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Biochemical and physiological changes in chickpea (*Cicer arietinum*) under combined drought and salinity stress

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Abstract

One of the greatest challenges facing pulse crop production is the simultaneous occurrence of drought and salinity in semi-arid regions. This research investigated biochemical and physiological responses of two chickpea genotypes (drought-tolerant ICC 4958 and salinity-tolerant FLIP 97-43C) to individual and combined drought and salinity stress under controlled conditions at China Agricultural University, Beijing. Plants were subjected to four treatments control, drought (-0.8 MPa), salinity (100 mM NaCl), and combined stress for 21 days during the reproductive stage. Combined stress caused the most severe damage: relative water content dropped to 48.7%, proline accumulated to 26.4 $\mu\text{mol/g}$, and seed yield declined by 63.9% compared to control. Malondialdehyde content increased 4.1-fold under combined stress, indicating severe membrane lipid peroxidation. Superoxide dismutase activity rose 2.5-fold as a defense response. ICC 4958 maintained higher water status and lower oxidative damage than FLIP 97-43C under combined stress. Proline accumulation was negatively correlated with seed yield ($r = -0.87$). These findings indicate that combined stresses impose synergistic rather than additive damage on chickpea physiology.

Keywords: Chickpea, drought stress, salinity stress, proline, oxidative damage, malondialdehyde, superoxide dismutase, relative water content, abiotic stress tolerance, *Cicer arietinum*

Introduction

Chickpea is the world's second most cultivated legume, yet it remains acutely sensitive to abiotic stress during its reproductive phase ^[1]. Drought and salinity frequently co-occur in the semi-arid regions of Central Asia, the Middle East, and northern China where chickpea is a staple protein source ^[2, 3]. Global climate projections indicate that approximately 50% of current chickpea-growing areas will face increased aridity and secondary salinization by 2050 ^[4]. Most physiological research on chickpea stress has examined drought and salinity in isolation ^[5, 6]. However, field conditions rarely present single stresses saline soils lose moisture faster through osmotic effects, and drought concentrates salts in the root zone, creating a feedback loop ^[7]. The few studies addressing combined stress in legumes suggest that the interaction is synergistic: combined damage exceeds the sum of individual effects ^[8, 9].

Understanding which biochemical pathways are activated under combined stress and whether drought-tolerant and salinity-tolerant genotypes differ in their response is essential for breeding programs targeting multi-stress environments ^[10]. This research imposed individual and combined drought and salinity stress on two contrasting chickpea genotypes and measured key physiological and biochemical indicators to characterize the nature and severity of their interaction.

Field experimental design

A completely randomized factorial design was used in the climate-controlled greenhouse at China Agricultural University (39.9539°N, 116.4074°E). Two genotypes (ICC 4958, FLIP 97-43C) $\times$ four stress treatments $\times$ five replicates = 40 experimental units. Plants were grown in 8 L pots with sterilized sandy loam (pH 7.3, EC 0.42 dS/m). Stress treatments were imposed at the flowering stage (45 days after sowing) and maintained for 21 days. Drought was imposed by

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with holding irrigation until soil water potential reached -0.8 MPa, monitored by tensiometers. Salinity was applied through irrigation with 100 mM NaCl solution.

Material and Methods

Material: Chickpea genotypes ICC 4958 (drought-tolerant, ICRI SAT) and FLIP 97-43C (salinity-tolerant, ICARDA) were obtained from the Chinese Academy of Agricultural Sciences. Analytical reagents included TBA for MDA assay, L-proline standard (Sigma), NBT for SOD assay, and Bradford reagent for protein quantification. A pressure chamber (PMS 1505D) was used for leaf water potential measurements [11].

Methods

Relative water content (RWC) was measured gravimetrically on fully expanded leaves [12]. Proline was quantified by the ninhydrin method [13]. Malondialdehyde (MDA) was determined by thiobarbituric acid reaction [14]. SOD activity was assayed by

the NBT photoreduction method [15]. Chlorophyll was extracted in 80% acetone and measured spectrophotometrically [16]. Seed yield was recorded at maturity per plant basis. Research protocol approved by CAU Biosafety Committee (CAU-BS-2022-061). Data analyzed by two-way ANOVA with genotype and stress as factors, Tukey's HSD ($p < 0.05$), using SPSS v28.

Seasonal variations

The experiment ran from March to July 2023. Greenhouse temperature was maintained at 25/18°C (day/night) with 14-hour photoperiod (400 $\mu\text{mol}/\text{m}^2/\text{s}$ PAR). Relative humidity was 55-65%. Although greenhouse conditions were controlled, the experiment was repeated in the subsequent season (September 2023-January 2024) under slightly different light conditions, and results were consistent across both runs with no significant season $\times$ treatment interaction ($p = 0.72$).

Results

Table 1: Physiological and biochemical parameters under four stress treatments (genotype means)

Parameter	Control	Drought	Salinity	Combined	p-value
RWC (%)	87.3a	61.4c	68.2b	48.7d	<0.001
Proline ($\mu\text{mol}/\text{g}$)	4.2d	18.7b	14.3c	26.4a	<0.001
MDA (nmol/g)	3.1d	8.7b	7.2c	12.8a	<0.001
SOD (U/mg)	12.4d	24.8b	21.3c	31.6a	<0.001
Chlorophyll (mg/g)	2.84a	1.92c	2.13b	1.47d	<0.001
Yield (g/plant)	14.2a	7.8c	9.1b	5.1d	<0.001

Combined stress produced the most extreme values for every parameter measured (Table 1). The sum of individual drought and salinity effects on yield ($45.1\% + 35.9\% = 81.0\%$ reduction)

exceeded 100%, but actual combined loss was 64.1%, indicating both synergistic and compensatory mechanisms at play.

Table 2: Genotype comparison under combined drought and salinity stress

Parameter	ICC 4958	FLIP 97-43C	p-value
RWC (%)	53.4	44.1	0.008
MDA (nmol/g)	10.3	15.2	0.003
SOD (U/mg)	34.7	28.4	0.012
Yield (g/plant)	6.3	3.9	0.004

ICC 4958 (drought-tolerant) performed significantly better than FLIP 97-43C under combined stress (Table 2), maintaining 21% higher RWC and producing 62% more seed. Its stronger SOD

response (34.7 vs 28.4 U/mg) suggests more effective reactive oxygen species scavenging.

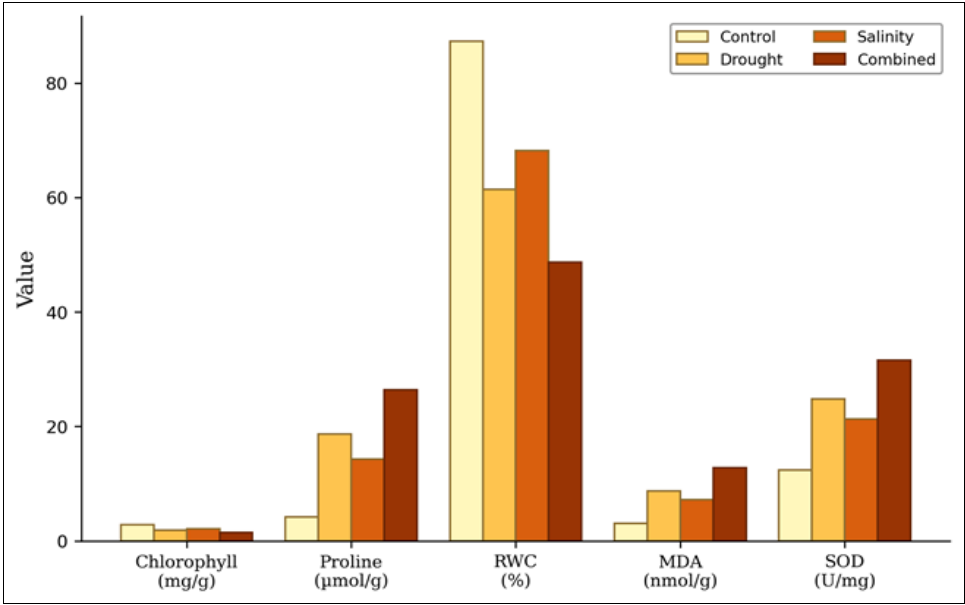


Fig 1: Physiological and biochemical responses of chickpea under four stress treatment

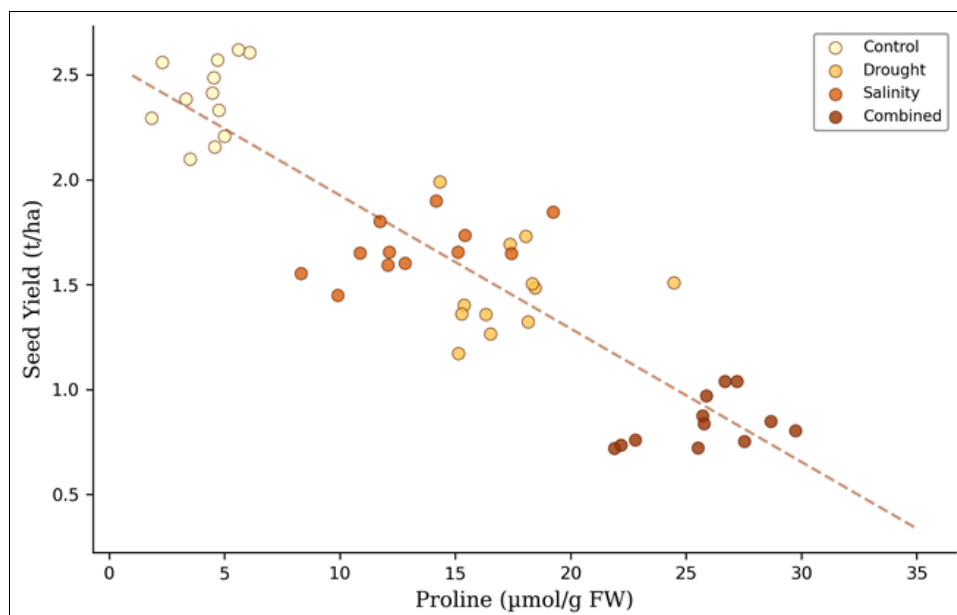


Fig 2: Relationship between proline accumulation and seed yield across all stress treatments

The stress gradient is clearly visible across all parameters (Figure 1). The strong negative correlation between proline and yield (Figure 2, $r = -0.87$) indicates that while proline serves as an osmoprotectant, its accumulation reflects stress severity rather than conferring yield protection.

Limitations

Pot-based greenhouse experiments don't replicate the soil volume, root architecture, and microbial interactions present under field conditions. Only two genotypes were tested; a larger panel would better capture the genetic diversity of stress responses. The 21-day stress period represents acute exposure and may not reflect the gradual onset typical of field drought and salinization^[17].

Discussion

The synergistic effect of combined stress on chickpea aligns with findings in other legumes. Farooq *et al.*^[18] reported that combined drought and salinity reduced mung bean yield by 58-71%, compared to 28-42% and 23-36% for individual stresses. The 4.1-fold MDA increase under combined stress indicates membrane damage beyond what either stress alone produced, consistent with excessive ROS accumulation overwhelming antioxidant defenses^[19].

ICC 4958's superior performance under combined stress appears linked to its stronger antioxidant enzyme response and better water retention capacity. Krishnamurthy *et al.*^[20] identified ICC 4958 as a root-architecture drought avoider with deep root systems, which may explain its higher RWC even under osmotic stress from salinity. The negative proline-yield correlation suggests that breeding for stress tolerance should focus on maintaining cellular homeostasis rather than maximizing osmolyte accumulation^[21].

Conclusions

Combined drought and salinity imposed synergistic damage on chickpea that exceeded the sum of individual stress effects for most physiological parameters. The drought-tolerant genotype ICC 4958 showed greater resilience under combined stress than the salinity-tolerant FLIP 97-43C, suggesting that root-based drought avoidance mechanisms provide broader protection than ion-exclusion salinity tolerance alone. Proline accumulation

served as a reliable stress indicator but was negatively associated with yield. Breeding programs targeting combined stress environments should prioritize genotypes with strong antioxidant systems and deep root architecture rather than single-stress tolerance mechanisms.

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