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Research Article

Role of Osmolyte Application in Regulating Physiological and Biochemical Traits for Osmotic Adjustment in Wheat Under Drought Stress

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Abstract

Wheat (*Triticum aestivum* L.) yield is severely limited by drought, which disrupts water status, photosynthesis, osmotic balance and antioxidant defenses. This study evaluated the efficacy of foliar-applied glycine betaine (GB; 50 and 100 mM) and putrescine (Put; 0.1 and 1 mM) at anthesis and post-anthesis in modulating physiological and biochemical traits across four wheat genotypes (MP 3288, GW 11, GW 451, GJW 463) under field-imposed drought. A split-plot design with three replicates was used, comparing well-watered control, drought stress, and drought plus osmolyte treatments. Key parameters relative water content (RWC), chlorophyll retention, free amino acids (FAA), total soluble sugars (TSS), proline, malondialdehyde (MDA), chlorophyll stability index (CSI), and yield components, were measured at anthesis and grain filling. Drought reduced RWC, chlorophyll content, and yield, while increasing FAA, TSS, proline and MDA. Foliar GB (100 mM) and Put (1 mM) effectively restored RWC toward control levels, preserved chlorophyll, enhanced osmolyte accumulation, limited lipid peroxidation and maintained chlorophyll stability. These treatments also recovered seed yield close to well-watered control. GW 11 exhibited the highest intrinsic drought tolerance, whereas GJW 463 showed the greatest relative gain from osmolyte sprays. The results demonstrate that timely foliar application of GB and Put at critical reproductive stages confers osmotic adjustment, membrane protection, and antioxidative reinforcement, thereby mitigating drought-induced yield losses in wheat.

Keywords: Drought, Osmolytes, Glycine betaine, Putrescine, Proline

1. Introduction

Wheat (*Triticum aestivum* L.) is one of the world's most important staple crops, cultivated on nearly 17% of the global arable land and providing food for about 40% of the world's population (Farcas *et al.,* 2021). India ranks second after China in wheat production and has recorded remarkable gains in yield over the past four decades. However, water scarcity remains a persistent challenge, causing yield reductions ranging from 25% to 85%, depending on the intensity and duration of drought (Singh *et al.,* 2024). Water deficit is a major constraint to agricultural productivity across many regions of the world, as it adversely affects plant growth, physiology, and metabolism (Moorthy *et al.,* 2024). The reduction in growth under water stress is largely due to decreased biomass accumulation resulting from impaired physiological and biochemical processes. Drought tolerance in plants is a

complex trait governed by multiple adaptive mechanisms, including cuticle thickness, stomatal regulation, root architecture, hormonal balance, osmotic adjustment, and antioxidant defense (Szegletes *et al.,* 2000). Water stress leads to a cascade of metabolic, physiological, and biochemical alterations such as changes in growth dynamics (Ashraf and Harris, 2013), cellular water status, membrane stability (Bai *et al.,* 2006), pigment composition, and photosynthetic efficiency (Ekmekçi *et al.,* 2005). Among various adaptive mechanisms, the accumulation of osmolytes or compatible solutes plays a pivotal role in maintaining cellular homeostasis under stress conditions. These compounds are small, highly soluble in water, and non-toxic even at high intracellular concentrations (Bohnert *et al.,* 1995). Osmolyte accumulation, often referred to as osmotic adjustment, is a well-recognized trait used in breeding programs and molecular approaches to enhance drought tolerance (Pandya *et al.,* 2023; Zhang *et al.,* 2018). The plant kingdom synthesizes a diverse range of osmolytes, including amino acids, sugars, polyols, and betaines (Rhodes and Hanson, 1993).

Among these, glycine betaine (GB) and putrescine (Put) have emerged as key molecules in mitigating the adverse effects of abiotic stresses. Glycine betaine, a quaternary ammonium compound synthesized in several plant species, contributes significantly to stress tolerance by stabilizing the photosynthetic apparatus and chloroplast membranes under drought (Raza *et al.,* 2012; Wang *et al.,* 2014). It is also involved in the reduction of reactive oxygen species (ROS) accumulation, protection of membrane integrity, activation of stress-responsive genes, and stabilization of protein structures, thereby preserving enzyme functionality. Putrescine, a low-molecular-weight polyamine, acts as a growth regulator and signaling molecule involved in numerous physiological and developmental processes (Li *et al.,* 2023). Under water-deficit conditions, elevated putrescine levels contribute to osmotic adjustment, maintenance of chlorophyll content, membrane stabilization, and regulation of ion balance (El-Beltagi *et al.,* 2024; Sharma, 1999). The exogenous application of glycine betaine and putrescine has been reported to improve plant performance under drought by enhancing photosynthetic activity, osmotic regulation, and antioxidant responses (Farooq *et al.,* 2009). Considering these aspects, the present study was undertaken to elucidate the role of exogenously applied glycine betaine and putrescine in modulating the accumulation of compatible osmolytes and their contribution to drought tolerance in wheat under field conditions.

2. Material and Methods

The experiment was carried out during the *rabi* season of 2016–17 at the Wheat Research Station, Junagadh Agricultural University, Junagadh (21°31′ N latitude, 70°33′ E longitude; 83 m above sea level). Seeds of four wheat (*Triticum aestivum* L.) cultivars MP 3288, GW 11, GW 451, and GJW 463 were used for the study. The field trial followed a split-plot design with six main treatments and four cultivars as sub-plot treatments, each replicated three times. The main plot treatments included: (1) control (normal irrigation), (2) drought stress, (3) drought stress + 50 mM glycine betaine (GB), (4) drought stress + 100 mM GB, (5) drought stress + 0.1 mM putrescine (Put), and (6) drought stress + 1.0 mM Put. Each sub-plot measured 1.0 × 1.6 m^2.

Crop management followed standard agronomic practices, with seeds sown at a rate of 120 kg ha^{-1} and rows spaced 20 cm apart. Drought stress was induced by withholding irrigation throughout the crop growth period, except for an initial pre-sowing irrigation to ensure uniform germination. The intensity of drought was assessed through measurements of flag leaf relative water content (RWC) at anthesis and post-anthesis stages, along with visual observations of leaf rolling, wilting, and chlorosis. Control plots received 9–10 irrigations throughout the growing season to maintain optimum soil moisture.

For foliar applications, glycine betaine solutions (50 mM and 100 mM) were prepared in distilled water with 0.1% TWEEN-20 as a surfactant, following the method of Ma *et al.* (2006). The GB sprays were applied twice at anthesis and post-anthesis stages. Similarly, putrescine dihydrochloride (Sigma, USA) was dissolved in distilled water, adjusted to pH 7.0 using 1 M NaOH, and supplemented with 0.1% TWEEN-20 before foliar application at 0.1 mM and 1.0 mM concentrations. Control and drought-stressed plots (unsprayed treatments) received distilled water containing 0.1% TWEEN-20 at the same growth stages to maintain uniformity. One week after each foliar spray, leaf samples were collected from each treatment. The youngest fully expanded leaf at anthesis and the flag leaf at post-anthesis were excised from ten randomly selected plants per plot and used for biochemical analyses.

2.1. Relative water content

Relative water content (RWC) of the wheat leaves was determined following the procedure described by Barrs and Weatherley (1962) at both anthesis and post-anthesis stages. Fresh leaf samples were first weighed to record their fresh weight (FW) and then immersed in distilled water for 4 hours to achieve full turgidity. After removing the samples, excess surface moisture was gently blotted off, and the turgid weight (TW) was measured. Subsequently, the leaves were oven-dried at 60 °C until a constant weight was attained to record the dry weight (DW). The RWC was then calculated using the standard formula:

RWC = 100 × (Fresh weight – Dry weight)/(Turgid weight – Dry weight).

2.2. Metabolites

Chlorophyll content was determined following the method of Arnon (1949). Proline concentration was estimated as per Bates *et al.* (1973). Fresh leaf tissue was homogenized in 5 mL of 3% aqueous sulfosalicylic acid and centrifuged at 5000 × *g* for 5 minutes. The supernatant was mixed with equal volumes of glacial acetic acid and acid ninhydrin reagent, and the mixture was heated in a water bath at 100 °C for color development. After cooling, 5 mL of toluene was added, and the chromophore-containing organic phase was separated. Absorbance was measured at 528 nm using a spectrophotometer, and proline concentration was quantified using a standard calibration curve. Free amino acid content was estimated according to the procedure of Yemm and Cocking (1955), and the results were expressed on a fresh weight basis.

2.3. Chlorophyll stability index

Estimation of chlorophyll stability index was determinate using method given by Murthy and Majumdar (1962). Chlorophyll stability index (CSI) is a rapid method and forms one of the indices for estimating resistance to dehydration. Chlorophyll stability index was measured in terms of total chlorophyll content. Chlorophyll stability during periods of drought is characteristic of drought resistance in plant. In this study the recorded observation in respect of chlorophyll stability index. It will be calculated as CSI = 100 x (Cs/Cc), here Cs, total chlorophyll content of stressed plant and Cc, total chlorophyll content of control plants.

2.4. MDA (Malondialdehyde) content

Lipid peroxidation in plant tissues was assessed by estimating the malondialdehyde (MDA) content following the procedure of Heath and Packer (1968). The absorbance of the reaction mixture was recorded, and the MDA concentration was determined using an extinction coefficient of 155 mM^{-1} cm^{-1}. The results were expressed as micromoles of MDA per gram of fresh weight (µmol MDA g^{-1} FW).

2.5. Yield and yield attributes

Observation on number of grains per spike, 1000 grain weight, biomass per plant and grain yield were recorded at maturity and during harvest of the crop.

2.6. Statistical analysis

The experimental data were analyzed using analysis of variance (ANOVA) suitable for a split plot design. Two-way ANOVA was performed using OPSTAT software and Microsoft Excel. The significance of treatment effects was assessed at the $p \leq 0.05$ level. Means and standard errors were calculated, and interaction effects between main plot treatments (drought and osmolyte applications) and subplot treatments (wheat varieties) were evaluated. Significant F-values were interpreted to determine the effects of treatments and their interactions on the measured parameters.

3. Results and Discussion

The foliar application of glycine betaine (GB) and putrescine (Put) significantly influenced relative water content (RWC) and chlorophyll pigments (Chl-a, Chl-b, and total chlorophyll) in wheat genotypes under drought stress imposed during anthesis and grain-filling stages (Fig. 1a–h). Drought stress (WS) markedly reduced RWC and chlorophyll content in all genotypes compared with the control, indicating the adverse effects of water deficit on plant water status and photosynthetic pigments. However, exogenous application of GB and Put effectively alleviated these reductions, with varying responses among genotypes.

Drought stress caused a noticeable decline in RWC across all wheat genotypes, reflecting impaired tissue hydration and osmotic imbalance (Fig. 1a, b). The foliar application of GB (50 and 100 mM) and Put (0.1 and 1 mM) significantly improved RWC compared to untreated drought-stressed plants, suggesting enhanced osmotic

adjustment. The highest RWC was recorded under GB@100 mM and Put@1 mM treatments, particularly in MP 3288 and GW 451 genotypes, implying their superior drought tolerance. GB acts as a compatible solute that stabilizes cellular osmotic potential and membrane integrity (Biswal *et al.,* 2025), while Putrescine enhances stomatal regulation and protects membranes through polyamine-mediated ROS scavenging (Raziq *et al.,* 2022; Hassan *et al.,* 2020). Similar findings were reported by Qayyum *et al.* (2021), who observed improved leaf water status in wheat following GB and polyamine applications under terminal drought stress.

Water stress significantly reduced chlorophyll-a, chlorophyll-b, and total chlorophyll content in all genotypes during both anthesis and grain-filling stages (Fig. 1c–h). This reduction is attributed to oxidative degradation of chlorophyll molecules and impaired biosynthesis of photosynthetic pigments under water deficit (Dalal, 2021). However, foliar supplementation with GB and Put remarkably restored chlorophyll levels, especially at higher concentrations (GB@100 mM and Put@1 mM). Among the genotypes, MP 3288 and GW 463 maintained higher chlorophyll content under stress, indicating better photosynthetic stability.

The restoration of chlorophyll under GB and Put treatments may result from their role in protecting chloroplast membranes, enhancing antioxidant enzyme activities, and maintaining nitrogen assimilation pathways (Basit *et al.,* 2025; Shemi *et al.,* 2021). Glycine betaine stabilizes thylakoid membranes and photosystem II efficiency under osmotic stress, thereby preventing pigment loss (Ali *et al.,* 2020). Putrescine, being a polycationic molecule, binds with negatively charged membrane phospholipids and chloroplast proteins, reducing oxidative injury and promoting pigment stability (Lakshmi and Beena, 2023). These protective roles are consistent with previous reports showing that polyamines and betaines improve chlorophyll retention and photosynthetic efficiency under abiotic stresses in cereals (Li *et al.,* 2024).

Overall, the combined data demonstrate that GB and Put application effectively mitigates drought-induced declines in RWC and chlorophyll content through osmotic adjustment, membrane stabilization, and enhanced antioxidative defense. These responses were more pronounced during anthesis than grain-filling, suggesting stage-dependent tolerance mechanisms. Genotypic differences in response indicate that physiological resilience under drought can be enhanced through exogenous osmolyte and polyamine applications.

Foliar application of glycine betaine (GB) and putrescine (Put) substantially modulated free amino acids (FAA), proline accumulation, lipid peroxidation (MDA) and chlorophyll stability index (CSI) in all wheat genotypes under water stress (WS) imposed at anthesis and grain-filling (Fig. 2a–h).

Water deficit induced an increase in FAA in all genotypes (Fig. 2a, b), reflecting stress-driven proteolysis and accumulation of soluble amino acids that contribute to osmotic adjustment. Foliar GB and Put further modulated FAA pools: low-to-moderate increases were observed with GB@50 mM and Put@0.1 mM, whereas GB@100 mM and Put@1 mM tended to maintain or modestly elevate FAA relative to WS alone. These shifts likely indicate enhanced nitrogen remobilization and activation of amino-acid–based osmoprotective pathways when osmolytes or polyamines are supplied exogenously (Amiri *et al.,* 2024).

Proline content increased markedly under WS at both anthesis and grain filling (Fig. 2c, d), consistent with its role as a primary osmoprotectant. Application of GB and Put further raised proline levels beyond WS alone, particularly at the higher doses (GB 100 mM, Put 1 mM), suggesting stimulation of proline biosynthesis and/or suppression of catabolism. Enhanced proline accumulation contributes to osmotic balance, stabilizes proteins and membranes, and helps maintain cellular redox state under drought (Atta *et al.,* 2024). In several genotypes the anthesis stage showed larger proline responses than grain filling, indicating stage-dependent capacity for osmotic adjustment.

MDA concentrations rose under WS in all genotypes (Fig. 2e, f), indicating increased membrane lipid peroxidation and oxidative stress. Foliar GB and Put significantly lowered MDA relative to WS, with reductions proportional to treatment strength. The decline in MDA suggests that GB and Put reduce ROS accumulation and/or enhance antioxidant defenses (SOD, CAT, APX), and they stabilize membranes against peroxidative damage effects previously reported for compatible solutes and polyamines in cereals under water stress (Isgandarova *et al.,* 2024). The concurrent increases in proline and FAA alongside decreased MDA

point to coordinated biochemical protection that preserves cellular function during drought.

CSI decreased under drought, reflecting pigment degradation and` loss of chloroplast integrity (Fig. 2 g, h). GB and Put sprays significantly improved CSI compared with WS, with GB@100 mM and Put@1 mM showing the most pronounced effects. Improved CSI likely results from membrane stabilization, reduced lipid peroxidation and maintenance of antioxidant systems that together protect chlorophyll and thylakoid structure (Mokhtari *et al.,* 2024; Ishfaq *et al.,* 2024). Improvements were generally larger when treatments were applied at anthesis, suggesting that timely foliar application during sensitive reproductive stages better preserves photosynthetic apparatus.

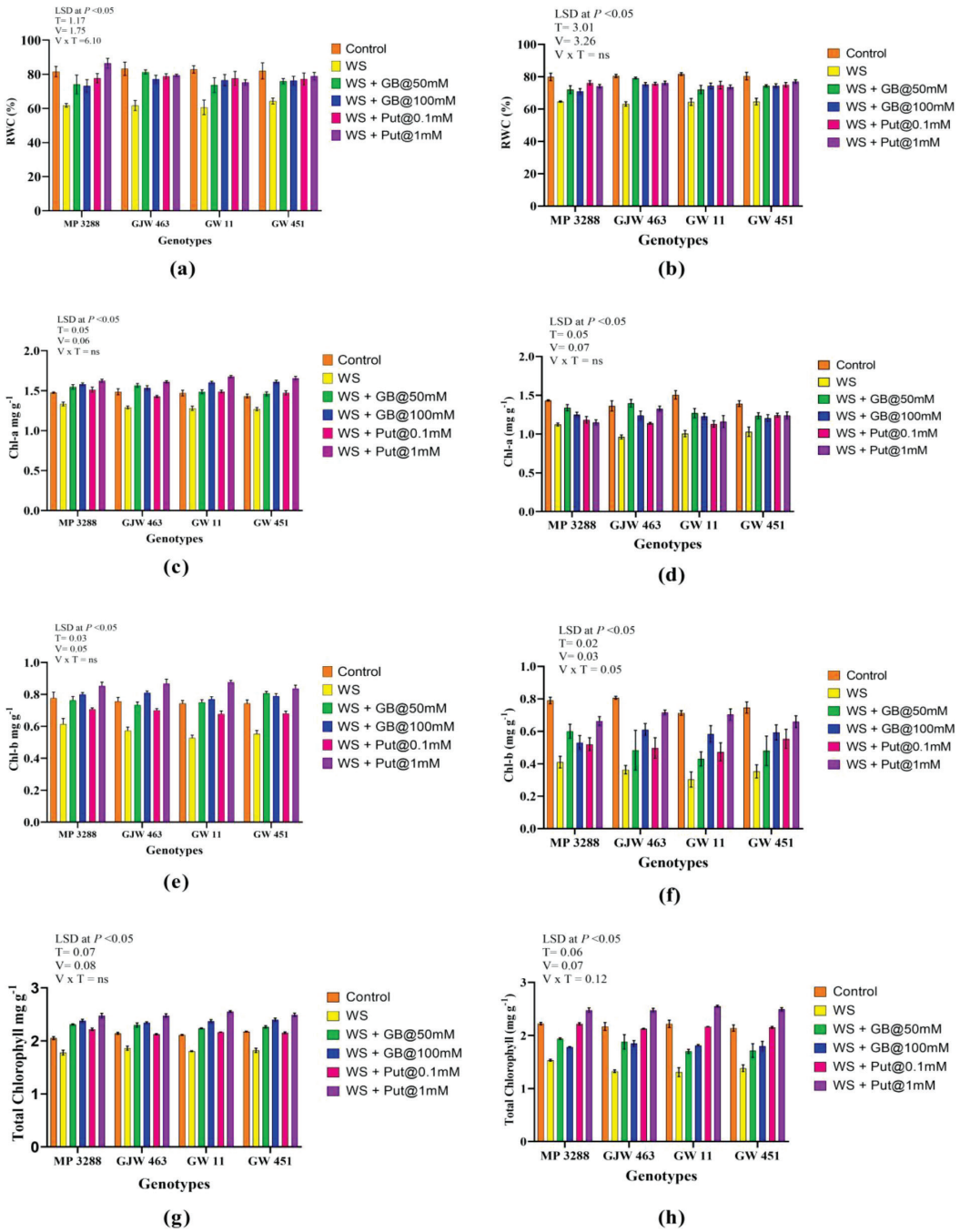

Fig. 1. Influence of foliar application of glycine betaine (GB) and putrescine (put) on (a) and (b) RWC, (c) and (d) Chl-a content, (e) and (f) Chl-b content and (g) and (h) Total Chlorophyll content in wheat genotypes under drought stress during anthesis and grain filling

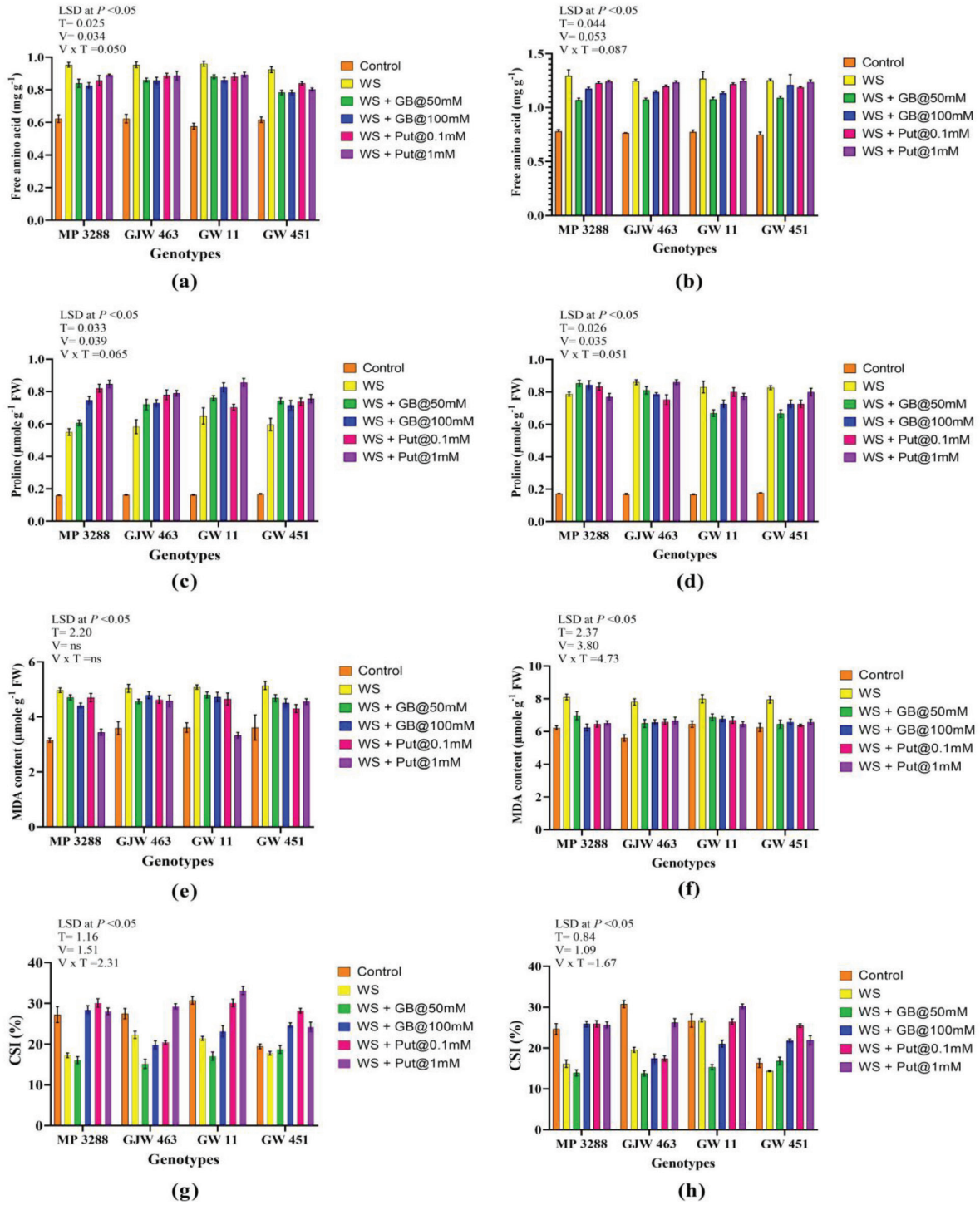

Fig. 2. Influence of foliar application of glycine betaine (GB) and putrescine (put) on (a) and (b) free amino acid (FAA), (c) and (d) Proline, (e) and (f) MDA content and (g) and (h) chlorophyll stability index (CSI) in wheat genotypes under drought stress during anthesis and grain filling

The influence of foliar-applied glycine betaine (GB) and putrescine (Put) on yield and yield-attributing traits of four wheat genotypes under drought stress is summarized in Table 3. Among genotypes, GW 11 exhibited the highest seed yield per plant (17.30 g) and thousand-grain weight (TGW; 40.95 g), while GJW 463 recorded the lowest values for both traits (15.36 g and 36.68 g, respectively). MP 3288 produced the greatest number of effective tillers per meter (143.40), followed by GW 451 (130.10) and GJW 463 (131.85). Genotypic differences were

significant for seed yield per plant, effective tillers, TGW and biological yield ($p \leq 0.05$), but non-significant for seed yield per plot (NS).

Under treatments, the water-stressed control (WS) showed marked reductions in all parameters compared to the well-watered control: seed yield per plant declined by 14.1 %, tiller number by 12.2 %, and seed yield per plot by 30.4 %. Foliar application of GB at both 50 mM and 100 mM ameliorated drought effects, with GB@100 mM restoring seed yield per plant to 16.86 g and TGW to 39.50 g. Putrescine treatments (0.1 mM and 1 mM) were similarly effective: Put@1 mM recovered seed yield per plant to 16.99 g and seed yield per plot to 2.58 kg, significantly higher than WS ($p \leq 0.05$). Treatment effects were significant for seed yield per plant, effective tillers and seed yield per plot ($p \leq 0.05$), but non-significant for TGW.

The superior performance of GW 11 under drought aligns with its inherent drought-tolerance traits, such as deeper root systems and efficient stomatal regulation. MP 3288's high tiller number underpins its capacity for compensatory growth, which partly buffered yield losses under stress (Mutanda *et al.*, 2024). Foliar GB significantly mitigated drought-induced yield reductions. GB stabilizes photosynthetic machinery and osmotic balance, thereby sustaining grain filling under water deficit (Ashraf & Foolad, 2007). The 100 mM GB dose was more effective than 50 mM, consistent with dose–response patterns reported in wheat (Ashraf and Harris, 2013). Putrescine treatments also conferred stress tolerance, likely *via* membrane protection and modulation of antioxidative enzymes (Kusano *et al.*, 2008). Li *et al.* (2025) demonstrated approximately 20 % RWC improvement with foliar Put, which supports our findings of yield recovery close to non-stress levels. The non-significant variety × treatment interaction for most traits suggests that GB and Put benefits were broadly applicable across genotypes. However, slight genotypic variation in response magnitude indicates potential for breeding programs to combine osmolyte-responsive alleles with high-yield backgrounds. Overall, foliar application of GB (100 mM) and Put (1 mM) at anthesis effectively alleviates drought stress, maintaining yield components and final yield. Integrating such foliar sprays with drought-tolerant genotypes like GW 11 could enhance wheat productivity under drought conditions.

Table 3: Influence of foliar application of glycine betaine (GB) and putrescine (put) on yield and yield attributing parameters of wheat genotypes under drought stress during anthesis and grain filling

	Seed yield per plant (g)	Effective tillers/ meter	Seed Yield (kg per plot)	TGW (g)	Biological yield per plot (kg)
Genotype					
MP 3288	16.22	143.40	2.36	39.42	1.21
GJW 463	15.36	131.85	2.27	36.68	0.98
GW 11	17.30	128.37	2.33	40.95	1.07
GW 451	16.69	130.10	2.34	39.01	1.08
Treatments					
Control	17.06	138.76	2.80	39.66	1.21
WS	14.66	121.90	1.95	37.30	1.00
WS + GB@50mM	15.94	134.40	1.99	39.22	0.99
WS + GB@100mM	16.86	136.62	2.00	39.50	1.13
WS + Put@0.1 mM	16.86	135.05	2.57	39.18	1.10
WS + Put@1 mM)	16.99	133.85	2.58	39.23	1.07
LSD ($p \leq 0.05$)					
Varieties (V)	1.30	10.77	NS	2.78	0.08
Treatment (T)	0.98	7.76	0.13	NS	0.08
V x T	1.95	15.50	0.25	NS	0.16

WS = Water stress (drought); GB = Glycine betaine; Put = Putrescine; NS = Non-significant at $p \leq 0.05$.

Conclusion

Foliar application of glycine betaine (100 mM) and putrescine (1 mM) at anthesis and early grain filling substantially enhances wheat's resilience to drought by improving leaf water relations, preserving chlorophyll integrity, and strengthening biochemical defenses. Treated plants maintained higher relative water content and chlorophyll stability under water deficit, while osmolyte and antioxidant systems were bolstered, evidenced by increased soluble sugars, free amino acids, proline levels, and reduced lipid peroxidation. These physiological and biochemical adjustments translated into a near-complete recovery of seed yield and yield components compared to well-watered controls. Notably, genotypes such as GW 11, which exhibited relatively better performance under drought, benefited further from osmolyte sprays, suggesting that combining foliar applications with more responsive genotypes offers a pragmatic strategy to safeguard wheat productivity in drought-prone environments.

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Author Contributions

CK and AM conceived and designed the research. CK and AM conducted the experiment and collected the data. CK and AM did the statistical analysis. AM wrote the primary draft of the manuscript. CK and AGP reviewed and improved the manuscript.

Conflict of Interest

The authors declare there are no conflict of interest.

Ethical Approval

The article doesn't contain any study involving ethical approval.

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No

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