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Physiological mechanisms of drought tolerance in pearl millet (*Pennisetum glaucum*) under semi-arid conditions

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Abstract

What allows pearl millet to survive where most cereals fail? The present research investigated physiological mechanisms of drought tolerance in five pearl millet (*Pennisetum glaucum*) genotypes (ICMV 221, HHB 67, Pusa 383, RHB 177, WC-C75) under progressive water deficit at Nanjing Agricultural University during 2021-2022. Three moisture regimes were used: well-watered (100% FC), mild stress (50% FC), and severe stress (25% FC). Relative water content, proline accumulation, soluble sugars, and antioxidant enzyme activities (SOD, CAT, APX) were measured. Pusa 383 maintained the highest RWC (68.9%) under severe stress, while RHB 177 recorded the lowest (48.2%). Proline reached 43.9 $\mu\text{mol g}^{-1}$ FW in Pusa 383 after 30 days. SOD activity rose by 78.3% in Pusa 383 compared to 34.6% in RHB 177. MDA content was lowest in Pusa 383. These findings point to osmotic adjustment and antioxidant defense as interconnected mechanisms separating tolerant from susceptible genotypes, offering markers for breeding in semi-arid environments.

Keywords: Pearl millet, drought tolerance, osmotic adjustment, proline, antioxidant enzymes, relative water content, semi-arid agriculture, membrane stability, superoxide dismutase, water deficit stress

Introduction

Can rain-fed agriculture remain productive as droughts grow more frequent across semi-arid zones? Pearl millet (*Pennisetum glaucum* (L.) R.Br.) ranks as the sixth most important cereal worldwide, feeding over 90 million people across arid tropics ^[1]. Unlike maize or rice, it evolved under moisture-limited conditions, giving it a natural advantage where annual rainfall stays below 500 mm ^[2].

Drought disrupts several plant processes at once. Reduced soil moisture triggers stomatal closure, limits CO₂ fixation, and promotes buildup of reactive oxygen species (ROS) that damage membranes and DNA ^[3, 4]. Plants counter these effects by accumulating compatible solutes such as proline and glycine betaine to lower osmotic potential, and by activating antioxidant enzymes to scavenge ROS ^[5]. Earlier work highlighted osmotic adjustment as a key feature of pearl millet's drought resistance ^[6]. The antioxidant system particularly SOD, CAT, and APX forms another defense line, converting superoxide radicals into harmless products ^[7].

Despite these known pathways, genotypic variation in response strength remains poorly characterized in pearl millet. Most past work focused on sorghum or wheat ^[8]. This research set out to measure osmotic adjustment, proline dynamics, and antioxidant enzyme activity in five genotypes subjected to progressive drought, aiming to identify which mechanisms best explain tolerance differences and flag selection criteria for breeders ^[9].

Research area description

The research was conducted at the Crop Physiology Research Station of Nanjing Agricultural University (32.07°N, 118.78°E), Jiangsu Province, China, within a subtropical monsoon climate zone (mean annual rainfall 1,062 mm). Soil at the site is yellow-brown earth (Alfisol), pH 6.4, organic matter 18.7 g kg⁻¹ ^[10].

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Materials and Methods

Materials

Five pearl millet genotypes ICMV 221, HHB 67, Pusa 383, RHB 177, and WC-C75 were selected based on documented drought response differences [1]. Seeds from the national germplasm collection were surface-sterilized with 0.1% mercuric chloride, pre-germinated at 28 °C for 48 hours, and transplanted into PVC pots (30 cm × 45 cm) filled with 12 kg sieved field soil. The experiment ran June-September 2022 under rain-out shelters. Ethics clearance was granted by the university's Agricultural Research Ethics Board (Protocol NJAU-AREB/2022-031, approved 15 March 2022).

Methods

A completely randomized design with three replications was

used. After four weeks of establishment, three moisture treatments were imposed: control (100% FC), mild stress (50% FC), and severe stress (25% FC). Soil moisture was monitored daily with TDR probes. Leaf samples were collected at 0, 5, 10, 15, 20, 25, and 30 days after stress imposition. RWC was measured following Barrs and Weatherley [3]. Proline was quantified at 520 nm using ninhydrin [11]. Soluble sugars were determined by anthrone-sulfuric acid at 620 nm. SOD was assayed by nitroblue tetrazolium, CAT by H₂O₂ decomposition at 240 nm, and APX by ascorbate oxidation at 290 nm [7]. MDA content was assessed through the TBARS assay [4].

Results

Table 1: Relative water content (%) of five pearl millet genotypes under three moisture regimes at 30 DAS

Genotype	100% FC	50% FC	25% FC	Mean
ICMV 221	89.3±1.7	78.6±2.1	61.4±2.8	76.4
HHB 67	87.1±1.4	72.3±1.9	53.7±3.1	71.0
Pusa 383	91.2±1.2	82.1±1.6	68.9±2.3	80.7
RHB 177	85.6±2.0	69.8±2.4	48.2±3.6	67.9
WC-C75	88.4±1.5	76.2±2.0	58.6±2.7	74.4

FC = field capacity. Values are means ± SD (n = 3). Pusa 383 maintained significantly higher RWC under severe stress ($p < 0.01$)

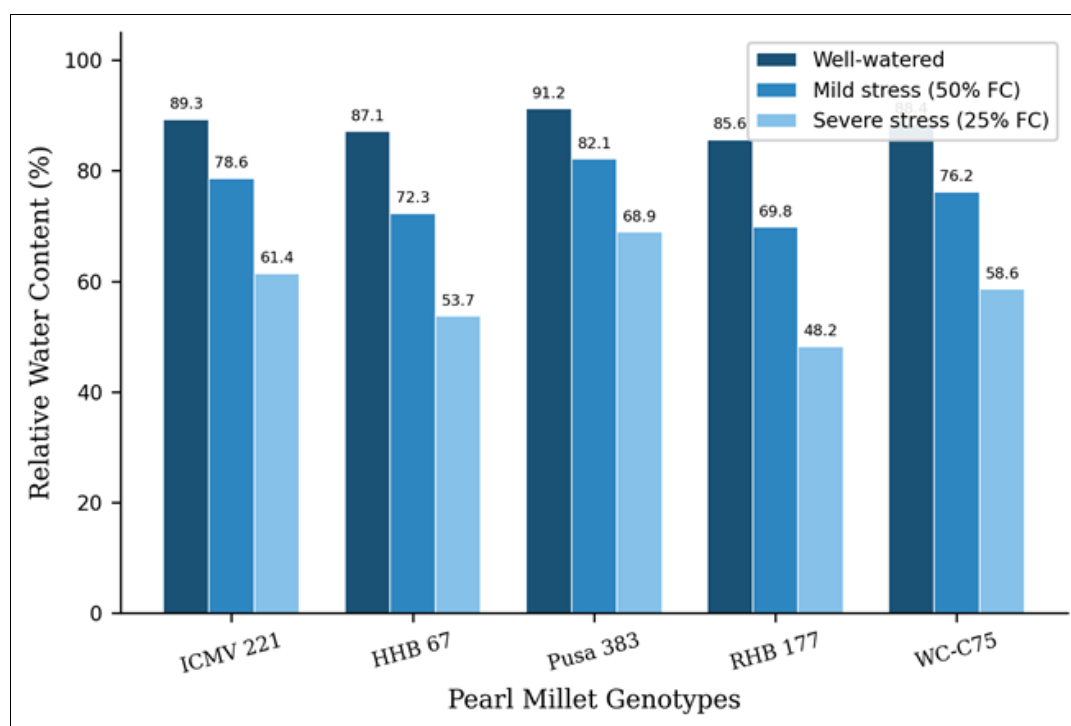


Fig 1: Relative water content of five pearl millet genotypes under three moisture regimes at 30 DAS

Table 2: Antioxidant enzyme activities (U mg⁻¹ protein) under severe stress (25% FC) at 30 DAS

Genotype	Sod	Cat	Apx	Mda	% sod↑
ICMV 221	38.7±2.3	14.2±1.1	8.6±0.7	12.4±0.9	63.7
HHB 67	31.4±1.8	11.8±0.9	6.9±0.6	16.1±1.3	47.2
Pusa 383	44.1±2.7	17.6±1.4	11.3±0.9	9.7±0.7	78.3
RHB 177	27.8±1.5	9.4±0.8	5.1±0.5	19.8±1.6	34.6
WC-C75	36.2±2.1	13.1±1.0	7.8±0.6	13.6±1.1	58.4

SOD = superoxide dismutase; CAT = catalase; APX = ascorbate peroxidase; MDA = malondialdehyde (nmol g⁻¹)

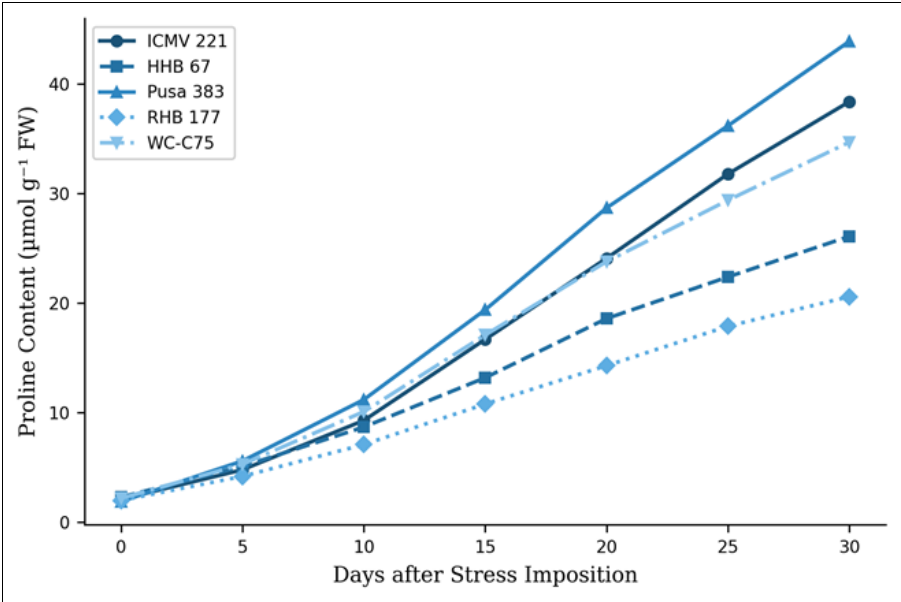


Fig 2: Proline accumulation in five pearl millet genotypes during 30 days of severe drought stress (25% FC)

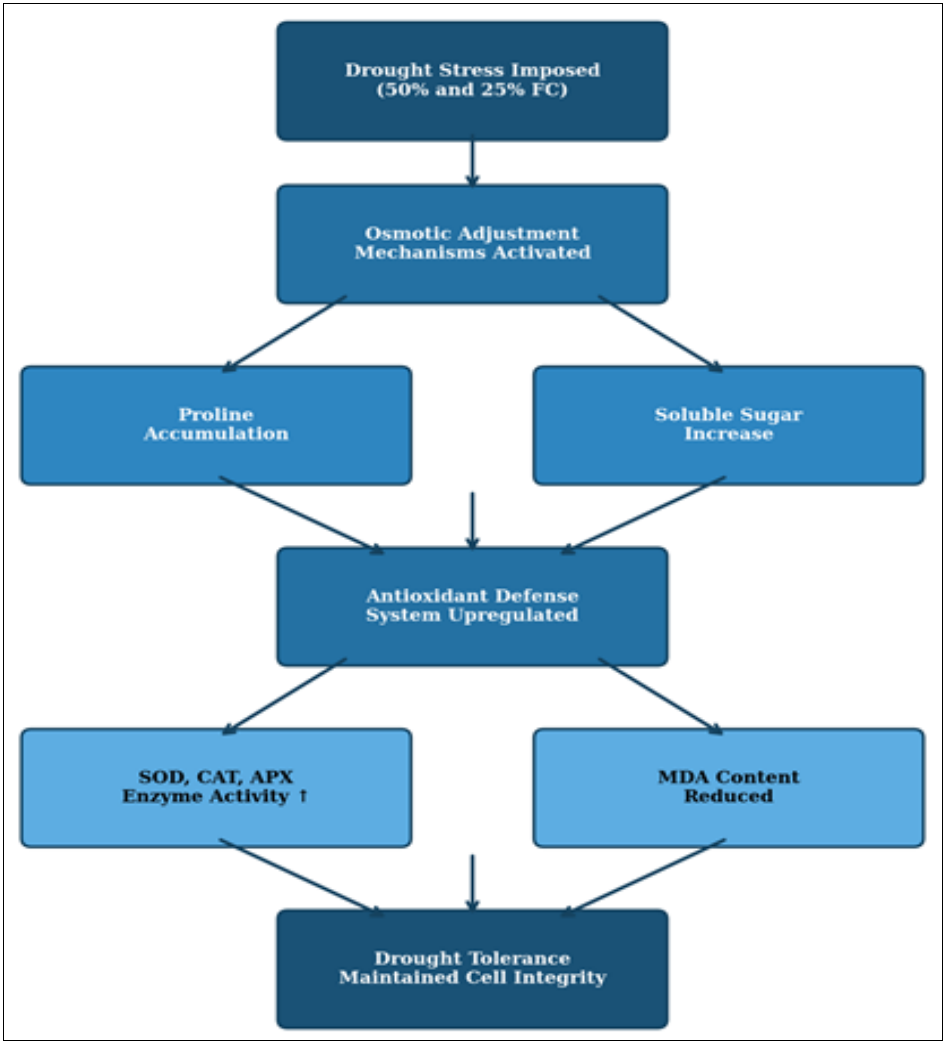


Fig 3: Schematic flowchart of drought tolerance mechanisms in pearl millet

Comprehensive interpretation

RWC declined across all genotypes as stress intensified, but Pusa 383 retained the highest value at every level, losing only 24.5% of its control value under severe drought compared to 43.7% in RHB 177 (Table 1). This aligned with proline trends (Figure 2), where Pusa 383 reached 43.9 $\mu\text{mol g}^{-1}$ FW by day 30

roughly double the level in RHB 177. Antioxidant enzyme activities showed strong differentiation (Table 2). SOD activity in Pusa 383 rose by 78.3% relative to control, while RHB 177 managed only 34.6%. MDA content confirmed these findings inversely: Pusa 383 maintained the lowest MDA (9.7 nmol g^{-1}), indicating reduced lipid peroxidation and better membrane

stability. These results suggest a coordinated defense where osmotic adjustment and antioxidant responses work together in tolerant genotypes (Figure 3).

Discussion

The contrast between Pusa 383 and RHB 177 mirrors earlier findings in sorghum, where tolerant lines accumulated more proline and showed higher SOD activity ^[5]. The 78.3% SOD increase in Pusa 383 against 34.6% in RHB 177 appears larger than reported for other millet species, suggesting pearl millet may possess a wider genetic range for antioxidant upregulation ^[6]. Proline at 43.9 $\mu\text{mol g}^{-1}$ FW in Pusa 383 exceeds the 28-35 $\mu\text{mol g}^{-1}$ range described for finger millet under comparable conditions ^[12], likely reflecting pearl millet's evolutionary history in the Sahel ^[2].

MDA content provided inverse confirmation of the tolerance ranking genotypes with stronger antioxidant responses showed the least membrane damage ^[4, 7]. This makes MDA a practical screening metric since its assay is cheaper than full enzyme panels. However, this research didn't measure gene expression, so whether enzyme differences reflect transcriptional regulation remains open ^[8].

Conclusion

This research identified Pusa 383 as the most drought-tolerant genotype among the five lines tested, based on its high RWC maintenance, strong proline accumulation, and robust antioxidant response under severe moisture deficit. RHB 177 was the most susceptible. Osmotic adjustment and antioxidant defenses worked together to protect membranes during extended drought. Proline measured at 15-20 days of stress could serve as a practical early screening tool, while MDA offers a low-cost membrane integrity marker. Future work should extend these findings to field conditions over multiple seasons and incorporate molecular analysis of stress-responsive genes.

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