MSI), photosynthetic efficiency (F_v/F_m), chlorophyll content, and proline accumulation, were recorded a week after stress imposition.

Relative water content (RWC): Completely developed third leaf from the top of each plant was collected across all treatments to determine RWC. The fresh weight (FW) was recorded quickly after detachment whereas the turgid weight (TW) was measured after keeping the leaves floated in distilled water for four hours at room temperature. Subsequently, the leaves were dried in an oven at 60°C for 48 h to obtain their dry weight (DW). The estimation of leaf RWC was done using the equation:

$$\text{RWC (\%)} = (\text{FW} - \text{DW}) / (\text{TW} - \text{DW}) \times 100$$

Photosynthetic efficiency: It indicates the plant photosynthetic performance and usually investigated by chlorophyll fluorescence. In this study, completely developed third leaves from the top of each plant within each replication were sampled at approximately 09:00 h. The samples were immediately shifted to a dark room and allowed to acclimate for one hour to achieve dark stabilization. Chlorophyll fluorescence measurements were obtained using a Handy FluorCam imaging fluorometer (P.S.I., Brno, Czech Republic). Fluorescence signals were captured by a high-sensitivity charge-coupled device (CCD) camera and processed using the FluorCam software (version 7, 1.2.5.3). Initial measurements of dark-adapted fluorescence (F_0) were taken using non-actinic measuring flashes provided by super-bright LEDs. This was followed by an 800 ms saturating light pulse [2,500 μmol (photon)/m²/s¹] from a halogen lamp to record the maximum fluorescence (F_m). The maximum quantum efficiency of photosystem II (PSII), expressed as F_v/F_m , was calculated using the formula:

$$F_v/F_m = (F_m - F_0) / F_m$$

Where F_v (variable fluorescence) is the difference between F_m and F_0 .

Leaf membrane stability index (MSI): It was assessed using the fully developed third leaf from the top of four plants per replication for each treatment. The collected leaves were cut into 1 cm² discs, and 0.5 g of these discs

were kept in test tubes containing 10 mL of double-distilled water, with samples set up in duplicate. One set of tubes was incubated at 40°C for 30 min, while the second set was placed in a boiling water bath for 15 min at 100°C. Further, electrical conductivity was recorded for both sets using a conductivity meter (Adawa-260, Thermo Scientific, USA), and denoted as C₁ (40°C) and C₂ (100°C), respectively. The MSI was then calculated using the below equation:

$$MSI (\%) = [1 - (C_1 / C_2)] \times 100$$

Similarly, proline content of leaves were quantified following the procedure laid out by Bates *et al.* (1973). Further, plants were harvested at physiological maturity by manually cutting the above ground plant parts and sun-drying them to 13% moisture content. Seeds were threshed by hand, and the weight of both seed and husk per plant was recorded for each pot and treatment. Other yield attributing parameters such as panicle length, panicle weight, 1000-seed weight and seed yield were also recorded for each treatment to assess the impact of stress treatments on specific growth stages.

The study followed a CRD with plants assigned to treatments and positioned within the controlled environment chamber through random selection. Physiological parameters were recorded from randomly chosen plants within each replication across all treatments. Data from both experimental years were pooled and analysis was carried out using general linear models (GLMs) in the “Agricolae” package of R. Standard error of the mean (SE) indicated variability present in the data and Least Significant Difference (LSD) test at a 5% probability level was used to determine statistical significance between the treatment means.

RESULTS AND DISCUSSION

Combined drought and heat stress was found to be more detrimental to quinoa plants than either stress alone, affecting all parameters irrespective of growth stages. However, morphological parameters were most affected by stress imposed at vegetative stage while physio-chemical parameters were affected by stress imposed at flowering and grain filling stage.

Morphological parameters: Plant height ranged from 34–76.8 cm during the growth cycle, with the vegetative

stage being most sensitive to stress. Drought + heat had the greatest impact during this stage, resulting a significant 55.7% reduction in plant height followed by heat (44.67%) and drought (37.5%) compared to the control (76.8 cm). Stress imposed at flowering and grain filling also followed a similar trend with lower reduction values under respective stress treatments (Table 1). Similar trend was also followed for stem girth and shoot biomass. These outcomes closely align with the results obtained from pot experiments conducted at the International Center for Biosaline Agriculture in the UAE, as reported by Abdelaziz and Redouane (2020). Heat stress has been reported to induce notable alterations in various aspects of plant growth and development. These findings are similar with that of Ruiz-Carrasco *et al.* (2011), who cited comparable alterations in these parameters when quinoa plants were subjected to high temperatures. Hussain *et al.* (2019) also demonstrated that the combined drought and heat stress in maize led to a substantial decrease in these growth indicators.

Physio-chemical parameters: Relative water content values varied from 37.70–63.44% among the treatments across the growth stages (Table 2). Stress imposed at grain-filling stage was most detrimental showing the highest RWC reduction of 33% followed by 19 and 12% reduction by heat and drought, respectively as compared to control RWC value (62%). Stress imposed at flowering and vegetative stages also followed the same trend of treatments with slightly lower values. Similarly, photosynthetic efficiency (F_v/F_m) values varied from 0.30–0.78. Stress imposed at grain filling stage was most detrimental with D + H resulting in highest reduction of F_v/F_m (60.19%) followed by heat (54.44%) and drought (44.70%) treatments as compared to the control plants (F_v/F_m values of 0.75). When stress was imposed during flowering, drought and heat led to a 55.81% reduction, followed by heat (43.91%) and drought (39.24%) compared to control F_v/F_m of 0.78. The combination of drought and heat resulted a 54.99% reduction followed by heat (45%) and drought (40.60%) compared to control F_v/F_m of 0.75 when the stress was imposed during vegetative stage. The MSI also followed a similar trend with values ranging from (57.23–86.27%) (Table 2). Similarly, the proline content ranged from 46–257 µg/g FW, with maximum increase (80.79%) in D+H stress imposed during vegetative stage followed by H (37.75%) and D (16.22%) as compared to

Table 1 Effect of treatments imposed at various growth stages on quinoa morphology

Treatments	Plant height (cm) at harvest			Stem girth (cm) at harvest			Shoot biomass (g/plant) at harvest		
	Stress imposition stages*								
	V	F	G	V	F	G	V	F	G
Control (C)	76.8 ^a	76.8 ^a	76.8 ^a	1.54	1.54	1.54	9.82	9.82	9.82
Drought (D)	48 ^b	63.8 ^b	70.5 ^b	0.44	0.70	1.31	2.75	3.83	6.85
Heat (H)	42.5 ^c	59.5 ^c	65.5 ^c	0.40	0.66	1.22	2.09	3.11	4.98
Drought + heat (D + H)	34 ^d	56.3 ^d	61.1 ^d	0.29	0.55	1.04	1.7	2.24	3.75

*Stress imposed at quinoa growth stages viz. V, Vegetative stage; F, Flowering stage; G, Grain filling stage. Values with different letters indicate a significant difference with $p < 0.05$.

control (Table 2). Considering proline content of leaves, grain filling stages was least affected by stress treatment imposition. It is well known that occurrence of drought and heat stress primarily affects plants by damaging cell membranes, thereby adversely affecting the normal cell functions. Drought stress predominantly impacts photosynthetic pigments and thylakoid membrane integrity, whereas heat stress primarily disrupts chlorophyll biosynthesis, leading to a decline in chlorophyll levels. Elevated temperatures further impair photosynthesis by damaging pigments, reducing Photosystem II activity, and hindering ribulose biphosphate (RuBP) regeneration. Consistent with these physiological mechanisms, our research found that the combined effect of drought and heat stress resulted in significantly greater reductions in relative water content and membrane stability compared to either stress alone (Pradhan *et al.* 2022).

Yield and yield attributes: Panicle length varied from 8.18–17.56 cm. Occurrence of drought + heat stress during the grain filling stage, resulted in the highest reduction (53.38%) followed by heat stress (44.48%) and drought stress (33.45%), compared to the control panicle length of 17.56 cm (Fig. 1A). Similarly, for panicle weight per plant, drought + heat stress imposed during grain filling stage caused the most significant decrease (64.14%), followed by heat (42.07%) and drought (35.24%), compared to the control weight of 36.25 g (Fig. 1B). The 1000-seed weight also followed a similar trend (Fig. 1C). Quinoa seed yield was maximum in control treatments with 1,106 kg/ha. However,

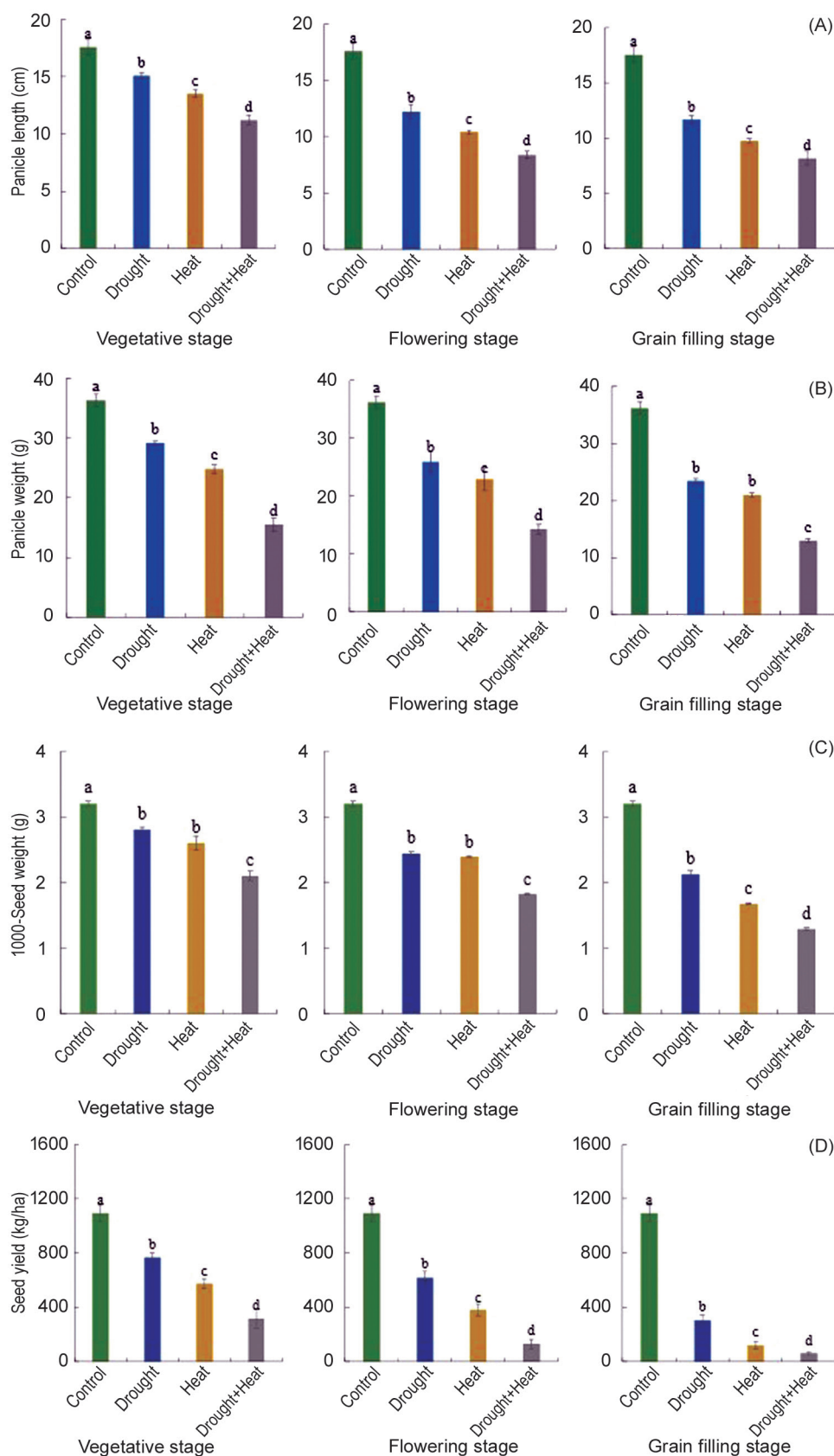


Fig. 1 Effect of stress treatments imposed at different growth stages on A) Panicle length; B) Panicle weight; C) 1000-seed weight and D) Seed yield of quinoa.

Vertical bars represent means values \pm SE (n=8). Bars with different letters indicate significant differences ($p < 0.05$).

Table 2 Effect of stress treatments imposed at different growth stages on relative water content (RWC), photosynthetic efficiency (F_v/F_m), membrane stability index (MSI), and proline content of quinoa

Treatments	RWC (%)			F_v/F_m			MSI (%)			Proline ($\mu\text{g/g f wt}$)		
	Stress imposition stages*									V	F	G
	V	F	G	V	F	G	V	F	G			
Control (C)	49.12 ^a	63.44 ^a	62.62 ^a	0.76 ^a	0.79 ^a	0.75 ^a	75.78 ^a	86.27 ^a	81.23 ^a	46.03 ^d	57.28 ^d	69.91 ^d
Drought (D)	47.90 ^b	56.56 ^b	55.19 ^b	0.44 ^b	0.46 ^b	0.42 ^b	71.12 ^b	82.19 ^b	75.69 ^b	149.18 ^c	162.48 ^c	167.56 ^c
Heat (H)	45.24 ^c	52.34 ^c	50.67 ^c	0.43 ^c	0.45 ^c	0.34 ^c	64.67 ^c	75.28 ^c	68.13 ^c	200.79 ^b	209.34 ^b	217.62 ^b
Drought + heat (D + H)	37.70 ^d	43.98 ^d	41.91 ^d	0.34 ^d	0.35 ^d	0.31 ^d	57.63 ^d	68.74 ^d	60.96 ^d	239.65 ^a	245.78 ^a	256.48 ^a

*Stress imposed at quinoa growth stages viz. V, Vegetative stage; F, Flowering stage; G, Grain filling stage. Values with different letters indicate a significant difference with $p < 0.05$.

individual or combined imposition of drought and heat stress had a substantial impact on seed yield (Fig. 1D).

Occurrence of the combination of drought and heat stress during grain-filling stage, resulted in a significant 94.2% reduction in seed yield, whereas reductions of 88.2% and 71.2% occurred during flowering and in the vegetative stage, respectively. Heat stress alone also led to decreased seed yield, with reductions of 89%, 65%, and 47.5% during grain filling, flowering, and the vegetative stage, respectively. Furthermore, drought stress when imposed alone resulted reductions of 72%, 43.2%, and 29.7% during grain filling, flowering, and the vegetative stage, respectively. This aligns with reports from Geerts *et al.* (2008), Al-Naggar *et al.* (2017) and Jamali *et al.* (2020), collectively emphasising the individual adverse impacts of water scarcity and high temperature on quinoa's seed production as there is a discernible decrease in seed yield and 1000-seed weight, accompanied by adverse effects on various yield-related attributes such as panicle length and weight, and the number of panicles per plant. Our observations also corroborate with the findings reported by Reguera *et al.* (2018), Hinojosa *et al.* (2019), Tovar *et al.* (2020), and Matías *et al.* (2021) highlighted that as stress levels intensify in quinoa, there was a corresponding decline in seed yield and various yield-related parameters.

Based on the study, it can be concluded that quinoa plants exposed to stress conditions exhibited reduced plant height, leaf area, stem girth, and shoot biomass as compared to those under control conditions. However, morphological parameters were severely impacted by stress imposed at vegetative stage while physiological and biochemical parameters were affected by stress imposed at flowering and grain filling stage. Combined drought and heat stress, in particular, had the most severe negative impacts on quinoa growth and development. Drought, heat, and drought + heat stress impaired the physiological health of quinoa plants, as indicated by reductions in PS-II efficiency (F_v/F_m), RWC, MSI, and proline content. Reduction in quinoa seed yield was 82, 67 and 48% in drought + heat, heat and drought stress, respectively as compared to control (1106 kg/ha). These findings highlight the vulnerability of quinoa to environmental stresses and emphasize the importance of understanding and mitigating the challenges posed by

marginal environments to ensure food security and crop sustainability.

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