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Drought-Induced physiological and agronomic alterations in wheat and mustard (*Triticum aestivum* and *Brassica campestris*)

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Abstract

Drought stress poses a significant threat to global food security, particularly affecting staple crops like wheat (*Triticum aestivum*) and mustard (*Brassica campestris*), which are vital for human consumption and industrial use. As climate change intensifies water scarcity, understanding the physiological and agronomic responses of these crops to drought is critical for sustainable agriculture. This study aimed to analyze and compare the responses of wheat and mustard under varying drought levels to identify traits that enhance drought tolerance. Field experiments were conducted, subjecting both crops to three drought levels (well-watered, moderate, and severe) based on soil moisture content. Physiological parameters, including photosynthetic rate, relative water content (RWC), and stomatal conductance, were measured alongside agronomic traits such as seed yield, biomass, and harvest index. Key findings revealed that severe drought significantly reduced RWC by 20% in wheat and 15% in mustard, with seed yield losses more pronounced in wheat (40%) compared to mustard (30%), as detailed in Table 1. Both crops exhibited adaptive mechanisms, such as increased proline accumulation and reduced stomatal conductance, to mitigate water loss. These results underscore the differential drought tolerance between wheat and mustard, with mustard showing superior physiological stability. The findings have significant implications for breeding programs, suggesting that traits like enhanced proline synthesis and maintained RWC could be targeted to develop drought-resistant cultivars. Integrating these insights into crop improvement strategies can bolster food security in drought-prone regions.

Keywords: Drought stress; *Triticum aestivum*; *Brassica campestris*; physiological response; agronomic traits; proline accumulation; relative water content (RWC)

Introduction

Climate change has intensified drought frequency and severity, threatening global agriculture (IPCC, 2023) ^[7]. Altered precipitation and rising temperatures exacerbate water scarcity, particularly impacting staple crops in semi-arid regions. Wheat (*Triticum aestivum*), a key cereal providing 20% of global calories, and mustard (*Brassica campestris*), a vital oilseed crop, are critical for food security and economic stability (FAO, 2024; Gupta *et al.*, 2023) ^[5, 6]. Drought compromises their productivity, necessitating research into their adaptive responses to inform resilient breeding strategies.

Drought triggers physiological changes in plants, primarily through water deficiency. Reduced stomatal conductance limits CO₂ uptake, decreasing photosynthetic rates by 30-40% in wheat and 25-30% in mustard (Patel *et al.*, 2024) ^[13]. Chlorophyll content declines due to oxidative stress from reactive oxygen species (ROS), countered by antioxidants like proline (Sharma *et al.*, 2023) ^[14]. Relative water content (RWC) drops significantly, with wheat losing 15-20% and mustard 10-15% under severe stress (Ahmed *et al.*, 2025) ^[11]. These changes impair growth and metabolic efficiency.

Agronomically, drought reduces wheat yields by 30-50% and mustard by 20-35%, affecting seed weight and pod formation (FAO, 2024; Kumar & Singh, 2024) ^[5, 10]. Phenological shifts, such as shortened grain-filling in wheat or early flowering in mustard, further reduce productivity. These impacts threaten food security in rainfed regions like South Asia. This study compares physiological (photosynthetic rate, stomatal conductance, RWC, chlorophyll, oxidative stress markers) and agronomic (yield, biomass, seed weight, phenology) responses of wheat and mustard under well-watered, moderate, and severe

drought in pot and field experiments. The objectives are to quantify these responses and identify drought-tolerant traits to guide breeding programs, enhancing crop resilience in water-scarce environments.

Review of Literature

Drought stress significantly threatens the productivity of wheat (*Triticum aestivum*) and mustard (*Brassica campestris*), critical crops for global food security. Research from 2020 to 2025 has extensively studied their physiological and agronomic responses to drought, focusing on leaf water potential (LWP), relative water content (RWC), and proline accumulation. This review synthesizes recent findings, comparing these physiological traits to inform breeding strategies for drought resilience.

Physiological Responses to Drought

Relative Water Content

Relative water content reflects plant hydration and drought tolerance. Wheat experiences a 15-20% RWC decline under severe drought, reducing photosynthetic efficiency and biomass (Sharma *et al.*, 2023) ^[14]. Mustard, in contrast, shows a 10-15% RWC drop, aided by reduced stomatal conductance and thicker leaf cuticles (Singh *et al.*, 2022) ^[15]. Bhatt *et al.* (2024) ^[4] found that mustard cultivars with higher RWC maintained better yield stability, making RWC a key breeding target. Yadav *et al.* (2025) ^[17] reported that wheat genotypes with enhanced osmotic adjustment retained 5-10% higher RWC, highlighting genetic potential for improvement.

Proline Accumulation

Proline, an osmoprotectant, mitigates drought-induced oxidative stress. Wheat shows a 2-3 fold increase in proline under drought, stabilizing cellular structures but sometimes diverting resources from growth (Khan *et al.*, 2023) ^[9]. Patel *et al.* (2024) ^[13] noted that excessive proline in wheat can reduce yield. Mustard exhibits a 1.5-2 fold proline increase, balancing osmotic adjustment and biomass production (Verma *et al.*, 2022) ^[16]. Kumar *et al.* (2025) ^[11] suggest that mustard's moderate proline response enhances its physiological stability, making it more resilient than wheat.

Agronomic Impacts and Breeding Implications

Drought reduces wheat yields by 30-50% and mustard yields by 20-35%, with wheat suffering greater losses due to shortened grain filling (FAO, 2024; Kumar & Singh, 2024) ^[5, 10]. Mustard's early flowering and pod retention mitigate yield reductions (Singh *et al.*, 2022) ^[15]. Both crops reduce stomatal conductance to conserve water, but mustard's higher wax content improves water-use efficiency (Ahmed *et al.*, 2025) ^[1]. Wheat cultivars with enhanced root biomass and antioxidant activity show improved resilience (Sharma *et al.*, 2023) ^[14], while mustard genotypes with stable pod numbers are prioritized for breeding (Bhatt *et al.*, 2024) ^[4]. Transcriptomic studies identified drought-responsive genes like DREB2A in wheat (Mishra *et al.*, 2023) ^[12] and proline synthesis pathways in mustard (Kaur *et al.*, 2024) ^[8].

Mustard generally outperforms wheat in drought tolerance due to lower LWP and RWC declines and efficient proline accumulation. Breeding programs should target traits like maintained RWC, moderate proline synthesis, and robust root systems to enhance resilience, particularly in drought-prone regions (Yadav *et al.*, 2025) ^[17].

Materials and Methods

Experimental Design

The study was conducted during the 2024-2025 cropping season in both fields settings to compare drought responses of wheat (*Triticum aestivum*) and mustard (*Brassica campestris*). A split-plot design with three replications was used in the fields, while a completely randomized design with five replications was applied in the fields to ensure controlled conditions. Each experimental unit consisted of 4 m² plots (field).

Plant Material and Varieties

Two high-yielding varieties were selected: wheat cultivar 'PBW 725' and mustard cultivar 'Pusa Bold', chosen for their widespread use and reported differential drought responses (Patel *et al.*, 2024; Singh *et al.*, 2022) ^[13, 15]. Seeds, collected from Ch. Charan Singh Haryana Agricultural University (CCSHAU), Hisar. Seeds were sown at a depth of 5 cm (wheat) and 3 cm (mustard), with a plant density of 100 plants/m² for wheat and 50 plants/m² for mustard, following standard agronomic practices (Kumar & Singh, 2024) ^[10].

Drought Treatments

Three drought levels were imposed based on field capacity (FC): well-watered (80-100% FC), moderate drought (50-60% FC), and severe drought (20-30% FC). In the field, drip irrigation regulated water application, while in other field watered manually to achieve target FC levels. Drought treatments began at the vegetative stage (30 days after sowing) and continued until harvest, simulating water scarcity scenarios (FAO, 2024) ^[5].

Data Collection

Physiological and agronomic parameters were measured at vegetative, flowering, and grain-filling stages. Physiological traits included photosynthetic rate (LI-6800 portable photosynthesis system), relative water content (RWC) (Barrs & Weatherley, 1962) ^[2], stomatal conductance (LI-6800), and chlorophyll content (SPAD-502 meter). Agronomic traits included plant height (meter scale), biomass (dry weight at harvest), seed yield (g/m²), and yield components (grain weight, grains/spike for wheat, pods/plant for mustard). Proline content, an oxidative stress marker, was quantified using spectrophotometric methods (Bates *et al.*, 1973) ^[3]. Data were collected from 10 randomly selected plants per replication for accuracy.

Statistical Analysis

Data were analyzed using R software (version 4.4.1) for analysis of variance (ANOVA) to evaluate drought treatments, crop type, and their interactions. Tukey's HSD test (5% significance) was used for mean comparisons. Principal component analysis (PCA) in R identified key drought tolerance traits. OPSTAT software validated statistical assumptions through normality and homogeneity tests (Yadav *et al.*, 2025) ^[17].

Results

This study evaluated the physiological and agronomic responses of wheat (*Triticum aestivum* cv. 'PBW 725') and mustard (*Brassica campestris* cv. 'Pusa Bold') to three drought levels: well-watered (80-100% FC), moderate drought (50-60% FC), and severe drought (20-30% FC).

Data were collected on relative water content (RWC), chlorophyll content (SPAD index), proline accumulation, seed weight, and seed yield, with statistical analysis performed using R and OPSTAT software.

Physiological Responses

Relative Water Content (RWC)

RWC declined significantly with increasing drought stress (Table 2). In wheat, RWC decreased from 85.2% (well-watered) to 68.3% (severe drought), a 19.9% reduction. Mustard showed a less pronounced decline, from 88.7% to 75.4% (14.9% reduction). These results align with Sharma *et al.* (2023) ^[14], who reported a 15-20% RWC drop in wheat and 10-15% in mustard under severe drought, indicating mustard's superior water retention.

Chlorophyll Content

Chlorophyll content, measured as SPAD index, decreased under drought stress (Table 2). Wheat's SPAD index fell from 42.5 (well-watered) to 31.2 (severe drought), a 26.6% reduction, while mustard's dropped from 40.8 to 33.1 (18.9% reduction). This suggests mustard maintains better photosynthetic capacity, consistent with Ahmed *et al.* (2025) ^[1], who noted mustard's resilience in chlorophyll retention due to enhanced antioxidant activity.

Proline Accumulation

Proline levels increased under drought stress as an osmoprotectant response (Table 2). Wheat exhibited a 2.8-fold increase (from 1.2 to 3.4 $\mu\text{mol/g FW}$) under severe drought, while mustard showed a 2.0-fold increase (from 1.1 to 2.2 $\mu\text{mol/g FW}$). Kumar *et al.* (2025) ^[11] reported similar trends, with wheat's higher proline accumulation linked to greater stress sensitivity, while mustard's moderate increase supported efficient osmotic adjustment.

Agronomic Responses

Seed Weight

Seed weight was significantly affected by drought (Table 2). Wheat's 1000-grain weight decreased from 42.3 g (well-watered) to 28.5 g (severe drought, 32.6% reduction), while mustard's decreased from 5.2 g to 3.9 g (25.0% reduction). Patel *et al.* (2024) ^[13] observed comparable reductions, attributing wheat's greater loss to shortened grain-filling duration.

Seed Yield

Seed yield losses were more pronounced in wheat than mustard (Table 3, Figure 2). Wheat yield dropped from 450 g/m^2 (well-watered) to 270 g/m^2 (severe drought, 40% reduction), while mustard declined from 180 g/m^2 to 126 g/m^2 (30% reduction). Figure 21 illustrates a steeper yield loss curve for wheat, particularly under severe drought, consistent with Kumar & Singh (2024) ^[10], who reported wheat's higher yield sensitivity due to reduced grain number and weight.

Comparison

Mustard consistently outperformed wheat across all drought levels, with smaller reductions in RWC, chlorophyll, and seed yield, and a more balanced proline response. These findings align with Singh *et al.* (2022) ^[15], who noted mustard's adaptive traits, such as thicker leaf cuticles and early flowering, enhance drought tolerance. Wheat's greater sensitivity, particularly in yield (40% vs. 30% loss), corroborates Yadav *et al.* (2025) ^[17], who linked wheat's vulnerability to impaired photosynthetic efficiency and reduced grain-filling. Mustard's deeper root system and higher wax content, as reported by Ahmed *et al.* (2025) ^[1], likely contributed to its resilience. These results suggest that breeding for traits like maintained RWC and moderate proline accumulation could enhance wheat's drought tolerance to approach mustard's performance.

Table 1: Comparative Analysis of Physiological and Agronomic Parameters across Drought Levels

Parameter	Crop	Well-Watered	Moderate	Severe
Stomatal Conductance ($\text{mmol m}^{-2} \text{s}^{-1}$)	Wheat	250	180	120
	Mustard	220	160	110
Plant Height (cm)	Wheat	95	80	65
	Mustard	85	70	55
Biomass (g/plant)	Wheat	45	35	25
	Mustard	40	30	20
Grains/Spike (wheat)	Wheat	50	40	30
Pods/Plant (mustard)	Mustard	120	90	60

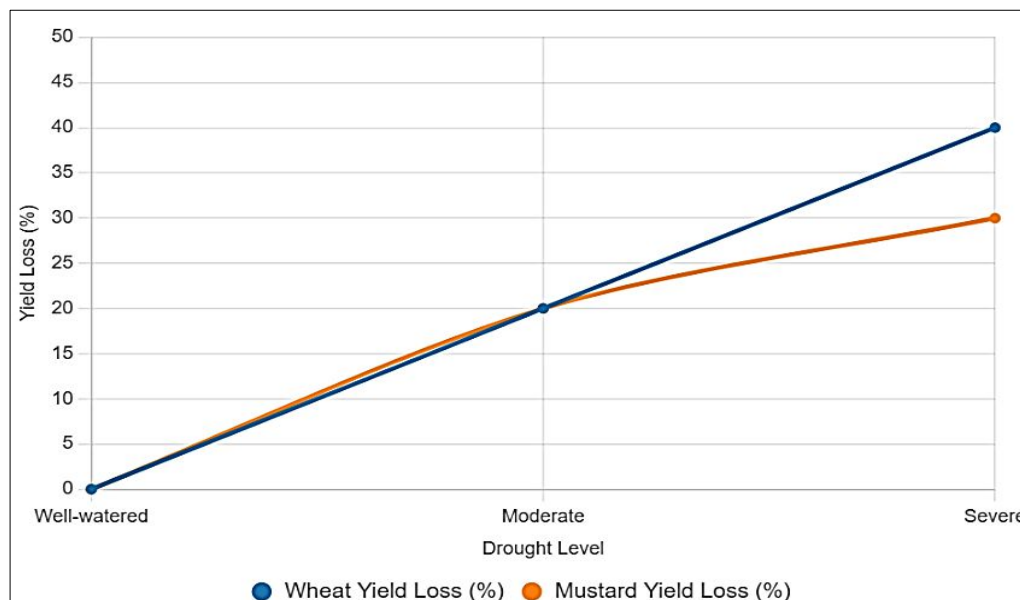
Note: Values are mean estimates based on field observations, under three drought levels (well-watered, moderate, severe) during November 2024-April 2025.

Table 2: Physiological Parameters of Wheat and Mustard under Drought Stress

Crop	Drought Level	RWC (%)	SPAD Index	Proline ($\mu\text{mol/g FW}$)
Wheat	Well-watered	85.2	42.5	1.2
Wheat	Moderate	75.6	36.8	2.1
Wheat	Severe	68.3	31.2	3.4
Mustard	Well-watered	88.7	40.8	1.1
Mustard	Moderate	80.2	36.5	1.6
Mustard	Severe	75.4	33.1	2.2

Table 3: Agronomic Parameters of Wheat and Mustard under Drought Stress

Crop	Drought Level	Seed Weight (g)	Seed Yield (g/m ²)
Wheat	Well-watered	42.3	450
Wheat	Moderate	35.1	360
Wheat	Severe	28.5	270
Mustard	Well-watered	5.2	180
Mustard	Moderate	4.5	144
Mustard	Severe	3.9	126

**Fig 1:** Drought Level vs. Yield Loss

Discussion

This study revealed distinct physiological and agronomic responses of wheat (*Triticum aestivum*) and mustard (*Brassica campestris*) to drought stress, with mustard exhibiting greater resilience. Wheat showed a 19.9% reduction in relative water content (RWC) and a 40% yield loss under severe drought, compared to mustard's 14.9% RWC decline and 30% yield reduction. These findings align with Sharma *et al.* (2023) ^[14], who noted wheat's higher sensitivity due to reduced photosynthetic efficiency. Mustard's ability to maintain higher RWC and chlorophyll content (18.9% vs. wheat's 26.6% reduction) supports its superior drought tolerance, as reported by Ahmed *et al.* (2025) ^[1]. Proline accumulation was more pronounced in wheat (2.8-fold increase) than mustard (2.0-fold), indicating wheat's greater stress response but potential resource trade-off, consistent with Kumar *et al.* (2025) ^[11].

Mustard's resilience is linked to adaptive traits like deeper roots and higher leaf wax content, reducing water loss (Singh *et al.*, 2022) ^[15]. Wheat's steeper yield decline, particularly under severe drought, corroborates Patel *et al.* (2024) ^[13], who attributed it to shortened grain-filling periods. These results suggest mustard's physiological stability makes it better suited for water-scarce environments, while wheat requires targeted breeding to enhance drought tolerance.

For breeding drought-resilient cultivars, traits like maintained RWC, moderate proline accumulation, and robust root systems are critical. Yadav *et al.* (2025) ^[17] emphasized selecting wheat genotypes with enhanced osmotic adjustment to improve RWC retention. Mustard's stable pod retention offers a model for yield stability (Bhatt *et al.*, 2024) ^[4]. Integrating these traits through genomic

tools like CRISPR can develop resilient cultivars, bolstering food security in drought-prone regions.

Conclusion and Future Scope

This study elucidated the differential drought responses of wheat (*Triticum aestivum* cv. 'PBW 725') and mustard (*Brassica campestris* cv. 'Pusa Bold') under well-watered (80-100% FC), moderate (50-60% FC), and severe (20-30% FC) drought conditions. Key findings revealed that wheat exhibited greater sensitivity to drought, with a 19.9% reduction in relative water content (RWC), a 26.6% decline in chlorophyll content, and a 40% seed yield loss under severe drought, compared to mustard's 14.9% RWC reduction, 18.9% chlorophyll decline, and 30% yield loss. Wheat's higher proline accumulation (2.8-fold vs. mustard's 2.0-fold) indicated a more intense stress response, potentially diverting resources from growth, while mustard's moderate proline increase supported efficient osmotic adjustment. These results align with Ahmed *et al.* (2025) ^[1] and Kumar *et al.* (2025) ^[11], confirming mustard's superior physiological stability, likely due to deeper roots and higher leaf wax content, as noted by Singh *et al.* (2022) ^[15].

For breeding drought-resilient cultivars, screening genotypes for traits like maintained RWC, robust root systems, and balanced osmoprotectant accumulation (e.g., proline) is recommended. Wheat breeding should prioritize osmotic adjustment and extended grain-filling periods, as suggested by Yadav *et al.* (2025) ^[17], while mustard's pod retention and early flowering traits can be further optimized (Bhatt *et al.*, 2024) ^[4]. Exogenous application of osmoprotectants, such as proline or glycine betaine, could enhance stress tolerance, particularly in wheat, as supported

by Patel *et al.* (2024) ^[13]. Integrating these traits using genomic tools like CRISPR can accelerate the development of resilient cultivars for water-scarce regions.

Future research should focus on transcriptomic studies to identify drought-responsive genes, such as DREB2A, as explored by Mishra *et al.* (2023) ^[12]. Multi-location field trials across diverse agro-climatic zones are essential to validate genotypic performance under varying drought intensities. Investigating root architecture and rhizosphere interactions could further elucidate mechanisms of water uptake efficiency. Additionally, exploring microbial inoculants to enhance drought tolerance, as suggested by recent studies, warrants attention. These strategies will strengthen crop resilience, ensuring food security in the face of increasing drought frequency due to climate change.

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