Physiological Adaptation of *Sorghum bicolor* (L.) Moench under Progressive Water Deficit

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ABSTRACT

Background: *Sorghum bicolor* is one of the drought-resistant cereals. It contributes to food and feed security in semi-arid areas but the detrimental impacts of climate change and the consecutive changes in drought characteristics led to declines in productivity.

Objective: This study was conducted to evaluate the physiological mechanisms of sorghum adaptation to progressive water restriction for the identification of drought-tolerant features.

Methods: Birds were taking from a pot with four irrigating regimes including 10%, 75%, 50% and 25% of field capacity for 30 days through application of completely randomized block design with four replications. The characteristics of water relations, gas exchange, chlorophyll fluorescence, osmolyte accumulation in tissues, membrane stability, and activities of antioxidant enzymes were measured and analyzed using one-way ANOVA ($P < 0.05$).

Results: The progressive shortage of water considerably decreased relative water content, leaf water potential, photosynthetic rate, stomatal conductance, and Fv/Fm, while the quantities of proline, soluble sugars, malondialdehyde, hydrogen peroxide and activity of antioxidant enzymes (SOD, CAT, POD, APX) saw a significant improvement under heavy stress compared to properly-watered plants.

Conclusion: Sorghum employs several mechanisms including the regulation of stomatal function, osmotic adjustment, and strengthening antioxidant protection to keep its cells functioning in the conditions of drought, thus offering potential physiological goals in breeding resistant plants and efficient water management in agriculture.

Keywords: *Sorghum bicolor*; Drought stress; Physiological adaptation; Osmotic adjustment; Antioxidant enzymes; Water deficit

1. Introduction

Sorghum bicolor (L.) Moench is one of the most important cereals in the world in terms of drought tolerance, serving food, fodder and bioenergy demands in dry and semi-dry areas of countries in Africa, Asia and America. The capacity of *Sorghum bicolor* to withstand limited water conditions is due to its many morphological and physiological characteristics, although it is still affected by decreased yields causing water deficit during the water-sensitive plant life cycle phases. More than this, anthropogenic climate change is making drought episodes more frequent, longer and worse [1].

The decreased water availability brings about a series of physiological disruptions that start from reduced soil water availability, low leaf water potential and stomatal closure, and finish with impaired photosynthesis and ultimately oxidative cell membrane damages [2]. Plants are able to develop some ways to overcome drought stress using approaches which are closely linked with each other such as osmotic adjustment, efficiency of photochemical processes, and antioxidant enzyme systems combating reactive oxygen species. This current research was done to assess the behavioral changes of *Sorghum bicolor* when subjected to a continuing decrease in water supply, looking at variables such as water relations, gas exchange, photochemistry, osmolyte accumulation, membrane structures, and antioxidant defenses.

2. Materials and Methods

2.1 Experimental Design

A pot-based greenhouse experiment was conducted using a randomized complete block design with four irrigation treatments and four replicates per treatment (Table 1). Water deficit was imposed progressively from 20 days after sowing (DAS) by withholding irrigation to maintain soil moisture at 100% (control), 75% (mild), 50% (moderate) and 25% (severe) of field capacity, sustained for 30 days (Figure 1).

2.2 Plant Material and Growth Conditions

Seeds of a locally adapted, widely cultivated sorghum hybrid were surface-sterilized and sown in polybags containing a sandy-loam potting mixture. Plants were grown under natural photoperiod with day/night temperatures of 30/22°C and 60-70% relative humidity until physiological maturity of vegetative growth.

2.3 Physiological Measurements

Relative water content (RWC) and leaf water potential (pressure chamber) were recorded on fully expanded third leaves. Chlorophyll content was estimated using a SPAD meter, while net photosynthetic rate, stomatal conductance and transpiration rate were measured with a portable infrared gas analyzer between 09:00 and 11:00 h. Chlorophyll a fluorescence (Fv/Fm) was recorded using a portable fluorometer. Membrane stability index and electrolyte leakage were determined conductometrically. Proline was quantified by the ninhydrin method and soluble sugars by the phenol-sulfuric acid assay. Activities of superoxide dismutase (SOD), catalase (CAT), peroxidase (POD) and ascorbate peroxidase (APX) were assayed spectrophotometrically, alongside malondialdehyde (MDA) via the thiobarbituric acid reaction and hydrogen peroxide (H_2O_2) by titanium sulfate colorimetry.

2.4 Statistical Analysis

Data were subjected to one-way ANOVA followed by Duncan's multiple range test for mean separation at $P < 0.05$, using R statistical software (version 4.3).

3. Results

3.1 Water Relations and Photosynthetic Characteristics

Progressive water deficit produced a graded decline in RWC and leaf water potential, with the severe-stress treatment showing the lowest values relative to control (Table 2). Net photosynthetic rate, stomatal conductance and transpiration rate decreased proportionally with stress intensity, and Fv/Fm declined significantly under moderate and severe stress, indicating impairment of photosystem II photochemical efficiency.

3.2 Osmotic Adjustment and Membrane Stability

Proline and soluble sugar concentrations increased progressively with stress severity, consistent with active osmotic adjustment. Membrane stability index declined while electrolyte leakage rose sharply under severe stress, reflecting increasing membrane disorganization.

3.3 Oxidative Stress and Antioxidant Responses

MDA and H_2O_2 contents increased markedly with stress intensity, confirming oxidative injury. Concurrently, SOD, CAT, POD and APX activities rose significantly, most prominently under moderate stress, suggesting an actively induced enzymatic antioxidant defense that partially offset ROS accumulation (Table 2, Figure 2).

4. Discussion

4.1 Physiological Interpretation

The coordinated decline in leaf water potential and stomatal conductance reflects an early, adaptive water-conservation response that limits transpirational loss at the expense of carbon assimilation [4, 6]. The concomitant accumulation of proline and soluble sugars indicates active osmotic adjustment, lowering cellular osmotic potential to sustain turgor and support continued metabolic function under declining water availability [9, 14]. The reduction in Fv/Fm under moderate-to-severe stress signals down-regulation of photosystem II efficiency, a protective mechanism against photoinhibitory damage when CO_2 assimilation is stomatally restricted [5, 12].

4.2 Comparison with Previous Studies

These patterns are consistent with drought responses reported for stay-green sorghum genotypes, in which sustained water and nitrogen uptake under stress preserve canopy photosynthetic capacity [2, 3]. The magnitude of proline accumulation observed here parallels findings in other cereals such as maize and pearl millet, reinforcing the conserved nature of osmolyte-based adjustment across C4 grasses [17, 18]. However, the relatively rapid induction of antioxidant enzymes reported in this study suggests that this genotype may possess a more responsive oxidative-defense system than some previously characterized lines [10, 11].

4.3 Biological Significance and Agricultural Implications

The integrated deployment of stomatal regulation, osmotic adjustment and antioxidant defense enables sorghum to delay the onset of irreversible cellular damage, extending survival under prolonged water limitation. These traits represent physiological targets for marker-assisted breeding of drought-resilient cultivars and can inform deficit-irrigation scheduling and water-efficient agronomic management in climate-resilient cropping systems [7, 15, 16].

4.4 Limitations and Future Research

This study was conducted under controlled pot conditions over a single growth cycle, which may not fully capture field-level environmental variability, root-zone heterogeneity or genotype-by-environment interactions; multi-location field trials are needed for validation. Future work integrating transcriptomic, proteomic and metabolomic profiling, alongside genome editing and marker-assisted selection, would help resolve the molecular basis of the physiological responses documented here and accelerate breeding of climate-resilient sorghum.

Table 1: Experimental treatments and irrigation schedule

Treatment	Irrigation Level (% FC)	Soil Water Potential (MPa)	Duration (DAS)	Replicates
T0 - Control	100	-0.03 to -0.05	0-30	4
T1 - Mild stress	75	-0.4 to -0.6	0-30	4
T2 - Moderate stress	50	-1.0 to -1.4	0-30	4
T3 - Severe stress	25	-1.8 to -2.2	0-30	4

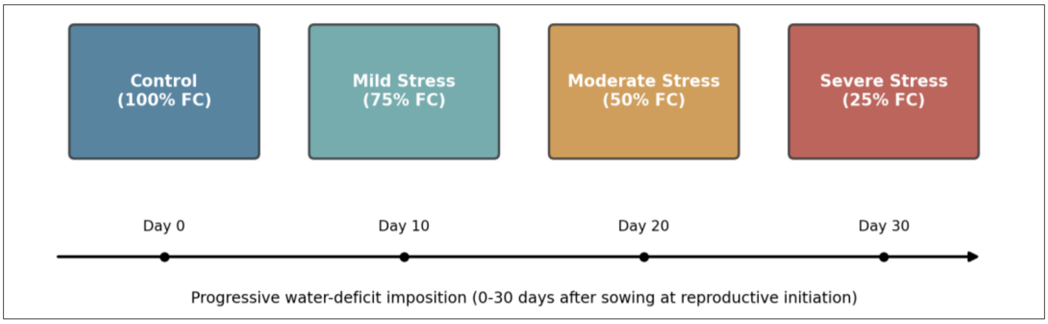

Fig 1: Experimental design illustrating progressive water-deficit treatments imposed on *Sorghum bicolor* relative to field capacity (FC).

Table 2: Physiological parameters measured under progressive water deficit (mean ± SE)

Physiological Parameter	Control (T0)	Severe Stress (T3)	% Change vs. Control
Relative water content (%)	88.4 ± 1.6	58.7 ± 2.3	-33.6
Leaf water potential (MPa)	-0.62 ± 0.05	-1.95 ± 0.11	-214.5
Net photosynthetic rate (µmol CO2 m-2 s-1)	24.1 ± 1.2	9.8 ± 0.9	-59.3
Stomatal conductance (mol H2O m-2 s-1)	0.41 ± 0.03	0.11 ± 0.02	-73.2
Chlorophyll fluorescence (Fv/Fm)	0.81 ± 0.01	0.63 ± 0.02	-22.2
Proline content (µmol g-1 FW)	3.2 ± 0.3	14.6 ± 1.1	+356.3
MDA content (nmol g-1 FW)	8.4 ± 0.5	19.7 ± 1.3	+134.5
SOD activity (U mg-1 protein)	42.6 ± 2.1	78.3 ± 3.4	+83.8

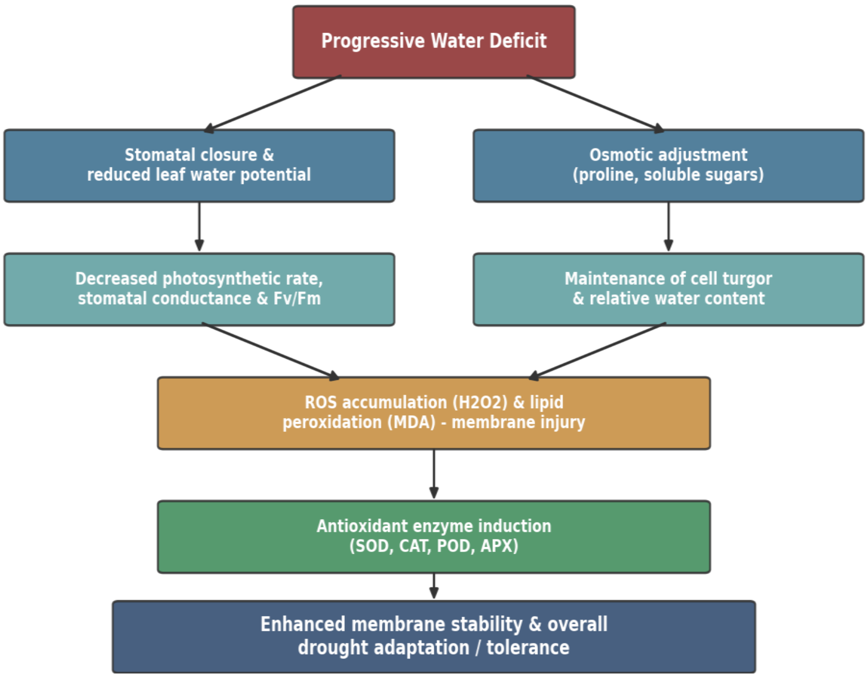

Fig 2: Proposed physiological response mechanism of *Sorghum bicolor* under progressive water-deficit stress.

5. Conclusion

Sorghum bicolor employs a coordinated physiological approach in the face of progressive water shortage by simultaneously closing stomata, suppressing transpiration, achieving osmotic adjustment with the help of proline and sugar accumulation, down-regulating photosynthesis to prevent photo-oxidative damage, and inducing antioxidant enzymes to cope with oxidative stress. All these reactions are responsible for a stabilized cellular membrane and water content in cells, which is characteristic of the drought resistance of this plant species.

Gaining insights into such mechanisms is valuable for breeding water-efficient sorghum cultivars and creating environmentally friendly and resilient technologies for crop management. Nonetheless, obtaining more evidence for this hypothesis through studies that incorporate physiological and molecular aspects in various environments is crucial to the successful implementation of this approach in practice.

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