AATCC Journal

12 April 2026: Received
11 May 2026: Revised
15 June 2026: Accepted
13 July 2026: Available Online

https://aatcc.peerjournals.net/

Review Article Open Access

Osmoprotectant-mediated stress mitigation in rainfed field crops: Biosynthetic cascades, physiological mechanisms, and agronomic intervention frameworks for drought-resilient production systems

P.O. Bhutada*[1], B.V. Asewar[2], I.A.B. Mirza[3], M. Jagatap[4], and S. A. Shinde[5]

Department of Agronomy, Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani – 431402 (M.S.), India

ABSTRACT

India's rainfed agricultural matrix, encompassing 51.12% of the net sown area and sustaining livelihoods of approximately 80% of small and marginal farmers, confronts escalating abiotic stress pressures chiefly drought, heat, and secondary osmotic derangements that compromise physiological homeostasis and depress field-crop productivity. The accelerating frequency of terminal heat events and erratic monsoon distribution necessitates bio-molecular interventions that extend beyond conventional agronomic management. Rainfed farming is mainly affected by irregular rainfall, dry spells, drought and high temperature, which often lead to inadequate soil moisture and unstable crop yields. Moisture stress during critical growth stages can reduce photosynthesis, nutrient uptake and reproductive growth. Hence, there is a need for simple, climate-resilient management practices that help crops withstand water and heat stress under rainfed conditions.

Objectives: *This systematic review synthesizes empirical evidence from 2008–2024 to characterize the biosynthetic pathways, intracellular compartmentalization, and protective mechanisms of the five canonical osmoprotectant groups, and to evaluate their agronomic efficacy across wheat, maize, coarse cereals, and oilseed crops under field and controlled-environment conditions.*

Critical Synthesis: *Osmoprotectants — encompassing amino acids (proline, glycine), quaternary ammonium compounds (glycine betaine), tertiary sulphonium compounds (dimethylsulphoniopropionate), soluble sugars (trehalose, sucrose), and polyhydric alcohols (mannitol, pinitol) — operate through convergent physiological axes: osmotic adjustment, membrane lipid bilayer stabilization via preferential exclusion, antioxidant enzyme upregulation, and transcriptional modulation of heat- and drought-responsive gene networks. Foliar-delivered proline at 20–50 mM increased wheat grain yield by 2.88–52.88% under graduated drought intensities [15]. Salicylic acid at 100 ppm augmented maize grain yield by 78.8% over drought-stressed controls at the vegetative stage, while also enhancing relative water content and membrane stability index [29] Glycine betaine mitigated heavy-metal-coupled oxidative stress in wheat through antioxidant enzyme gene activation [14]; Sharma et al., 2024).*

Agronomic Implications: *Stage-specific foliar application — calibrated to vegetative rather than terminal-reproductive windows — maximizes protective efficacy. Integration of nano-formulated osmoprotectants (SNP-NP at 90 ppm) with salicylic acid represents a scalable frontier for semi-arid dryland production systems. Systemic adoption of osmoprotectant spray protocols within the rainfed crop calendar could yield 20–40% water savings while sustaining productivity benchmarks.*

Keywords: *Compatible solutes; Glycine betaine; Proline biosynthesis; Drought tolerance; Salicylic acid; Rainfed agriculture; P5CS/P5CR pathway; Reactive oxygen species scavenging, dry spell, osmoprotectant.*

1. INTRODUCTION

1.1 Structural Significance of Rainfed Agriculture in India

India occupies a position of global agricultural primacy, contributing approximately 13% of the national GDP and sustaining around 55% of the country's workforce. Within this agrarian framework, rainfed systems constitute the load-bearing architecture: they account for 51.12% of the net sown area (71.7 million hectares), generate roughly 40% of total food grain output, support two-thirds of the national livestock inventory, and underpin the food security of approximately 40% of the human population[6]. The socioeconomic significance is amplified further by the fact that approximately 80% of India's small and marginal farming households derive their livelihoods directly from rainfed agriculture.

Sectoral disaggregation reveals the disproportionate rainfed dependency among critical commodity crops: coarse cereals (sorghum, pearl millet, finger millet) rely on rainfall for 85% of their cultivated area; pulses for 83%; oilseeds for 70%; and cotton for 65%. This structural concentration in high-value protein and oilseed crops makes rainfed system productivity central not only to caloric security but also to nutritional and economic self-sufficiency. An authoritative projection by [6] affirms that even if India's entire identified irrigation potential were developed and deployed, approximately 40% of the net cultivated area would remain permanently under rainfed conditions a geographic and hydrological reality that will persist as the dominant food production domain through 2050.

*Corresponding Author: **Pritam O. Bhutada***
*Email Address: **pritambhutada1@gmail.com***

DOI: https://doi.org/10.21276/AATCCReview.2026.14.03.32

The country must provision food for a population approaching 1.5 billion people, requiring an incremental 5–6 million tonnes of additional food grain annually. With over 70% of the rural population lacking reliable access to food and non-food commodities due to infrastructural deficits and under-employment [8], the pressure on rainfed systems is not merely agronomic but structurally developmental.

1.2 Bio-physical Constraints and the Stress Architecture of Rainfed Ecosystems

Rainfed ecosystems confront acompounding and interconnected stress architecture. Total degraded land in India spans 120.7 million hectares, of which water erosion affects 73.3 Mha, wind erosion 12.4 Mha, salinity and alkalinity 6.64 Mha, and soil acidity 5.7 Mha. This resource degradation creates chronic background stress even in years of adequate rainfall. Superimposed upon this base degradation are episodic climatic perturbations - prolonged inter-seasonal droughts, terminal heat waves during grain filling, erratic rainfall distribution within the growing season, and flooding events in low-lying rainfed tracts each of which triggers characteristic secondary stress cascades.

Among abiotic stressors, drought holds primacy as the single most consequential yield-limiting factor.

It does not operate as an isolated physiological insult; rather, drought stress initiates a cascade of secondary osmotic, oxidative, and ionic stresses (Table 1). Water deficit reduces soil water potential, triggering osmotic stress that limits water influx into root cells. The resulting turgor loss compromises stomatal conductance, suppresses CO_2 assimilation, and curtails photosynthetic efficiency. Concurrently, restricted mineral nutrient translocation depresses enzyme-mediated metabolic processes, while the disruption of electron transport chains elevates reactive oxygen species (ROS) production - particularly H_2O_2, superoxide anion (O_2^-), and hydroxyl radicals — generating oxidative damage to lipid membranes, nucleic acids, and catalytic proteins [29].

Heat stress, particularly terminal heat affecting the grain-filling stage in wheat and the silking–tasseling period in maize, produces analogous ROS-mediated damage while additionally causing protein misfolding, denaturation of starch biosynthetic enzymes, and direct disruption of thylakoid ultrastructure. Salinity stress involves an additional ionic dimension Na^+ toxicity and K^+ displacement that compounds the osmotic effect. These overlapping stress phenotypes are not independent; in rainfed systems, crops commonly experience drought-heat co-occurrence at reproductive stages, which are precisely the developmental windows most sensitive to yield formation.

Table 1: Abiotic stresses, induced secondary stresses, and characteristic plant responses

Abiotic Stress	Induced Secondary Stresses	Key Physiological and Biochemical Effects
Drought	Osmotic, heavy metal, oxidative stress	Turgor loss, ion leakage, ROS accumulation, chlorophyll depletion, CO_2 assimilation decline, protein denaturation, necrosis
Terminal heat (grain filling)	Osmotic, oxidative stress	Starch enzyme denaturation, protein misfolding, PS I/II disruption, reduced 1000-grain weight and grain fill duration
Salinity	Ionic imbalance, water deficit, osmotic and oxidative stress	Na^+ toxicity, K^+/Ca^{2+} depletion, thylakoid disorganization, reduced germination, premature senescence
Chilling/freezing	Nutritional imbalance, oxidative stress	PS II inhibition, cell dehydration, metabolic imbalance, retarded maturation
Flooding/waterlogging	Water and nutrient deficiency, oxidative stress	Anoxia, anaerobic metabolism, inhibited root respiration, ethylene overproduction
Heavy metals	Oxidative, nutrient stress	Enzyme structure disruption, stomatal conductance decline, net photosynthesis reduction, biomass inhibition

[29]

1.3 The Osmoprotectant Paradigm as a Resilience Strategy

Field crops have evolved, or been cultivated to possess, a spectrum of adaptive strategies to navigate the multi-axis stress architecture of rainfed environments. These strategies encompass avoidance mechanisms stomatal closure, leaf orientation, transpiration cooling, and phenological escape through early maturity and tolerance mechanisms: transcriptome reprogramming, free-radical scavenging, lipid membrane restructuring, heat shock protein induction, and osmoprotectant accumulation [41]. These tolerance mechanisms, osmoprotectant accumulation has attracted sustained scientific scrutiny because it operates simultaneously across multiple protective axes, is inducible at all developmental stages, and is amenable to exogenous supplementation through agronomically practical foliar intervention.

Osmoprotectants are low-molecular-weight, non-toxic, highly water-soluble organic compounds that accumulate in the cytoplasm of stressed plant cells.

Their defining functional property is compatibility with normal cellular biochemistry even at high concentrations a property that distinguishes them sharply from inorganic ionic solutes, which are osmotically effective but metabolically disruptive [43]. Beyond osmotic adjustment, osmoprotectants stabilize macromolecular structure, protect membrane integrity, quench ROS, and function as carbon and nitrogen repositories that supply respiratory substrates during recovery phases [13]. Their synthetic deployment through seed priming, root-zone application, or foliar spray constitutes one of the most physiologically well-grounded agronomic interventions available for dryland cropping systems.

2. CLASSIFICATION AND BIOSYNTHETIC PATHWAYS OF MAJOR OSMOPROTECTANTS

2.1 Systematic Classification

[13] formalized the taxonomy of osmoprotectants into five major structural-functional groups based on their functional chemistry.

Table 2: Systematic classification of osmoprotectants

Class	Sub-class	Representative Compounds
Amino acids	Protein amino acids	Proline, alanine, arginine, glycine, cysteine, aspartate
	Non-protein amino acids	γ-aminobutyric acid (GABA), pipecolic acid, citrulline, ornithine, polyamines (putrescine, spermine, spermidine)
	Amides	Glutamine, asparagine
Quaternary Ammonium Compounds (QACs)	Methylated prolines	Methyl proline, prolinebetaine, hydroxyprolinebetaine
	Betaines	Glycine betaine, β-alanine betaine, choline-O-sulphate
Tertiary Sulphonium Compounds	—	DMSP (dimethylsulphoniopropionate)
Sugars	Disaccharides	Sucrose, trehalose
	Polyfructans	Fructans
Polyhydric Alcohols	Acyclic polyols	Mannitol, sorbitol
	Cyclic polyols	Pinitol, ononitol

[13]

2.2 Proline: Biosynthetic Cascade and Compartmentalization

Among all osmoprotectants, proline (L-proline) has received the most sustained mechanistic scrutiny and is the most broadly deployed agronomically. It is a cyclic imino acid with unique conformational rigidity that confers structural stabilization to adjacent polypeptides and membrane phospholipids.

Biosynthetic Pathway: Proline is produced via two interconnected routes in higher plants:

The Glutamate Pathway (primary under osmotic stress): Glutamate is converted to glutamate-γ-semialdehyde (GSA) by Δ^1-pyrroline-5-carboxylate synthetase (P5CS), a bifunctional enzyme possessing both γ-glutamyl kinase and glutamic-γ-semialdehyde dehydrogenase activities. GSA spontaneously cyclizes to Δ^1-pyrroline-5-carboxylate (P5C), which is then reduced to proline by Δ^1-pyrroline-5-carboxylate reductase (P5CR). P5CS is the rate-limiting enzyme in this cascade, encoded by two genes in *Arabidopsis thaliana* and homologous species, while P5CR is encoded by a single gene [38] [19]. The availability of the precursor glutamate itself constitutes an upstream metabolic constraint on proline production [39].

The Ornithine Pathway (active under nitrogen-sufficient conditions): Ornithine, generated via the urea cycle, undergoes transamination by ornithine δ-aminotransferase (OAT) to form P5C/GSA, which then enters the terminal reduction step shared with the glutamate pathway.

Cellular Localization: Proline biosynthesis occurs in both the cytosol and the chloroplast, with the relative contribution of each compartment being stress-context-dependent [37]. The chloroplast is the primary locus of proline synthesis under severe osmotic stress because NADPH generated during photosynthetic light reactions drives the P5CR reaction. Proline-specific transporters (ProTs) facilitate inter-organellar and inter-tissue transport, enabling the redistribution of proline from source leaves to stress-exposed sinks during salinity or drought events [36].

Catabolism: In mitochondria, proline is oxidized back to glutamate via proline dehydrogenase (ProDH) and P5C dehydrogenase (P5CDH). This oxidative cycle releases electrons into the mitochondrial electron transport chain and produces NADH, providing energy for post-stress recovery. The reversibility of proline biosynthesis and catabolism makes it an especially versatile metabolic buffer.

Additional Metabolic Functions: Beyond osmotic adjustment, proline maintains cytoplasmic pH and sustains appropriate $NADP^+$/NADPH ratios that are essential for respiratory and photosynthetic electron flow [11].

Under extreme stress, elevated $NADP^+$ concentrations facilitate NADPH regeneration and supply ribose-5-phosphate for purine synthesis via the pentose phosphate pathway [37]. This dual function as osmoticum and redox buffer gives proline unique metabolic versatility among osmoprotectants.

2.3 Glycine Betaine: Choline-Mediated Biosynthesis and Chloroplast Accumulation

Glycine betaine (GB; N, N, N-trimethylglycine) is the archetypal quaternary ammonium compound and the most ecologically widespread osmoprotectant in plants facing dehydration stress. Its biosynthesis proceeds through a well-characterized two-step oxidative pathway:

Choline → Betaine Aldehyde → Glycine Betaine

The first oxidation step is catalyzed by choline monooxygenase (CMO), a ferredoxin-dependent enzyme localized in the chloroplast stroma. The second step is catalyzed by betaine aldehyde dehydrogenase (BADH), a NAD^+-dependent enzyme. Betaine aldehyde, the intermediate, is itself toxic and is rapidly converted to GB, which accumulates to high concentrations [28,4]. Choline availability is the primary rate-limiting constraint for GB production [12]. Choline biosynthesis occurs in the cytosol via phospho-base N-methylation, and the choline must subsequently be transported into the chloroplast for GB formation [12]. Variation in chloroplast choline availability among plant species explains the wide differences in intrinsic GB accumulation capacity [24]. Betaine accumulation is additionally regulated at the transcriptional level by the AmT1 and AmT2 transporter genes.

Chloroplast Compartmentalization: [28] established that approximately 30–40% of total leaf GB resides within chloroplasts, where it accumulates preferentially during salt stress to maintain osmotic adjustment in the stroma. This chloroplastic concentration is not accidental: GB directly protects Photosystem II (PS II) against dehydration, high ionic strength, and oxidative damage by stabilizing the oxygen-evolving complex and associated thylakoid membrane proteins [5].

2.4 Sugars and Fructans

Sucrose and trehalose accumulate in plant vacuoles and cytosol under stress. Trehalose — a non-reducing glucose disaccharide — is particularly effective in protecting cell membranes and proteins, functioning both as an immediate energy source and as a regulator of starch metabolism (Lunn et al., 2014). Fructans, polymers of fructose stored in vacuoles, provide carbohydrate buffering capacity that contributes to osmotic adjustment, though the full mechanistic basis of their tolerance effect remains under active investigation [40].

2.5 Polyhydric Alcohols: Mannitol, Sorbitol, and Pinitol

Mannitol and D-ononitol contribute to osmotic adjustment in numerous salt-tolerant species and interact productively with glutathione–ascorbate cycle enzymes to protect membrane complexes from ROS-induced damage [2]. Mannitol's role in halophytes extends to macromolecular structure stabilization and enhancement of ROS scavenging mechanisms [17].

Pinitol (3-O-methyl-D-chiro-inositol), the most frequently reported cyclic polyol in halophyte literature, is synthesized through the epimerization of ononitol by ononitolepimerase and inositol-O-methyltransferase, with upstream methylation of myo-inositol. An alternative pathway, proposed by [9] involves epimerization of myo-inositol preceding the methylation step. Six inositol isomers have been identified in plant tissues, with myo-inositol predominating; their methylated derivatives exhibit both protective and signaling properties at the cellular level [35].

2.6 DMSP and Tertiary Sulphonium Compounds

Dimethylsulphoniopropionate (DMSP) is the principal tertiary sulphoniumosmoprotectant, produced in high concentrations by marine macroalgae and certain salt marsh grasses. Its biosynthesis from methionine follows divergent pathways depending on the organism: in marine macroalgae, methionine is transaminated to 4-methylthio-2-oxobutyrate, while in higher plants, it is methylated to S-methyl methionine and then converted directly to dimethyl sulphonio propion aldehyde (DMSP-ald) through a transamination-decarboxylation mechanism [12]. DMSP's structural similarity to glycine betaine suggests functional overlap in osmoregulatory capacity; it can constitute up to 86% of total sulphur in green tissues, and its transit between cytoplasm and vacuoles allows dynamic osmotic buffering without net tissue-level concentration change [23].

3. CELLULAR AND PHYSIOLOGICAL MECHANISMS OF OSMOPROTECTANT ACTION

3.1 Osmotic Adjustment and Turgor Maintenance

The fundamental osmotic function of compatible solutes resides in their capacity to lower cytoplasmic water potential without disrupting the ionic balance required for enzyme activity. When the external soil water potential declines under drought or salinity, the accumulation of osmoprotectants in the cytosol and organelle matrices lowers intracellular osmotic potential to maintain a favorable inward water potential gradient. This preserves the turgor pressure that drives cell elongation, stomatal aperture regulation, phloem loading, and nutrient transport [18].

The cytoplasmic osmotic adjustment achieved by compatible solutes is functionally complementary to vacuolar ionic sequestration in halophytes, where Na^+ and Cl^- ions are compartmentalized in the vacuole while organic osmoprotectants balance osmotic potential in the cytosol. In glycophytes subjected to drought, osmoprotectant accumulation in the cytosol and chloroplast is the primary osmotic adjustment mechanism.

Importantly, osmotic adjustment by compatible solutes extends to preservation of the structural integrity of the photosynthetic apparatus. Chloroplast-localized GB, for instance, maintains the osmotic environment of the stroma that is necessary for the proper assembly and function of PS I and PS II supercomplexes. Correspondingly, studies of heat-tolerant wheat genotypes (WH730 and WH1218) under terminal heat stress showed significantly higher accumulation of total soluble sugars (47% and 39% increase respectively, over control), proline (7–15%), and glycine betaine (22–34%) compared to heat-sensitive genotypes, directly correlated with their superior grain-filling performance [32].

3.2 Membrane Lipid Bilayer Stabilization: The Preferential Exclusion Model

The molecular mechanism through which osmoprotectants stabilize membranes under dehydration stress is best explained by the preferential exclusion model (also called the preferential hydration theory). In this framework, compatible solutes are excluded from the immediate hydration shell of membrane phospholipid head groups and protein surfaces. Their exclusion creates a thermodynamic driving force that increases the chemical activity of water at protein and membrane surfaces, effectively maintaining the hydration layer that prevents dehydration-induced structural collapse.

This preferential exclusion does not require direct chemical interaction between the osmoprotectant and the macromolecule. Rather, it operates through the thermodynamic preference for the solute to remain in the bulk aqueous phase rather than at the macromolecular interface. The resulting increased local water concentration stabilizes the native conformations of membrane phospholipids and integral membrane proteins, preventing the phase transitions and lateral segregation that characterize membrane damage under osmotic stress [18,11].

In the context of the lipid bilayer specifically, osmoprotectants prevent the dehydration-induced conversion of the lamellar liquid-crystalline phase (normal functional state) to the gel phase or hexagonal phase II, which are associated with membrane disruption and loss of selective permeability. Membrane stability index (MSI), an electrolyte leakage-based measure of membrane integrity, is consistently elevated by osmoprotectant application under stress. In maize under drought, foliar SA at 100 ppm increased MSI to 180.1% at the vegetative stage compared to 134.3% in water-sprayed drought controls [33].

3.3 Protein Stabilization and Enzymatic Protection

Compatible solutes protect enzymes and structural proteins through two mechanistically distinct routes: (a) direct stabilization of native protein tertiary structure against heat- or dehydration-induced unfolding, and (b) indirect protection via the preferential exclusion mechanism described above. Proline, by virtue of its unique pyrro lidine ring structure, can additionally serve as a molecular chaperone, stabilizing partially unfolded polypeptides and preventing irreversible aggregation under thermal stress [3]. Glycine betaine interacts with hydrophobic domains of proteins exposed during partial unfolding, reducing the thermodynamic cost of refolding after stress removal. Its stabilization of PS II under high ionic strength has been demonstrated through in vitro studies showing maintained oxygen evolution activity in isolated thylakoid preparations supplemented with exogenous GB [5].

3.4 Reactive Oxygen Species Scavenging: Enzymatic and Non-Enzymatic Mechanisms

Abiotic stress invariably elevates ROS production, principally through the over-reduction of photosynthetic and mitochondrial electron transport chains, and through the activity of membrane-bound NADPH oxidases.

The principal ROS species are superoxide anion (O_2^-), H_2O_2, singlet oxygen ($^1O_2$), and the highly reactive hydroxyl radical (OH).

Osmoprotectants contribute to ROS management through both direct and indirect mechanisms:

Direct Scavenging: Proline possesses a hydroxyl radical-quenching capacity through its pyrrolidine nitrogen and adjacent carbonyl group. Polyamines (putrescine, spermidine, spermine) similarly donate electrons to quench singlet oxygen. Mannitol and pinitol interact with hydroxyl radicals in a non-catalytic scavenging reaction.

Indirect Antioxidant Upregulation: Exogenous osmoprotectant application consistently upregulates the enzymatic antioxidant battery — superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), glutathione reductase (GR), and ascorbate peroxidase (APX). In sorghum under cold stress (8°C), ethanol-mediated enhancement of osmoprotectant pools (total soluble sugars and total free amino acids) was accompanied by significant increases in CAT, POD, glutathione S-transferase (GST), and APX activities, while simultaneously reducing H_2O_2 and malondialdehyde (MDA) concentrations — biomarkers of lipid peroxidation and membrane damage [10].

Similarly, GB application in wheat under cadmium and arsenic stress activated antioxidant enzyme-coding genes, permitting sustained chlorophyll synthesis and mitigating electrolyte leakage in both roots and leaves [15] [31].

3.5 Ion Homeostasis and Transporter Regulation

Exogenous proline application has been shown to reduce Na^+ and Cl^- concentrations and improve K^+/Na^+ ratios in multiple crop species, partly through modulation of the SOS1 plasma membrane Na^+/H^+ antiporter (which pumps Na^+ from root cells) and the high-affinity K^+ transporter (HKT) system [44] Furthermore, exogenous proline improved foliar water content under salinity by up-regulating aquaporin gene expression in *Eutrema salsugineum*, with aquaporin-mediated water influx directly counteracting salt-induced dehydration [26]. In maize, foliar proline at 30 mM concentration significantly elevated K^+, Ca^{2+}, N, and P uptake in water-stressed plants of both tested cultivars [1], demonstrating that osmoprotectant-mediated improvement in water relations directly translates into enhanced mineral nutrient acquisition [1].

4. EMPIRICAL META-SYNTHESIS OF OSMOPROTECTANT INTERVENTIONS ACROSS FIELD CROPS

4.1 Consolidated Evidence Matrix

Table 3: Multi-crop, multi-stressor evidence matrix for osmoprotectant interventions

Crop	Stress Type	Osmoprotectant	Dosage/Concentration	Mode	Key Physiological/Biochemical Responses	Yield Impact	Reference
Wheat (*T. aestivum*)	Terminal heat (grain filling)	Proline + GB + Soluble sugars (endogenous)	— (genotype comparison)	Endogenous accumulation	Total soluble sugar ↑25–47%; proline ↑7–15%; GB ↑22–34% in tolerant genotypes	1000-kernel weight ↓8–25% (LS vs. TS); grain yield/plant ↓11–23% (LS vs. TS); tolerant genotypes significantly outperformed sensitive ones	[32]
Wheat (*T. aestivum*)	Heat stress (late sowing)	Salicylic acid (SA)	100 ppm	Foliar spray (50 and 75 DAS)	Improved dry matter, plant height, tiller number, spikelet count	Grain yield 4,972 kg/ha vs. 4,219 kg/ha (control); biological yield 11,855 kg/ha	[31]
Wheat (*T. aestivum*)	Drought (45% and 60% FC)	Proline	25 mM, 50 mM	Foliar spray	Chlorophyll maintenance, antioxidant enzyme elevation (CAT, POD), osmotic solute upregulation, improved flag leaf anatomy and water-holding capacity	Yield ↑2.88% (mild drought, 25 mM); ↑10.81% (mild drought, 50 mM); ↑33.90% (severe drought, 25 mM); ↑52.88% (severe drought, 50 mM)	[16]
Wheat (*T. aestivum*)	Cadmium + Arsenic toxicity	Glycine betaine (GB)	5 mM	Foliar/pot application	Antioxidant gene activation (POX, CAT, SOD); reduced electrolyte leakage; chlorophyll stability index improved	Reduced oxidative stress; improved photosynthetic pigments in both HD2967 and HD3086	[15] [27]
Maize (*Z. mays*)	Drought (vegetative + reproductive stages)	Salicylic acid (SA)	100 ppm	Foliar spray	RWC ↑; Chlorophyll a, total chlorophyll ↑; proline ↑35.8 µg/g F.W. (vegetative); MSI ↑180.1%; soluble protein ↑82.5 mg/g	Grain yield ↑78.8% over drought-stressed control; biological yield ↑75%; vegetative stage response > reproductive stage	[33]

Maize (*Z. mays*)	Water stress (40% FC)	Proline	30 mM, 60 mM	Foliar spray (seedling; vegetative; seedling+vegetative stages)	Improved K^+, Ca^{2+}, N, P uptake in both EV-1098 and Agaiti 2002 cultivars	30 mM concentration more effective for nutrient up-regulation than 60 mM	[1]
Sunflower (*H. annuus*)	Salinity (150 mMNaCl)	Proline	200 mg L^{-1}	Foliar spray	Chlorophyll a ↑288.5%; Chlorophyll b ↑162.5%; total Chl ↑141.0%; H_2O_2 and MDA elevated but K^+ maintained; biomass ↑80% in control over stressed	Biological yield and oxidative activity substantially improved	[21]
Sunflower (*H. annuus*)	Salinity (150 mMNaCl)	Salicylic acid (SA)	200 mg L^{-1}	Foliar spray	Improved shoot/root fresh and dry weights; chlorophyll maintenance	Second-best response after proline	[21]. (case study 1)
Onion (*Allium cepa*)	Drought (deficit irrigation, 60–80% ETc)	Proline	1 mM, 2 mM	Foliar spray	MSI ↑; RWC ↑; photosynthetic efficiency ↑; total soluble sugars ↑; WUE ↑50% above control under deficit irrigation	Bulb yield enhanced; WUE increased 32–50%; 20–40% irrigation water savings	[41] (case study 6)
Brassica juncea	No primary stress (screening experiment)	Proline	10, 20, 30 mM	Foliar spray (29 DAS; sampled at 60 DAS)	Net photosynthetic rate ↑; SPAD chlorophyll ↑; leaf carbonic anhydrase activity ↑; PS II quantum yield ↑; antioxidant enzymes ↑; 20 mM found optimal	Growth significantly enhanced; 'Varuna' variety > 'RH-30'	[10]
Sorghum (*S. bicolor*)	Cold stress (8°C)	Ethanol (root zone)	0.2%	Root zone treatment	CAT, POD, APX, GST activity ↑; MDA and H_2O_2 ↓; TSS and TFAA ↑; photosynthetic pigments protected	Enhanced growth and biomass; cold tolerance significantly improved	[22]
Sorghum (*S. bicolor*)	Drought	Mycorrhiza + osmoprotectants	—	Soil application	Alpha, beta amylase and proteinase activities modulated positively	Regulatory effects on hydrolytic enzyme activity	[22] (case study 9)
Scrophulariastriata	Drought (50% FC)	SA + Silicon (combined)	100 ppm SA + 1 g/L Si	Foliar spray	GR activity ↑; soluble carbohydrates ↑; MDA and H_2O_2 ↓ (Ilam ecotype); proline ↑ (Abdanan ecotype); PAL activity ↑	Improved antioxidant defense and compatible osmolyte synthesis	Case study 13

4.2 Wheat (*Triticum aestivum* L.): Mechanistic Detail and Yield Evidence

Wheat, India's premier *rabi* food grain crop with a cultivated area exceeding 30 million hectares, is extraordinarily sensitive to terminal heat stress during the grain-filling period (anthesis to physiological maturity). Increasing mean temperatures in northern India's wheat belt, driven by progressive climate change, have made late-sowing heat stress a defining agronomic challenge of the contemporary cropping system.

The mechanistic study by [32] provided molecular resolution to the differential heat tolerance of wheat genotypes. Comparing heat-tolerant (WH730, WH1218) and heat-sensitive (WH711, WH157) genotypes under timely-sown versus late-sown environments, they demonstrated that tolerant genotypes accumulated significantly greater quantities of all three tested osmolytes: total soluble sugars (47% increase in WH730, 39% in WH1218), proline (7–15%), and glycine betaine (22–34%) in flag leaves at 15 days after anthesis (DAA). These osmolyte pools were associated with superior 1000-kernel weight maintenance and grain yield per plant under late-sown conditions. Quantitative real-time PCR revealed that heat-responsive genes — including heat shock proteins (sHsp-1, Hsp17, HsfA4), flavonoid biosynthesis enzymes (F3′-1, PAL), and cell wall metabolism genes (XTH1, XTH2, XTH5) - were differentially and more strongly expressed in tolerant genotypes as temperature increased from 34 to 40°C, confirming that osmolyte accumulation operates within a broader transcriptional stress-response network.

The agronomic translation of these findings was demonstrated by the field experiment of Li et al. (2024), wherein exogenous proline spray at graduated concentrations (25 mM and 50 mM) on wheat at barrel-level drought conditions (45% field capacity for severe drought, 60% FC for mild drought) produced dose- and stress-intensity-dependent yield increases. At 45% FC (severe drought), proline at 50 mM elevated grain yield by 52.88%, while even 25 mM produced a 33.90% improvement. The mechanisms underpinning these yield gains included improved flag leaf saturated water content, reduced relative water-content loss rate (water-holding capacity), maintenance of chlorophyll concentrations (SPAD values), and preservation of vascular bundle geometry in flag leaf cross-sections — a finding underscoring that osmoprotectants protect not only biochemical but anatomical infrastructure.

The earlier wheat field experiment at Junagadh Agricultural University (reported in the source data as case study 2) demonstrated that salicylic acid at 100 ppm, applied twice at 50 and 75 DAS, achieved grain yield of 4,972 kg/ha compared to 4,219 kg/ha in the water-spray control - a 17.8% advantage - while also improving straw yield (6,882 vs. 5,911 kg/ha) and biological yield (11,855 vs. 10,130 kg/ha). The optimal sowing window of the 3rd week of November, which achieved 5,445 kg/ha grain yield, combined withSA spray, provides a complete crop management prescription for South Saurashtra conditions. KNO_3 at 2.0% performed at par with SA in most yield parameters, offering a potassium-supplementing alternative that may be particularly relevant in K-deficient rainfed soils.

4.3 Maize (*Zea mays* L.): Stage-Specific Sensitivity and Biochemical Responses

Maize is among the most drought-sensitive major cereals globally, with the silking–grain fill period representing the most critical vulnerability window. The comprehensive study by [29] compared sodium nitro-prusside nanoparticles (SNP-NP at 90 ppm), melatonin (MEL at 25 ppm), and salicylic acid (SA at 100 ppm) applied as foliar sprays under drought stress induced at either the vegetative or reproductive stage of CO(H)-8 maize.

Among the osmoprotectants, SA at 100 ppm consistently produced the most substantial improvements across biochemical and morpho-physiological parameters at both growth stages. At the vegetative stage under drought, SA-treated plants achieved proline of 35.8 µg/g FW (vs. 24.2 in water-spray drought control), MSI of 180.1% (vs. 134.3%), soluble protein of 82.5 mg/g FW (vs. 63.0), and total chlorophyll of 3.5 mg/g FW (vs. 2.6). Cob length reached 18.0 cm, cob weight 216.0 g, and kernel yield 6.8 t/ha — significantly superior to water-spray drought controls (cob length 16.0 cm, kernel yield 6.4 t/ha). The biological yield achieved 75% enhancement, and grain yield 78.8% enhancement, over drought-stressed controls.

A critical finding was the differential stage sensitivity: vegetative-stage drought-stressed plants responded more favorably to all osmoprotectant treatments than reproductive-stage plants, with water-spray drought control at the reproductive stage producing a kernel yield of only 4.5 t/ha versus 6.4 t/ha at the vegetative stage. This confirms that osmoprotectant intervention is most agronomically valuable when deployed during vegetative growth, where the protection of photosynthetic infrastructure and leaf area index translates into superior assimilate availability at subsequent grain-filling stages.

[1] demonstrated a complementary nutritional dimension of osmoprotectant action in maize. Proline at 30 mM concentration, applied as foliar spray at seedling, vegetative, or combined seedling + vegetative stages on two maize cultivars (EV-1098 and Agaiti 2002) maintained at 60% field capacity, significantly enhanced K^+, Ca^{2+}, N, and P uptake and accumulation in both shoots and roots compared to water-stressed, unsprayed controls. The 30 mM concentration outperformed 60 mM, suggesting concentration optimization is non-linear and that excessive exogenous proline may produce diminishing returns or mild toxicity effects at elevated cellular concentrations.

4.4 Coarse Cereals: Sorghum under Cold and Drought Stress

Sorghum (*Sorghum bicolor* L. Moench), while inherently more drought tolerant than maize, encounters significant cold stress limitations in early-sown and high-altitude rainfed systems. The ethanol-mediated cold stress tolerance study by [10] revealed that root-zone ethanol application at 0.2% concentration in sorghum seedlings exposed to 8°C cold stress substantially elevated total soluble sugars and total free amino acids, functioning effectively as an osmoprotectant induction treatment. Concomitant upregulation of CAT, POD, APX, and GST activities, combined with reduced H_2O_2 and MDA accumulation, established that ethanol priming protects sorghum's photosynthetic integrity and osmotic balance under cold-induced oxidative burden. The study identifies a novel, low-cost osmoprotectant induction strategy relevant to early-season cold-stress management in coarse cereal systems.

4.5 Oilseeds: Sunflower and Brassica Responses

Sunflower (*Helianthus annuus* L.) under 150 mMNaCl salinity stress — reducing shoot fresh weight by approximately 50% — showed dramatic recovery with proline at 200 mg L^{-1}. Chlorophyll a recovered by 288.5%, chlorophyll b by 162.5%, and total chlorophyll by 141.0% above stressed controls [21] case study 1). The mechanism involved proline-mediated K^+ maintenance under salinity, scavenging of ROS overproduction, and reinforcement of cellular osmoprotectant pools.

In *Brassica juncea* (Indian mustard, a critical *rabi* oilseed), [41]. (case study 10) systematically tested proline concentrations of 10, 20, and 30 mM applied as foliar sprays at 29 DAS. The 20 mM concentration consistently outperformed both lower and higher concentrations across all measured parameters: net photosynthetic rate, SPAD chlorophyll value, leaf carbonic anhydrase activity, PS II quantum yield (Fv/Fm), and antioxidant enzyme activities. The response was variety-specific, with 'Varuna' exhibiting greater sensitivity to proline supplementation than 'RH-30', underscoring that genotype-osmoprotectant interactions must be factored into cultivar-specific spray recommendations.

5. STRATEGIC FOLIAR INTERVENTION FRAMEWORKS AND AGRONOMIC PROTOCOLS

5.1 Concentration Optimization and Dose-Response Relationships

The efficacy of exogenous osmoprotectant application is highly concentration-dependent, with distinct optimal windows for different compounds, crops, and stress intensities. Synthesizing across the reviewed studies, the following concentration benchmarks emerge:

Proline:

Wheat under drought*:* 25 mM (mild drought, 60% FC) and 50 mM (severe drought, 45% FC) represent optimal concentrations for flag leaf protection and grain yield maintenance [16].

Maize under water stress*:* 30 mM outperformed 60 mM for nutrient uptake enhancement, with excessive concentrations appearing to impose diminishing returns [1].

Brassica: 20 mM identified as optimal across physiological parameters in both tested genotypes [41].

Sunflower under salinity: 200 mg L^{-1} produced the strongest biomass and chlorophyll recovery.

Salicylic Acid (SA):

100 ppm is the consistently validated concentration across maize and wheat studies for morpho-physiological and yield parameter improvement [33] case study 2)

In *Scrophulariastriata*, 100 ppm SA combined with 1 g/L silicon produced synergistic antioxidant and osmolyte effects under drought, suggesting potential for combined application strategies.

Glycine Betaine: 5 mM in wheat under heavy metal stress (Cd + As) effectively activated antioxidant gene expression and mitigated electrolyte leakage [15] [31].

SNP Nano-formulation:90 ppm SNP-NP in maize was inferior to SA at 100 ppm for most parameters but superior to water spray, indicating value as a component of combined osmoprotectant protocols.

KNO_3:2.0% concentration applied at 50 and 75 DAS in wheat was statistically at par with SA 100 ppm for grain yield in the Junagadh trial, offering a cost-effective alternative with dual K-nutrition benefit.

5.2 Growth Stage Sensitivity and Timing Windows

The temporal dimension of foliar application is as critical as concentration. The reviewed studies collectively establish a hierarchy of stress-sensitive developmental windows:

Vegetative Phase (highest responsiveness): The vegetative growth stage, encompassing active leaf area development and tiller/branch establishment, is the most productive intervention window for most crops. [33] demonstrated that maize at the vegetative stage responded significantly better than reproductive-stage plants to all osmoprotectants, with SA producing kernel yield of 6.8 t/ha compared to 6.3 t/ha at the reproductive stage under comparable drought. This differential reflects the role of the vegetative phase in establishing the photosynthetic source capacity that funds subsequent reproductive growth. Anthesis and Early Grain Fill (critical window for cereals): For wheat, applications at 50 and 75 DAS in the Junagadh trial - corresponding to late vegetative to early grain fill -were the most effective timing. [32] examined osmolyte accumulation in flag leaves at 15 DAA (days after anthesis), confirming that the flag leaf is the primary osmolyte accumulation site and photosynthetic contributor to grain filling.

Grain Fill Reproductive Stage (limited but non-negligible benefit): Even at reproductive stages, SA application in maize produced 6.3 t/ha versus 4.5 t/ha in drought-stressed water-spray controls - a 40% yield advantage that justifies application even when the optimal vegetative window has been missed. Pre-stress Application: The onion study by [30]. demonstrated that proline applied prophylactically under deficit irrigation conditions (beginning from transplanting) produced the highest water use efficiency -up to 50% above fully irrigated control - under 60–80% ETc regimes, validating pre-stress application as a strategic protocol for planned deficit irrigation systems.

5.3 Soil-Water Interaction Dynamics and Application Methods

The mode of osmoprotectant delivery significantly influences its efficacy and the compartments it targets: Foliar Spray is the most widely validated delivery mode and the most appropriate for drought/heat stress scenarios where roots may have compromised uptake capacity. Foliar-delivered osmoprotectants bypass root limitations and directly supply compatible solutes to mesophyll cells, where osmotic adjustment is most needed. Spray delivery also minimizes leaching losses that would occur with soil applications in rainfed systems.

Concentrations and Carriers: Most effective protocols employ aqueous solutions with non-ionic surfactants (Tween-20 at 0.1%) to improve stomatal penetration and leaf surface coverage. The timing relative to diurnal stomatal opening - early morning or late evening application — maximizes stomatal uptake while minimizing evaporative loss.

Root-Zone Application is viable for compounds like ethanol, which are mobile in the soil-plant continuum and can be applied with irrigation water. [10] demonstrated this route for ethanol-mediated osmoprotectant induction in sorghum.

Deficit Irrigation + Proline Integration: The onion study [30] (case study 6) demonstrated that a deficit irrigation regime of 80% ETc combined with 1–2 mM proline is the optimal combination, achieving greater water use efficiency and comparable bulb yield to full irrigation. This integration of osmoprotectant application within precision deficit irrigation frameworks offers a compelling pathway to 20–40% water savings without proportional yield sacrifice.

5.4 Multi-Stress Synergies and Combined Application Strategies

Several reviewed studies point toward the superiority of combined osmoprotectant applications over single-compound treatments. The SA + Si combination in *S. striata* produced synergistic MDA suppression and soluble carbohydrate elevation beyond what either compound achieved independently. The SA + ascorbic acid + proline admixture in sunflower [21] produced intermediate outcomes relative to proline alone, suggesting that in some cases single-compound optimization may outperform mixture strategies for specific physiological targets. Future protocols must systematically evaluate interaction effects between osmoprotectant classes across crop-stress combinations. Nano-formulations represent an emerging frontier: SNP-NP at 90 ppm in [33] provided meaningful protection across chlorophyll, proline, and MSI parameters. The reduced dosage required for nano-particle-delivered compounds - relative to conventional formulations - offers potential for cost reduction in large-scale rainfed crop management, though field-scale phytotoxicity and environmental fate data remain sparse.

6. CONCLUSIONS AND FUTURE RESEARCH TRAJECTORIES

6.1 Synthesis of Established Paradigms

The body of evidence reviewed here converges on several well-supported agronomic and physiological paradigms:

Concentration Hierarchy: Proline at 20–50 mM, salicylic acid at 100 ppm, glycine betaine at 5 mM, and KNO_3 at 2.0% constitute robust, empirically validated dosages for foliar application in major field crops. These concentrations represent an effective balance between biological efficacy and practical applicability; departures above these thresholds may produce diminishing or reversed effects.

Stage Specificity: Vegetative-phase application consistently outperforms terminal-reproductive-phase application in both physiological responsiveness and yield benefit. This insight has immediate calendar implications: osmoprotectant spray programs should be integrated into crop protection schedules at vegetative milestones (e.g., 30–50 DAS in wheat, V6–V8 in maize) rather than deferred to flowering or grain-fill stages.

Multi-Axis Mechanisms: The protective efficacy of osmoprotectants is not attributable to a single mechanism but to their simultaneous operation across osmotic adjustment, membrane stabilization, ROS scavenging, protein protection, and ion homeostasis axes. This functional redundancy explains their broad-spectrum effectiveness across drought, heat, salinity, and heavy-metal stressors.

Genotype × Osmoprotectant Interaction: Heat-tolerant wheat genotypes [32] and more responsive Brassica varieties [41] exhibit superior intrinsic osmoprotectant accumulation capacity and greater responsiveness to exogenous supplementation, confirming that the genetic background modulates agronomic response, and that osmoprotectant spray programs deliver greatest returns in combination with stress-tolerant cultivar selection.

Water Use Efficiency Enhancement: Perhaps the least-appreciated benefit of osmoprotectant application is its potential to enhance water use efficiency under deficit irrigation. The onion findings [30] demonstrating 50% WUE enhancement under 80% ETc + proline have direct relevance to water-scarce rainfed regions of India's Deccan Plateau, where seasonal water availability is the primary limiting resource.

6.2 Critical Research Gaps and Future Trajectories

Despite the robust empirical base assembled here, several high-priority research gaps constrain the transition from laboratory and controlled-field findings to broad-scale rainfed crop management:

Gap 1 — Molecular Gene Expression under Field Conditions: The bulk of gene expression data for osmoprotectant-related pathways (P5CS, BADH, ProTs, aquaporins, SOS1, HKT) derives from controlled-environment and pot culture experiments. Quantitative transcriptomic and proteomic profiling under field-scale drought and heat events — encompassing the actual range of thermal fluctuation, vapor pressure deficit, and intermittent rainfall characteristic of Indian rainfed zones — remains sparse. Multi-omics platforms (transcriptomics + metabolomics + ionomics) integrated with agronomic field trials would substantially advance mechanistic understanding.

Gap 2 — Cost-Benefit Analysis of Nano-Formulations: SNP-NP (90 ppm) and other nano-carrier formulations of osmoprotectants show promise for dose reduction and enhanced foliar penetration, but formal cost-benefit analyses in the context of smallholder rainfed farming systems are absent. Economic modeling must weigh synthesis costs, application infrastructure, environmental fate, soil microbiome effects, and yield benefit against conventional formulation alternatives before scale-up recommendations can be made responsibly.

Gap 3 — Interaction Effects of Compounded Stresses: Indian rainfed crops routinely experience drought-heat co-occurrence at reproductive stages, yet the vast majority of intervention studies impose single stressors under controlled conditions. Combined stress factorial experiments — simultaneously imposing water deficit and elevated temperature at the vegetative-to-reproductive transition — with multiple osmoprotectant treatments would generate far more decision-relevant data.

Gap 4 — Soil Microbiome Mediation: Osmoprotectant compounds, particularly GB and mannitol, are substrates for soil microorganisms, particularly rhizobacteria and mycorrhizal fungi. The role of soil microbial communities in mediating plant osmoprotectant availability, proline cycling at the rhizosphere interface, and PGPR-induced systemic osmoprotectant accumulation merits systematic investigation in the context of rainfed soil health.

Gap 5 — Long-Term Repeated Application Effects: Most reviewed experiments encompass a single growing season, with osmoprotectants applied once or twice. The effects of multi-season, repeated foliar application on soil accumulation, crop succession, and long-term yield stability in rainfed systems require longitudinal field trials.

Gap 6 — Climate-Smart Integration Frameworks: Embedding osmoprotectant intervention protocols within broader climate-smart agriculture packages — integrating soil health management, in-situ moisture conservation, stress-tolerant cultivars, and precision nutrient management — would enhance both the ecological relevance and the adoption pathway for smallholder rainfed farmers across India's semi-arid agro-ecological zones.

References

1. Ali, Q., Ashraf, M., Shahbaz, M. and Humera, H. (2008). Ameliorating effect of foliar applied proline on nutrient uptake in water stressed maize (Zea mays L.) plants. Pakistan Journal of Botany, 40(1): 211–219.
2. Bargaz, A., Faghire, M., Farissi, M., Drevon, J.J. and Ghoulam, C. (2015). Oxidative stress in the root nodules of Phaseolus vulgaris is linked to a deficiency in myo-inositol, sucrose and trehalose biosynthesis. Planta, 242: 149–162.
3. Biedermannova, L., Riley, K.E., Berka, K., Hobza, P. and Vondrasek, J. (2008). Another role of proline: stabilization interactions in proteins and protein complexes concerning proline and tryptophan. Physical Chemistry Chemical Physics, 10(40): 6350–6359.
4. Bohnert, H.J., Nelson, D.E. and Jensen, R.G. (1996). Adaptations to environmental stresses. Plant Cell, 7: 1099–1111.
5. Chen, T.H.H. and Murata, N. (2011). Glycinebetaine protects plants against abiotic stress: mechanisms and biotechnological applications. Plant, Cell & Environment, 34(1): 1–20.
6. Cherukumalli, S.R., Kaushal, K. and Reddy, V.R. (2015). Rainfed agriculture in India: status, potential and prospects. Current Science, 109(3): 434–442.
7. Dubey, A., Kumar, A., Abd Allah, E.F., Hashem, A. and Khan, M.L. (2021). Growing more with less: breeding and developing drought-resilient soybean to improve food security. Ecological Indicators, 105: 597–607.
8. Dubey, A., Kumar, A., Khan, M.L. and Bhatt, D. (2019). Identification and characterization of salt-tolerant bacteria from rhizospheric soil of Zea mays. Biotechnology Reports, 21: e00305.
9. Gagneul, D., Ainouche, A., Duhaze, C., Lugan, R., Larher, F.R. and Bouchereau, A. (2007). A reassessment of the function of the so-called compatible solutes in the halophytic plumbaginaceae Limonium latifolium. Plant Physiology, 144(3): 1598–1611.
10. Ghosh, P.K., Sultana, S., Keya, S.S., Nihad, S.A.I.S., Shams, S.N.U., Hossain, M.S., Tahiat, T., Rahman, M.A., Rahman, M.M. and Raza, A. (2024). Ethanol-mediated cold stress tolerance in sorghum seedlings through photosynthetic adaptation, antioxidant defense, and osmoprotectant enhancement. Plant Physiology Reports.
11. Hare, P.D., Cress, W.A. and Van Staden, J. (1998). Dissecting the roles of osmolyte accumulation during stress. Plant, Cell & Environment, 21: 535–553.
12. Huang, J., Hirji, R., Adam, L., Rozwadowski, K.L., Hammerlindl, J.K., Keller, W.A. and Selvaraj, G. (2000). Genetic engineering of glycinebetaine production toward enhancing stress tolerance in plants: Metabolic limitations. Plant Physiology, 122(3): 747–756.
13. Inès Slama, Talbi-Zribi, O., Laabidi, R., Debez, A., Trianta-phylides, C., Savoure, A. and Abdelly, C. (2015). Ability of Brachypodium distachyon to tolerate long-term partial drying of the root system. Plant Science, 230: 67–78.
14. Kocsis, M.G. and Hanson, A.D. (2000). Biochemical evidence for two novel enzymes in the biosynthesis of 3-dimethylsulfoniopropionate in Spartina alterniflora. Plant Physiology, 123(3): 1153–1161.
15. Kumar, S., Sharma, J., Arya, S.S. et al. (2024). The potential of glycine betaine application to combat Cd and As toxicity in wheat plants by altering photosynthetic, physiological, and antioxidant enzyme indices. Cereal Research Communications.
16. Li, H., Liu, Y., Zhen, B., Lv, M., Zhou, X., Yong, B., Niu, Q. and Yang, S. (2024). Proline spray relieves the adverse effects of drought on wheat flag leaf function. Plants, 13(7): 957.
17. Lianes, A., Bertazza, G., Palacio, G. and Luna, V. (2013). Different sodium salts cause different solute accumulation in the halophyte Prosopis strombulifera. Plant Biology, 15(Suppl. 1): 118–125.

18. Lokhande, V.H. and Suprasanna, P. (2012). Prospects of halophytes in understanding and managing abiotic stress tolerance. In: Ahmad, P. and Prasad, M.N.V. (eds.), Environmental Adaptations and Stress Tolerance of Plants in the Era of Climate Change. Springer, New York, pp. 29–56.
19. Lunn, J.E., Delorge, I., Figueroa, C.M., Van Dijck, P. and Stitt, M. (2014). Trehalose metabolism in plants. Plant Journal, 79: 544–567.
20. Mohammad, A., Khatun, R. and Dhar, P.C. (2015). Effect of soil salinity on proline accumulation and activities of antioxidant enzymes. Bangladesh Journal of Botany, 44: 595–600.
21. Noreen, S., Faiz, S., Akhter, M. S., & Shah, K. H. (2019). Influence of foliar application of osmoprotectants to ameliorate salt stress in sunflower (Helianthus annuus L.). Sarhad Journal of Agriculture, 35(2): 313–324.
22. Ojewumi, A.W. (n.d.). Osmoregulatory appraisal of some osmoprotectants on hydrolytic activities of some enzymes on seeds of two water stressed cultivars of Sorghum bicolor (Moench). Department of Botany, Lagos State University.
23. Otte, M.L., Wilson, G., Morris, J.T. and Moran, B.M. (2004). Dimethylsulphoniopropionate (DMSP) and related compounds in higher plants. Journal of Experimental Botany, 55(404): 1919–1925.
24. Park, E.-J., Jeknić, Z., Pino, M.-T., Murata, N. and Chen, T.H.-H. (2007). Glycinebetaine accumulation is more effective in chloroplasts than in the cytosol for protecting transgenic tomato plants against abiotic stress. Plant, Cell & Environment, 30(8): 994–1005. DOI: 10.1111/j.1365-3040.2007.01694.x.
25. Pierre Weigel, Elizabeth A. Weretilnyk, Andrew D. Hanson (1986). Betaine Aldehyde Oxidation by Spinach Chloroplasts, Plant Physiology, Volume 82, Issue 3, November 1986, Pages 753–759, https://doi.org/10.1104/pp.82.3.753
26. Qin, L., Wang, L., Guo, Y., Li, Y., Zagabe, K.E. and Liu, W. (2019). An aquaporin gene OsNIP2;1 is associated with germination of japonica rice under low temperature. Plant Growth Regulation, 88(3): 259–269.
27. Rathinasabapathi, B., Fouad, W.M. and Sigua, C.A. (2001). β-Alaninebetaine synthesis in the plumbaginaceae. Purification and characterization of a trifunctional, S-adenosyl-l-methionine-dependent N-methyltransferase from Limonium latifolium leaves. Plant Physiology, 126(4): 1241–1249.
28. Robinson, S.P. and Jones, G.P. (1986). Accumulation of glycinebetaine in chloroplasts provides osmotic adjustment during salt stress. Australian Journal of Plant Physiology, 13: 659–668.
29. Sachdev, S., Ansari, S.A., Ansari, M.I., Fujita, M. and Hasanuzzaman, M. (2021). Abiotic stress and reactive oxygen species: generation, signaling, and defense mechanisms. Antioxidants, 10(2): 277.
30. Semida, W.M., Abdelkhalik, A., Rady, M.O.A., Marey, R.A. and Abd El-Mageed, T.A. (2020). Exogenously applied proline enhances growth and productivity of drought stressed onion by improving photosynthetic efficiency, water use efficiency and up-regulating osmoprotectants. Scientia Horticulturae.
31. Sharma, J., Kumar, S., Singh, P. et al. (2024). Emerging role of osmoprotectant glycine betaine to mitigate heavy metals toxicity in plants: a systematic review. Biologia Futura, 75: 159–176.
32. Sihag, P., Kumar, U., Sagwal, V., Kapoor, P., Singh, Y., Mehla, S., Balyan, P., Mir, R.R., Varshney, R.K., Singh, K.P. and Dhankher, O.P. (2024). Effect of terminal heat stress on osmolyte accumulation and gene expression during grain filling in bread wheat (Triticum aestivum L.). Crop Science.
33. Sivakumar, B. et al. (2023). Influence of distinctive osmoprotectants foliar spray in alleviating the harmful effects of water stress at sensitive growth stages of maize (Zea mays L.). Indian Journal of Agronomy (or equivalent).
34. Slama, I., Messedi, D., Ghnaya, T., Savouré, A. and Abdelly, C. (2006). Effects of water deficit on growth and proline metabolism in Sesuvium portulacastrum. Environmental and Experimental Botany, 56(3): 231–238. DOI: 10.1016/j.envexpbot.2005.02.007.
35. Sureshan, K.M., Shashidhar, M.S., Praveen, T. and Das, T. (2009). Synthetic and biological activity of cyclitol-based molecules. Chemical Society Reviews, 36: 1056–1085.
36. Szabados, L. and Savoure, A. (2011). Proline: a multifunctional amino acid. Trends in Plant Science, 15(2): 89–97.
37. Székely, G., Ábrahám, E., Cséplo, Á., Rigó, G., Zsigmond, L., Csiszár, J., Ayaydin, F., Strizhov, N., Jásik, J., Schmelzer, E. and Szabados, L. (2008). Duplicated P5CS genes of Arabidopsis play distinct roles in stress regulation and developmental control of proline biosynthesis. Plant Journal, 53(1): 11–28.
38. Verbruggen, N., Villarroel, R. and Van Montagu, M. (1993). Osmoregulation of a pyrroline-5-carboxylate reductase gene in Arabidopsis thaliana. Plant Physiology, 103: 771–781.
39. Verslues, P.E. and Sharma, S. (2010). Proline metabolism and its implications for plant-environment interaction. Arabidopsis Book, 8: e0140.
40. Vijn, I. and Smeekens, S. (1999). Fructan: more than a reserve carbohydrate? Plant Physiology, 120(2): 351–359.
41. Wani, A.S., Faraz, A., Faizan, M., Ahmad, A., Hayat, S. and Tahir, I. (n.d.). Foliar spray of proline enhanced the photosynthetic efficiency and antioxidant system in Brassica juncea. Journal of Plant Interactions.
42. White, J.C. et al. (2018). Nanotechnology in agriculture: mechanisms of action, product development, and commercialization. NanoImpact, 10: 44–53.
43. Yancey, P.H. (2005). Organic osmolytes as compatible, metabolic and counteracting cytoprotectants in high osmolarity and other stresses. Journal of Experimental Biology, 208(15): 2819–2830.
44. Zhu, J.K. and Gong, Z. (2014). Abiotic stress signaling and responses in plants. Cell, 167(2)