Osmoprotectant Accumulation and Membrane Stability in *Lens culinaris Medik*. under Moisture Stress

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ABSTRACT

Background: Lentil (*Lens culinaris Medik*.) is an important leguminous crop cultivated in the rain-fed agriculture systems where yield stabilization is hindered by moisture scarcity.

Objective: The purpose of this work was to measure osmoprotectant accumulation and their role in enhancing membrane stability of lentil varieties subjected to moisture stress.

Methods: The experimental plants consisted of two lentil varieties, grown under pot conditions in well-watered (100% field capacity) and moisture-stress (40% field capacity) conditions. The following indices were measured: relative water content (RWC); membrane stability index (MSI); electrolyte leakage (EL); proline; glycine betaine; soluble sugars; total soluble proteins; malonaldialdehyde (MDA); hydrogen peroxide; antioxidant enzyme activity. All data were processed using ANOVA and correlation statistics.

Results: Moisture stress reduced the RWC and MSI, and increased EL, MDA, and osmoprotectants amount. The accumulation of osmoprotectants was positively correlated with the MSI and antioxidant enzyme activity and negatively correlated with EL, indicating osmotic and antioxidant impact.

Conclusion: The accumulation of osmoprotectants is an important factor in membrane stabilization under drought, which can be used for identification of drought-resistant lentil varieties and developing drought-proof breeding.

Keywords: Lentil, Drought stress, Osmotic adjustment, Proline, Glycine betaine, Membrane stability index, Reactive oxygen species, Stress physiology.

Introduction

Lentil is one of the most cultivated pulses worldwide with its significance for protein content, nitrogen fixation and capability to grow in semi-arid conditions [1, 2]. With changes in climate patterns observed in rainfed areas, moisture stress has gained prevalence defining the abiotic barrier in lentil production and causing the decrease in the productivity by interfering with physiological processes of the plant [3, 4].

In physiological aspect, moisture stress leads to decreasing water potential and turgor of leaves which influences on stomatal conductance as well as photosynthetic process and chlorophyll [5, 6]. Osmotic adjustment is applied to combat stress as a process where compatible metabolites, such as proline, glycine betaine, soluble sugars and soluble proteins are established in the cytoplasm of the plant [7, 8]. In legumes, the process of osmotic adjustment is performed in cooperation with the antioxidant abilities, as stomatal closing triggered by drought leads to increased reactive oxygen species (ROS) production and damage membrane through lipid peroxidation [9, 10].

Membrane stability, normally evaluated via electrolyte leakage and the membrane stability index, is well-known as a useful physiological parameter of drought resistance as it summarizes the total effect of both osmotic and oxidative stress on the condition of the membrane phospholipid [11, 12]. Genotypes that have the ability to maintain membrane stability under deficit conditions are expected to have good recovery from stress and yield stability [13].

Even though the field of drought-resistant legumes breeding is developing, the quantitative relationship between osmoprotectants build-up and membrane stability in lentils is not well studied yet. Therefore, the aim of this research was to (i) assess the physiological and biochemical responses of lentil genotypes to moisture stress, and (ii) find out the relationship between the amount of osmoprotectants accumulated, enzyme activity and membrane stability.

Materials and Methods

Experimental Design: A pot experiment was conducted in a randomized complete block design (RCBD) with three replications, comprising two moisture regimes — well-watered control (100% field capacity, FC) and moisture stress (40% FC, imposed at the vegetative stage for 15 days) — applied to two lentil genotypes obtained from a certified regional gene bank.

Plant Material and Growth Conditions: Seeds were surface-sterilized, sown in loamy soil under controlled greenhouse conditions (25 ± 2 °C, 14 h photoperiod), and thinned to uniform seedling density prior to treatment imposition.

Physiological Measurements: RWC was determined from fresh, turgid, and dry leaf weights [14]. MSI and EL were assessed via electrical conductivity of leaf discs before and after boiling [11]. Total chlorophyll was estimated spectrophotometrically following acetone extraction [15].

Biochemical Analysis: Proline was quantified using the ninhydrin method [16]; glycine betaine by the periodide method [17]; soluble sugars by the anthrone method [18]; total soluble protein by the Bradford assay [19]; MDA content as a lipid peroxidation index via thiobarbituric acid reactivity [20]; and hydrogen peroxide spectrophotometrically [21]. Superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), and ascorbate peroxidase (APX) activities were assayed using standard enzyme kinetic protocols [22, 23].

Statistical Analysis: Data were subjected to two-way ANOVA, and means were compared using Duncan's multiple range test ($P < 0.05$). Pearson correlation and linear regression were used to examine relationships between osmoprotectants, antioxidant enzymes, and membrane stability indices.

Results and Performance Evaluation

Moisture stress significantly reduced RWC, MSI, and chlorophyll content relative to control, while EL and MDA increased markedly (Table 1), indicating stress-induced membrane destabilization and photosynthetic pigment degradation.

Table 1: Effect of moisture stress on physiological parameters of *Lens culinaris*.

Parameter	Control (100% FC)	Moisture Stress (40% FC)	% Change
RWC (%)	84.6 ± 1.8	58.3 ± 2.1	−31.1
MSI (%)	79.2 ± 1.5	51.7 ± 2.3	−34.7
Electrolyte Leakage (%)	18.4 ± 1.1	42.9 ± 2.0	+133.2
Total Chlorophyll (mg g^{-1} FW)	2.14 ± 0.09	1.42 ± 0.08	−33.6

Osmoprotectant concentrations rose substantially under stress (Table 2), with proline showing the largest relative increase, consistent with its role as a rapidly mobilized osmolyte. Antioxidant enzyme activities (SOD, CAT, POD, APX) increased concurrently, reflecting enhanced ROS-scavenging capacity.

Table 2: Osmoprotectant accumulation, antioxidant enzyme activity, and membrane stability under different moisture regimes.

Trait	Control	Moisture Stress
Proline (µg g^{-1} FW)	12.8 ± 0.6	38.5 ± 1.9
Glycine betaine (µmol g^{-1} DW)	4.1 ± 0.3	9.7 ± 0.5
Soluble sugars (mg g^{-1} DW)	21.3 ± 1.0	34.6 ± 1.4
MDA (nmol g^{-1} FW)	6.2 ± 0.4	14.8 ± 0.9
SOD (U mg^{-1} protein)	18.6 ± 1.2	31.4 ± 1.6
CAT (U mg^{-1} protein)	22.1 ± 1.5	35.9 ± 1.8

Pearson correlation analysis revealed a strong positive association between proline and glycine betaine accumulation and MSI ($r = 0.87$ and $r = 0.81$, respectively), and a strong negative correlation between these osmolytes and EL ($r = −0.83$), confirming their membrane-protective role. Antioxidant enzyme activity also correlated positively with MSI ($r = 0.79$), suggesting coordinated osmotic–antioxidative regulation. Genotypic comparison indicated differential stress responsiveness, with the genotype exhibiting higher constitutive osmoprotectant accumulation showing proportionally smaller declines in RWC and MSI, statistically significant at $P < 0.05$.

Discussion

The observed decline in RWC and concurrent rise in EL under-moisture stress reflect impaired cellular hydration and progressive loss of membrane semipermeability, both hallmark responses of drought-exposed legume tissue [3, 9]. The pronounced accumulation of proline and glycine betaine supports their established role as compatible solutes that maintain osmotic potential without interfering with enzymatic activity, thereby preserving turgor-dependent physiological processes [7, 8].

Mechanistically, osmoprotectants stabilize membranes through several complementary pathways: by preserving the hydration shell around membrane proteins and phospholipid head groups, by scavenging ROS directly, and by preventing protein denaturation during dehydration-induced macromolecular crowding [10, 12]. The strong inverse correlation between osmolyte accumulation and MDA content in this study reinforces the hypothesis that osmotic adjustment mitigates lipid peroxidation, a primary driver of membrane disintegration under oxidative stress [20].

Simultaneously, elevated SOD, CAT, and APX activities indicate an intact enzymatic antioxidant system capable of detoxifying superoxide radicals and hydrogen peroxide before they compromise membrane lipids [22, 23]. The coordinated rise of osmotic and antioxidative defenses aligns with findings in chickpea and other pulses, where membrane stability under drought was similarly attributable to combined osmoregulatory and redox-buffering mechanisms [13, 24].

These findings carry practical significance for lentil breeding: MSI and osmoprotectant profiling could serve as rapid, physiologically grounded selection criteria in early-generation drought screening, complementing yield-based selection that is often confounded by environmental variability [4, 13]. Given ongoing climate variability, incorporating such physiological markers into breeding pipelines may accelerate development of resilient cultivars for water-limited environments [25].

This study is limited by its pot-based, single-stress-duration design, which may not fully capture field-level drought dynamics involving fluctuating soil moisture and companion abiotic stresses. Future work should integrate transcriptomic and metabolomic profiling to identify regulatory genes governing osmolyte biosynthesis, alongside multi-environment field trials to validate physiological markers under natural drought conditions.

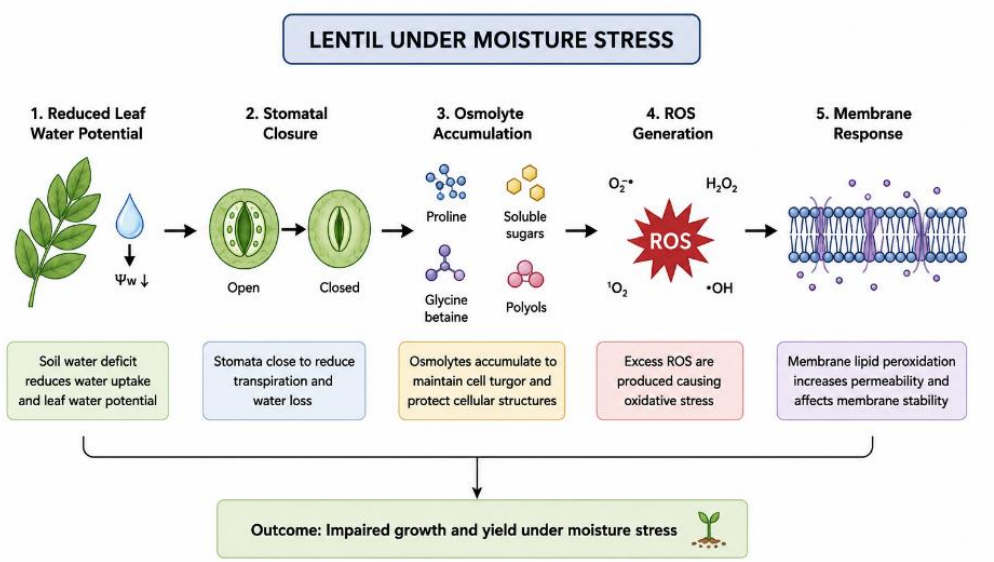

Fig 1: Schematic representation of physiological and biochemical responses of lentil under moisture stress, depicting sequential changes from reduced leaf water potential to stomatal closure, osmolyte accumulation, ROS generation, and membrane response.

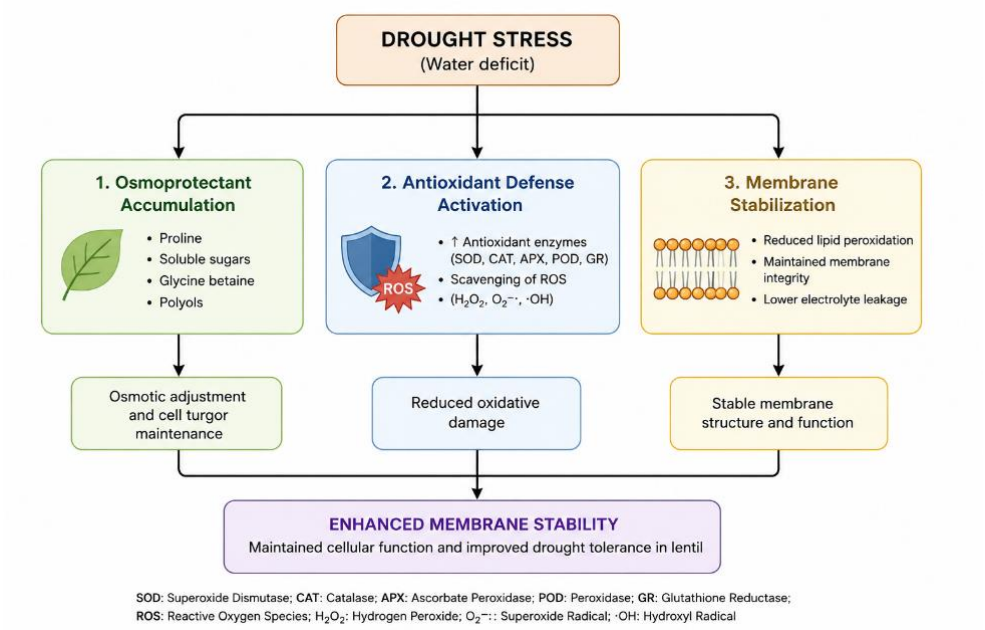

Fig 2: Proposed mechanism illustrating osmoprotectant accumulation, antioxidant defense activation, and consequent membrane stabilization during drought stress in lentil leaf cells.

Conclusion

Moisture stress led to a severe compromise of membrane integrity in lentil plants, as seen by a decline in RWC and MSI, and by an increase in electrolyte leakage and lipid peroxidation. The impact of moisture stress was alleviated partially due to the coordinated accumulation of proline, glycine betaine, and soluble sugars and increased activity of various antioxidant enzymes. The observed strong correlation between levels of osmotic protectants and membrane stability further shows that the process of osmoregulation is supported by an effective means of physiologically driven adaptation to drought conditions. The physiological parameters described offer an opportunity for their wide usage as selection criteria in breeding of lentil varieties resistant to drought. Recommendations for

further molecular and field investigations are included in the manuscript for achievement of a commercial progress by means of utilizing the information obtained in this study in breeding programs.

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