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# Foliar application of *Kappaphycus alvarezii* extract improves drought tolerance in wheat (*Triticum aestivum*) by modulating physiological and biochemical responses

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## ABSTRACT

The study was carried out during the winter (*rabi*) season of 2023–24 and 2024–25 at ICAR-Indian Agricultural Research Institute, New Delhi to reveal the potential of *Kappaphycus alvarezii* extract (KE), applied as Sagarika concentrate liquid in enhancing drought tolerance in wheat (*Triticum aestivum* L.). In this study, plants were subjected to foliar sprays of KE at varying concentrations (2.5%, 7.5%, and 10% v/v) and exposed to short-term drought stress (10 days) during initial vegetative phase (one-month old wheat seedlings). The results revealed that KE application significantly improved plant performance and physiological parameters under water stress conditions. The treatment combining irrigation with 10% KE ($T_5$) recorded the highest soil moisture content (29.17%) and shoots biomass (1.858 g DW/plant) while drought-stressed plants without KE ($T_2$) showed the lowest values. KE-treated plants retained higher relative water content, membrane stability and chlorophyll levels indicating improved stress tolerance. In addition, cellular oxidative stress markers such as electrolyte leakage and hydrogen peroxide accumulation were notably reduced in KE-applied treatments, confirmed through both biochemical analysis and DAB staining. These findings suggest that KE foliar application enhances drought resilience in wheat by alleviating oxidative stress and supporting physiological stability making it a sustainable and eco-friendly strategy for improving crop growth responses under water-deficit conditions.

**Keywords**: Abiotic stress, Drought, $H_2O_2$, Priming, Reactive radicals, Seaweed

Seaweeds and their derived products are widely used as agricultural biostimulants because they supply a wide range of plant growth regulators, essential macro and micronutrients and antioxidant compounds. These components stimulate plant growth, enhance tolerance to biotic and abiotic stresses and ultimately improve crop productivity (Pramanick *et al.* 2016, Elumalai *et al.* 2025). In wheat, foliar application of liquid seaweed fertiliser has been shown to increase plant growth, chlorophyll content and yield attributes (Shah *et al.* 2013).

Red algae, comprising nearly 6,000 spp. are commercially important owing to their cell wall polysaccharides such as agar, carrageenan and cellulose (Bixler and Porse 2011, Porse and Rudolph 2017). Among them, *Kappaphycus alvarezii* is a major source of carrageenan widely used in food, dairy and pharmaceutical industries (Eswaran *et al.* 2005). Biostimulant technologies developed by CSIR-Central Salt and Marine Chemicals Research Institute using *K. alvarezii* have demonstrated considerable potential as the seaweed sap contains key phytohormones including indole-3-acetic acid, kinetin, trans-zeatin and gibberellic acid (Prasad *et al.* 2010). Biostimulants obtained from seaweeds promote plant metabolic activity and improve nutrient absorption, transport and utilisation while simultaneously enhancing soil physical structure and biological activity by improving moisture-holding capacity and supporting beneficial microorganisms (Gopalakrishnan *et al.* 2025). With escalating global food demand driven by population growth, sustainable agronomic approaches are required to counter environmental stresses such as drought, salinity and temperature extremes (Agarwal *et al.* 2016). In this context, understanding stress tolerance mechanisms particularly the crosstalk among phytohormones such as abscisic acid, cytokinins and auxins offers promising avenues for improving crop resilience and productivity (Asada 2006, Ha *et al.* 2012).

As a result, seaweed extract based products (SWEPs) have emerged as important sustainable inputs in contemporary agricultural practices (Gautam *et al.* 2024, Hermans 2024, Vafa *et al.* 2024). In India, their production and use are actively encouraged under the Pradhan Mantri

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Matsya Sampada Yojana (Ranjan 2021). These products are derived from several marine algal genera including *Kappaphycus*, *Sargassum*, *Ascophyllum* and *Laminaria* and are known to contain natural growth regulators that positively influence plant physiological processes (Boukhari *et al.* 2020, Sani and Yong 2022). Seaweed extracts contain bioactive compounds including phenolics, betaines, carbohydrates, proteins, lipids, alginates and laminarins which collectively improve nutrient use efficiency and strengthen plant tolerance to abiotic stresses (Hernández-Herrera *et al.* 2024). These effects translate into improved biomass accumulation, yield, crop quality and nutritional value of the produce (Vafa *et al.* 2024). Foliar application of SWEPs influences stomatal regulation and leaf osmotic balance, thereby enhancing plant performance during periods of limited water availability (Carrasco-Gil *et al.* 2018). Soil application further enhances soil structure, aggregation, microbial activity and nutrient bioavailability through alginate-mediated chelation (Cardozo *et al.* 2007).

Wheat (*Triticum aestivum* L.) is a major global staple crop and meeting the food demands of a projected population of nine billion by 2050 will require substantial gains in productivity (Alexandratos and Bruinsma 2012). It is highly sensitive to drought stress during critical growth stages such as tillering, booting, heading, anthesis and grain filling, where water scarcity reduces radiation use efficiency and growth, leading to yield losses (Sharma *et al.* 2022). Seaweed extracts, particularly those derived from *K. alvarezii*, represent an environmentally sustainable option for improving wheat yield and grain quality under sub-optimal NPK fertilisation (Mondal *et al.* 2025). However, most studies on SWEPs have focused on horticultural crops and vegetables, with limited attention given to cereal crops during early vegetative stages (Layek *et al.* 2023, Mousavi *et al.* 2024).

Despite drought being a major constraint to wheat productivity, most studies emphasise its impact during reproductive stages while the early vegetative stage, which is critical for crop establishment remains underexplored (Ahmed *et al.* 2025). The physiological and biochemical basis of drought tolerance at the seedling stage remains poorly understood and reliable early screening traits are lacking (Ahmed *et al.* 2025). Moreover, the potential of seaweed extracts to enhance drought tolerance in wheat during early growth stages has not been adequately evaluated. This knowledge gap underscores the need for integrated studies aimed at identifying seedling-stage drought tolerance traits and assessing seaweed extract-based strategies to improve early drought resilience in wheat. Although the nutritional and hormonal components of seaweed extracts are well known, their biostimulatory mechanisms are not fully understood. This study evaluates the effectiveness of *Kappaphycus alvarezii* extract in mitigating short-term drought stress in wheat genotype HD2967 during the early vegetative stage, providing insights into seaweed-based strategies for improving early drought resilience.

## MATERIALS AND METHODS

*Plant growth conditions and stress treatments*: Seeds of the wheat genotype HD2967 (Mega variety) were procured from the National Seed Corporation, ICAR-Indian Agriculture Research Institute, New Delhi and sown in plastic pots (13 cm in diameter) filled with soil and farmyard manure (FYM) mixture in a 2:1 (v/v) ratio. The experiment was conducted under winter (*rabi*) season of 2023–24 and 2024–25 in the pot culture at ICAR-Indian Agriculture Research Institute, New Delhi. The mean values for both years were used for graphical representation. The experiment followed a completely randomised design (CRD) with 10 replications in each condition. Thinning was performed at the 3-leaf stage and 4 plants were maintained per pot. Plants were subjected to foliar spraying of *Kappaphycus alvarezii* extract (KE), Sagarika concentrate liquid applied at levels of 2.5%, 7.5% and 10% (v/v). The foliar treatments were implemented twice, at 30 and 34 days after sowing (DAS) ensuring that a fine mist covers the leaves evenly. After the second application, plants were subjected to drought stress by restricting water application for 10 days, starting at 34 DAS. This experiment was completed by 45 DAS. Eight treatment groups were established, $T_1$, Irrigated (IR) + foliar spray with water ($H_2O$); $T_2$, Drought (Dr) + $H_2O$; $T_3$, IR + 2.5% KE; $T_4$, IR + 7.5% KE; $T_5$, IR + 10% KE; $T_6$, Dr + 2.5% KE; $T_7$, Dr + 7.5% KE; $T_8$, Dr + 10% KE (Supplementary Table 1). Physiological traits were measured at 10 days after the second foliar treatment. Relative water content (RWC), membrane stability index (MSI) and total chlorophyll content (TCC) were analysed in wheat leaf (3$^{rd}$ fully expanded leave from top) at 10$^{th}$ day after stress. Plant biomass were recorded in terms of shoot and root dry weight at 10$^{th}$ DAS. Soil moisture content (SMC) was also determined at the 10$^{th}$ day of the stress (i.e. 45 DAS) stage using a gravimetric technique (Black 1965). Soil samples were taken from a depth of 10 cm from the middle of the earthen pots using a tube auger. The SMC values were recorded as 26.86%, 3.99%, 28.20%, 28.05%, 29.17%, 11.17%, 13.08% and 14.31% under $T_1$, $T_2$, $T_3$, $T_4$, $T_5$, $T_6$, $T_7$ and $T_8$ respectively (Supplementary Fig. 1).

*Analysis of growth parameters*: Morphological alterations in shoots and root were monitored by measuring the dry weights of shoots and roots from 45-day-old plants.

*Analysis of physiological parameters*: The healthy mature green leaf (3$^{rd}$ fully expanded leaf from the top of plant) of wheat plant was sampled after 10 days after stress under all the treatments. For extraction of chlorophyll pigments, the samples were incubated overnight in DMSO in the dark to record photosynthetic pigments. Optical density (OD) readings were taken at 445 nm, 645 nm and 663 nm to measure total chlorophyll content. The final pigment contents were noted based on the fresh tissue weight, following the procedure of Hiscox and Israelstam (1979). RWC was determined using Barrs and Weatherly (1962) method and membrane stability index (MSI) using Premachandran *et al.* (1990). Ion leakage and RWC were key indicators for setting critical time points for stress tolerance between treated and untreated plants. *In vivo* $H_2O_2$ (Hydrogen peroxide) monitoring was done using histochemical staining with 3, 3-diaminobenzidine (DAB), as per Shi *et al.* (2010) with minor modifications. For the localisation of $H_2O_2$, leaves were incubated in a 1mg/mL DAB solution prepared in 10 mM phosphate buffer (*p*H 7.8) and kept under illumination until brown spots appeared. Chlorophyll was bleached using a lactic acid-glycerol-ethanol solution, which decolourised the leaves, making the $H_2O_2$ presence visible as brown spots.

*Statistical analysis*: Each treatment was conducted in triplicate and the mean values, standard deviations and standard errors were computed. Analysis of variance (ANOVA) was performed, followed by Duncan's Multiple Range Test (DMRT) at a significance level of $p \leq 0.05$ to evaluate differences between the control and various stress treatments. Treatments showing statistically notable differences were denoted by distinct letters.

## RESULTS AND DISCUSSION

Biostimulants are gaining increasing attention for their ability to improve plant tolerance to abiotic stresses while contributing to more sustainable crop production systems (Kerchev *et al.* 2020). The present investigation highlights the positive role of *Kappaphycus alvarezii* extract (KE), a seaweed-based biostimulant in improving drought tolerance in wheat. A drought experiment was performed to determine pre-treatment of plants with KE as a spraying agent can induce tolerance against drought stress. Wheat plants were primed twice with KE at three different levels of i.e. 2.5%, 7.5% and 10% (v/v). The first foliar application was performed at 30 days after sowing (DAS) followed by a second application at 34 DAS. Subsequently, plants were subjected to water-limiting conditions from 34 to 45 DAS.

*Improved soil water status*: Soil water status was improved with KE application under both irrigated and non-irrigated conditions, as it improved soil physical and biological properties by increasing moisture retention and stimulating beneficial microbial populations (Gopalakrishnan *et al.* 2025). $T_1$ (IR + $H_2O$) and $T_2$ (Dr + $H_2O$) served as control treatments. Among the treatments, the maximum soil moisture retention was observed in $T_5$ (IR + 10% KE) and $T_4$ (IR + 7.5% KE) with values of 29.17% and 28.05% respectively. In contrast, the lowest soil moisture content was recorded in $T_2$ (Dr + $H_2O$) (Supplementary Fig.1).

*Improved morphological growth on KE application*: The foliar application of KE promoted shoot development under both control and stress conditions (Fig. 1). Treatments $T_3$ (IR + 2.5% KE), $T_4$ (IR + 7.5% KE) and $T_5$ (IR + 10% KE) exhibited significantly higher shoot biomass (g DW/plant) as compared to others. Among them, $T_5$ (IR + 10% KE) exhibited the highest shoot biomass with a mean value of 1.858 g DW/plant, indicating the most pronounced growth-promoting effect. In contrast, $T_2$ (Dr + $H_2O$) recorded the lowest mean (1.076 g DW/plant), suggesting that water spraying alone had a minimal impact on shoot biomass compared to other treatments. Notably, KE application under drought conditions positively influenced shoot biomass; however, the noted improvement was greater than that in $T_2$ but lower than that in $T_1$, $T_3$, $T_4$ and $T_5$. For root's dry weight (g DW/plant), treatments $T_1$, $T_3$, $T_4$, $T_5$, $T_6$, $T_7$ and $T_8$ were statistically similar except treatment $T_2$ (Dr + $H_2O$) which recorded the lowest (0.596 g DW/plant) value. These results highlight that KE application minimised or maintained root biomass reduction particularly under water limited conditions. Moreover, KE-treated plants under water limiting condition indicated more shoot biomass compared to that in $T_2$, indicating its potential role in mitigating drought-induced biomass reduction.

*KE foliar application enables wheat plants to overcome the effects of drought*: Plants primed in this manner exhibited greater drought tolerance compared to control KE-non-treated plants. Control plants started wilting after

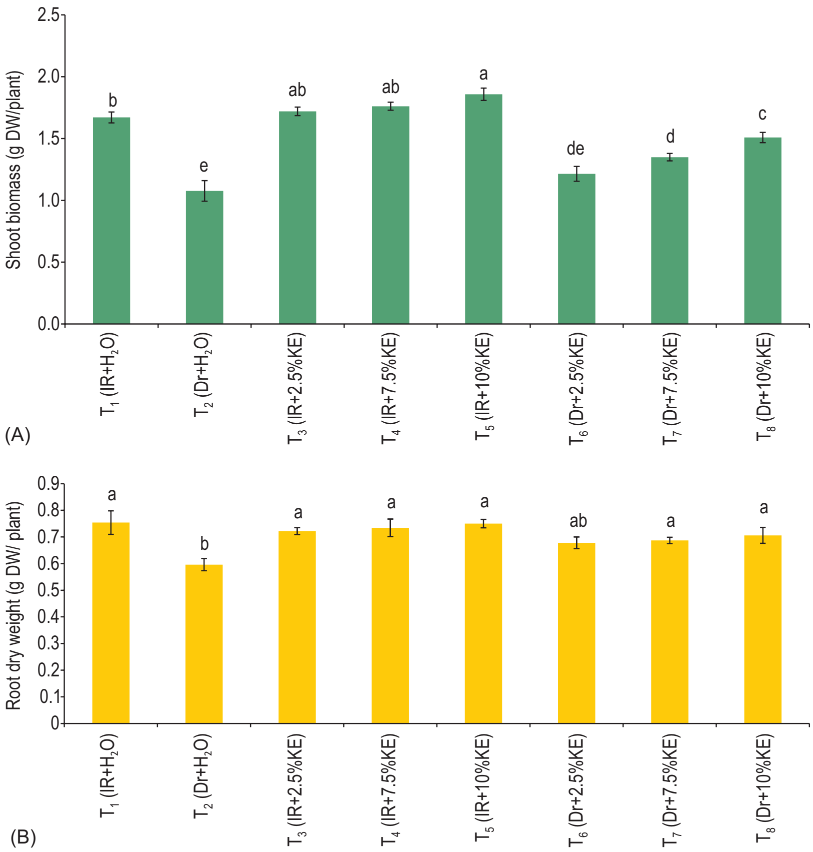


Fig. 1 Effect of two foliar applications of *Kappaphycus alvarezii* extract (KE) on (A) shoots and (B) roots biomass of wheat grown under well-watered and water limited conditions.
Values represent the mean of two growing seasons (2023–24 and 2024–25).

5 days of continuous drought and symptoms were more pronounced at 10 days of stress (Supplementary Fig. 2). In contrast, KE-treated plants showed improved tolerance to drought (Supplementary Fig. 2). Significant differences in development were observed including vegetative growth in terms of shoot and root DW (g/plant) and leaf greenness (total chlorophyll content) in K-sap treated than non-treated plants. Electrolyte leakage (EL) was also determined as a measure of cell membrane damage due to drought stress and RWC at $10^{th}$ days after stress. EL in K-sap treated plants was significantly lower ($p<0.01$) compared to non-treated plants subjected to drought stress (Fig. 2D). A severe decline in RWC was noticed in non-treated plants after 10 days of continuous drought, which was partially mitigated by K-sap application (Fig. 2A). Total pigment content was more in KE treated plants than non-treated plants under drought stress (Fig. 2B). A significant reduction in electrolyte leakage and hydrogen peroxide ($H_2O_2$) accumulation was observed, indicating better maintenance of membrane stability and reduced oxidative damage. The observed positive responses can be largely explained by the wide array of bioactive compounds contained in KE. According to Gopalakrishnan *et al.* (2025), KE contains plant growth regulators such as auxins, cytokinins and gibberellins along with essential amino acids, macro- and micronutrients and osmolytes like glycine betaine and choline. Together, these compounds act in a coordinated manner to activate plant defense mechanisms and enhance tolerance to environmental stress (Hilker *et al.* 2016).

*KE foliar application lowers hydrogen peroxide levels in plants*: $H_2O_2$ is an important stress molecule that accumulates to often toxic levels in many abiotic stresses (Noctor *et al.* 2014, Zhou *et al.* 2019), while moderate levels of $H_2O_2$ have a signaling function in plants (Smirnoff and Arnaud 2019). Thus, avoiding high levels of $H_2O_2$ is important to keep plants healthy and for maintaining growth. Based on the observed beneficial effect of KE on drought-stressed plants, it is considered that KE priming might affect $H_2O_2$ levels. Therefore, $H_2O_2$ levels were monitored by DAB staining in young wheat leaves ($3^{rd}$ fully developed leaf from top) from 45-day-old plants. In the irrigated condition; KE-treated leaves showed weaker DAB staining than non-KE treated plants, indicating lower $H_2O_2$ accumulation (Fig. 3). While in the absence of KE spray $H_2O_2$ levels strongly increased in drought stressed plants, this increase was significantly lower in KE-treated plants (Fig. 3). We concluded that KE priming improves tolerance to drought stress by preventing $H_2O_2$ accumulation. The lower accumulation of $H_2O_2$ detected in KE-treated plants indicates an improvement in antioxidant defense capacity which aligns with earlier findings that biostimulant application is known to strengthen antioxidant systems, preserve cellular integrity and support metabolic regulation under abiotic stress conditions (Noctor *et al.* 2014, Rouphael

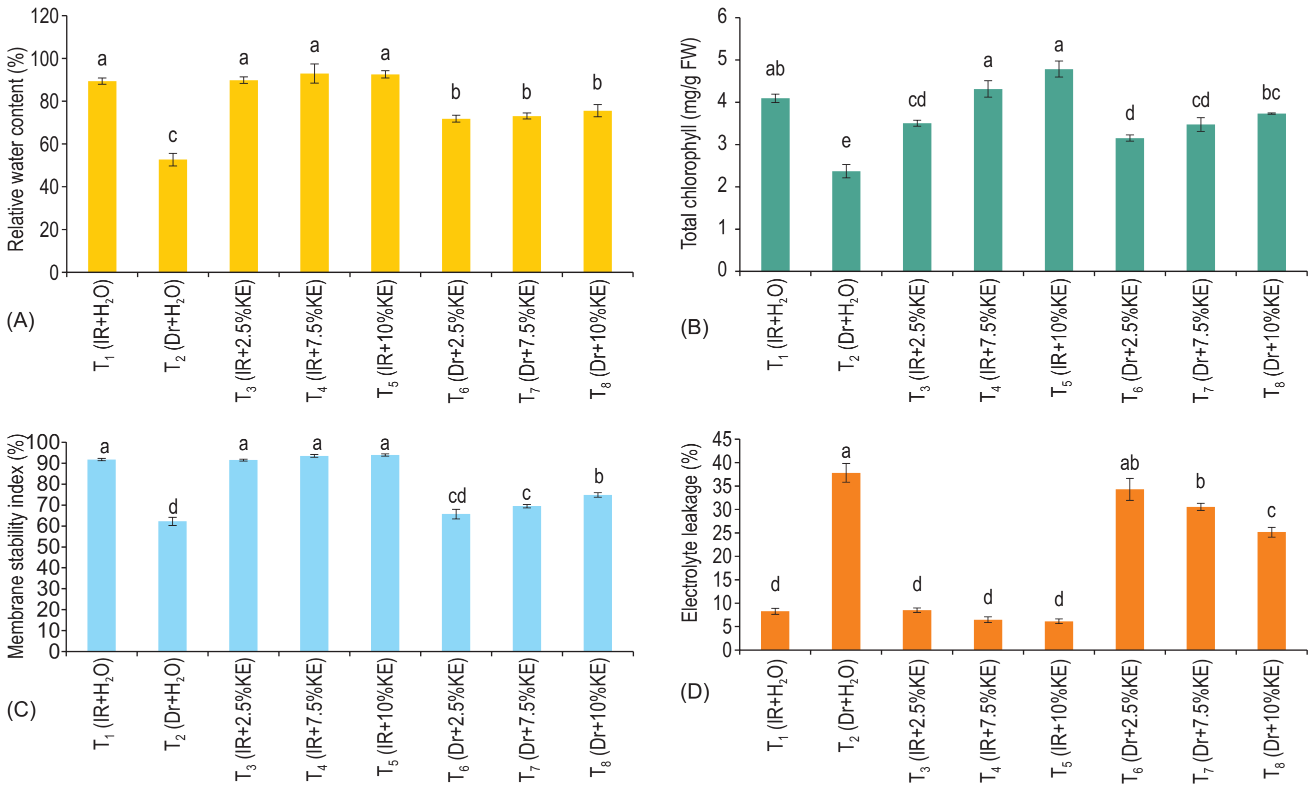


Fig. 2 Effect of two foliar applications of *Kappaphycus alvarezii* extract (KE) on (A) relative water content (%), (B) total chlorophyll content (mg/g FW), (C) membrane stability index (%) and (D) electrolyte leakage (%) in wheat plants grown under well-watered and water limited conditions.

Values represent the mean of two growing seasons (2023–24 and 2024–25).

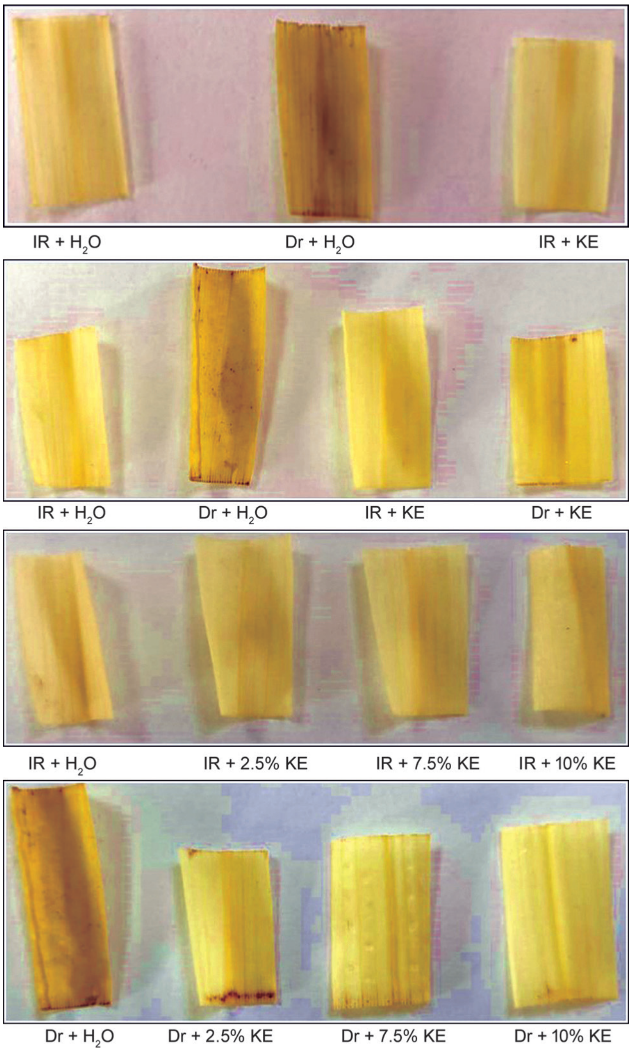


Fig. 3 Histochemical staining of ROS in leave using 3, 3′-diaminobenzidine.

and Colla 2020).

Plants treated with KE showed notable enhancement in physiological traits including higher relative water content, improved chlorophyll retention and increased shoot biomass under water-deficit conditions. The findings of this study are supported by similar reports in other crops which further validates the efficacy of *K. alvarezii* extract across species. In black gram, for instance a 5% foliar application of *Kappaphycus* sap was shown to improve photosynthetic activity and biomass accumulation, although concentrations beyond 10% had a detrimental effect indicating the need for precise dosage optimisation (Nunes *et al.* 2025). Similarly, studies in rice have shown that both foliar and root applications of KE promote photosynthetic efficiency, nutrient uptake (notably nitrogen and potassium) and biomass, especially under stress conditions. Root application in rice has been associated with longer-lasting physiological benefits and enhanced modulation of redox-related and stress-responsive signaling pathways (Castro *et al.* 2024). Glycine betaine, one of the key osmoprotectants found in KE, plays an important role in stabilising proteins, maintaining membrane integrity and supporting osmotic adjustment during drought stress (Ashraf and Foolad 2007). Its presence likely contributes to the improved drought tolerance seen in KE-treated wheat plants. Additionally, studies involving *Arabidopsis thaliana* treated with *Ascophyllum nodosum*-based biostimulants have shown enhanced meristem preservation and growth maintenance under drought, similar to the growth sustenance patterns observed in our wheat experiment (Rasul *et al.* 2021).

Overall, the findings of this study clearly demonstrate the potential of *Kappaphycus alvarezii* extract as an effective biostimulant for improving drought tolerance in wheat. The capacity of KE to improve water status, reduce oxidative damage and sustain plant growth under drought conditions highlights its relevance for climate-smart agricultural practices. However, to fully realize the benefits of KE in varied agricultural systems additional research is required to clarify the molecular mechanisms underlying its biostimulatory effects. Moreover, assessing its effectiveness across diverse wheat genotypes and environmental conditions will be essential for broader and long-term application.

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