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Effect of bentonite application on the morphological, physiological, and biochemical behaviour of *Triticum durum* Desf and *Triticum aestivum* L. cultivated in saline soils

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ABSTRACT

Soil salinity represents a major constraint to agricultural productivity in arid and semi-arid regions, severely affecting cereal growth and yield. This study evaluated the effect of mineral soil amendments using two types of bentonite, sodium bentonite from Mostaganem (B-Na) and calcium bentonite from Maghnia (B-Ca), on the morphological, physiological, and biochemical responses of durum wheat *Triticum durum* Desf. And bread wheat *Triticum aestivum* L. cultivated in saline soil 19 $dS.m^{-1}$ from the Relizane region (western Algeria). Greenhouse experiments were conducted using bentonite doses of 5% and 10% (w/w) to assess plant growth parameters, relative water content (RWC), chlorophyll pigments, and soluble sugar levels. Both bentonites enhanced wheat performance under saline conditions, with the 5% dose producing the most favourable effects. Notably, 5% B-Na significantly increased RWC, chlorophyll concentration, and soluble sugar content in *T. aestivum* compared with the saline control. Excessive amendment (10%) did not yield further benefits. These findings suggest that moderate application of bentonite can effectively alleviate salinity stress and improve physiological performance in wheat. The study emphasizes the importance of optimizing bentonite type and dose based on soil characteristics and crop sensitivity to salinity.

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1. INTRODUCTION

Soil salinity has emerged as one of the most pressing challenges to global agricultural productivity, particularly in arid and semi-arid regions where water scarcity and irregular rainfall patterns increasingly disrupt crop systems (Shalaby, 2024). Excessive accumulation of soluble salts in the soil profile alters osmotic balance, reduces water availability to plants, and disturbs ion homeostasis, thereby impairing physiological functions essential for growth and development (Benidire et al., 2020). When the osmotic potential of the soil solution decreases, plants experience water deficit even in moist conditions, leading to cellular dehydration and metabolic imbalance. As a result, salinity stress adversely affects all stages of plant development, from germination and seedling establishment to flowering, grain filling, and final yield (Al Khateeb et al., 2020; Hmissi et al., 2024; Puccio et al., 2023).

The morphological manifestations of salinity are well documented: reduced leaf expansion, shortened root systems, and lower shoot biomass (Moustafa et al., 2021; Shao et al., 2021; Zahra et al., 2020). These alterations restrict photosynthetic capacity and nutrient uptake, ultimately decreasing both fresh and dry matter accumulation (Chahal et al., 2022; Jia et al., 2022; Neji et al., 2021). High soil salinity has detrimental effects on photosynthesis (Shen et al., 2024), chlorophyll pigment synthesis (Salim, 2023) and causes a significant imbalance in mineral reserves (Horchani et al., 2025). To survive under these adverse conditions, plants have evolved diverse physiological and biochemical mechanisms, including osmolyte accumulation, antioxidant defense activation, and ion compartmentalization. However, the efficiency of these mechanisms varies widely among species and even among cultivars of the same species (Alavilli et al.,

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2023; Fellahi et al., 2024; Trivellini et al., 2023). Consequently, identifying genotypes capable of maintaining morphological integrity and metabolic balance under saline stress is a central goal of modern plant breeding and sustainable crop management (Aycan et al., 2024)

Wheat (*Triticum* spp.) holds a strategic position as a staple food crop, particularly in the Mediterranean basin, where it underpins food security and rural economies (Tsialtas et al., 2018). Despite its agronomic importance, wheat productivity is constrained by environmental stresses, especially drought and soil degradation (Al Khatib et al., 2025). Among abiotic stresses, salinity is one of the most detrimental factors affecting wheat physiology and yield (Farooq et al., 2024). Although wheat is considered moderately salt-tolerant ECe up to 7 dS m^{-1} (El-Ramady et al., 2024). Significant differences exist between species and cultivars regarding their ability to withstand saline environments. *Triticum durum* (durum wheat) and *Triticum aestivum* (bread wheat) are the two most cultivated species, yet they exhibit distinct physiological strategies under salinity, related to their differential ion transport, osmotic adjustment, and antioxidant capacity (Farooq et al., 2024).

In Algeria, wheat cultivation is of considerable socio-economic relevance, contributing approximately 250,000 tonnes annually (FAO, 2023). The country possesses a rich genetic diversity of local cultivars (Atoui et al., 2021), many of which have evolved to adapt to the harsh Mediterranean climate characterized by high evapotranspiration and poor soil fertility (Ababsa et al., 2023; Boudiar et al., 2019). Nevertheless, the progressive salinization of agricultural lands, exacerbated by inadequate irrigation practices, groundwater exploitation, and climate variability, threatens the sustainability of cereal production. In saline soils, the productivity of both durum and bread wheat is severely diminished, often failing to meet domestic demand. Therefore, identifying reliable indices of salt tolerance and understanding cultivar-specific responses are crucial to guide breeding programs and management practices (Chaurasia, 2024).

Beyond genetic selection, ecological and sustainable soil management approaches are increasingly recognized as complementary strategies to mitigate salinity effects. The use of soil amendments, particularly mineral conditioners (Kang et al., 2024; Meena et al., 2023), has gained attention as a means to improve soil structure, enhance cation exchange capacity, and facilitate nutrient retention (Bushron et al., 2025; Kumar et al., 2025). Among these amendments, bentonite, a naturally occurring clay primarily composed of montmorillonite, has shown promising results in restoring degraded soils (Iqbal et al., 2024). Belonging to the smectite group, bentonite exhibits remarkable swelling capacity, high surface area, and significant cation exchange potential (Abulimiti et al., 2023; Mohawesh & Durner, 2019). These physicochemical properties enable it to retain water efficiently (Rahmani et al., 2020), reduce nutrient leaching, and stabilize soil pH (Hoang et al., 2023; Ren et al., 2024)(Hoang et al., 2023; Ren et al., 2024). When applied to sandy or saline soils, bentonite improves aggregation, enhances nutrient availability, and creates a more favorable microenvironment for root development (Mi et al., 2021).

In Algeria, natural bentonite deposits are abundant and occur primarily in two forms, sodium bentonite and calcium bentonite. The two differ substantially in their physicochemical characteristics and agricultural potential. Calcium bentonite possesses a low swelling index but is rich in divalent cations (Ca^{2+}, Mg^{2+}), which can improve soil structure and promote cation exchange stability. In contrast, sodium bentonite exhibits a high swelling capacity and strong water retention ability due to the prevalence of Na^{+} and K^{+} ions (Muhammad & Siddiqua, 2022). Despite their availability, these bentonites remain largely underexploited in Algerian agriculture. Their potential to ameliorate saline soils and to enhance plant performance under salt stress conditions has not yet been systematically investigated.

The novelty of the present study lies precisely in this unexplored intersection: it constitutes one of the first comparative assessments of the effects of two distinct types of natural bentonite, sodium and calcium, on the morphological, physiological, and biochemical responses of two wheat species *(Triticum durum* and *Triticum aestivum*) cultivated under saline soil conditions in western Algeria. Unlike previous studies that have focused on the general use of soil amendments or single bentonite formulations, this research integrates multi-level analyses (morphological growth parameters, physiological traits, and biochemical indicators) to elucidate how different bentonite compositions influence wheat adaptation to salinity. Moreover, by employing soil collected from the Wilaya of Relizane, a region known for its saline-affected agricultural lands, the study directly addresses a problem of high local relevance with potential applicability to other Mediterranean environments facing similar constraints.

Specifically, the research aims to: (i) evaluate the impact of sodium and calcium bentonite incorporation into saline soils on the growth dynamics (plant length, number of leaves, fresh and dry biomass) of *T. durum* and *T. aestivum*; (ii) assess physiological parameters such as relative water content and chlorophyll and carotenoid concentrations, which are indicators of photosynthetic performance under stress; and (iii) quantify biochemical markers like soluble sugars, which reflect osmotic adjustment mechanisms. By integrating these complementary datasets, the study seeks to identify which type and dose of bentonite most effectively mitigates salinity stress and improves wheat performance.

Ultimately, this research contributes to the growing field of eco-efficient soil management, providing a practical, low-cost approach to enhance soil quality and crop resilience in saline environments. The findings are expected to advance current understanding of bentonite-plant-soil interactions and to inform both agronomic practices and policy strategies aimed at restoring productivity in degraded lands. In doing so, the study bridges a critical knowledge gap between soil mineral amendments and plant stress physiology, offering novel insights that align with global efforts toward sustainable agriculture and food security in salt-affected regions.

2. MATERIAL AND METHODS

2.1. Plant material and soil collection

Two cereal species commonly cultivated in Algeria were selected for this study: *Triticum durum* (local variety Sersou, also known as Siméto) and *Triticum aestivum* (variety Ain Abid, AS 81189). Seeds of both varieties were obtained from the Relizane Cereals and Pulses Cooperative (CCLS). The soil used for the experiment was collected from a wheat field in the municipality of El-Matmar, Wilaya of Relizane, located at a latitude of 35.7309° N, a longitude of 0.461769° E, and an altitude of 59 m above sea level. Samples were taken from three different points approximately 30 m apart, at a depth of 20–30 cm, and then combined to form a composite sample. The soil was air-dried, gently crushed, and sieved to remove coarse particles while preserving its natural physicochemical properties. Based on electrical conductivity measurements (19 $dS \cdot m^{-1}$), the soil was classified as highly saline.

2.2. Seed preparation and experimental design

Seeds were surface-sterilized in 2% sodium hypochlorite solution for 5 minutes, rinsed thoroughly with distilled water, and pre-germinated in plastic trays irrigated with distilled water. Uniform seedlings were transplanted into 1.2 kg capacity plastic pots, with one seedling per pot.

The growth substrate consisted of a homogeneous mixture of the saline soil from El-Matmar and natural bentonite at different inclusion levels. Two types of bentonite were tested, sodium bentonite and calcium bentonite, each incorporated at 5% and 10% (w/w) of the total substrate. Each treatment was replicated ten times for both wheat species. Pots were arranged in a completely randomized block design (CRBD) under controlled greenhouse conditions at the Faculty of Science and Technology, University of Relizane. Plants were irrigated twice weekly using tap water to maintain moderate moisture levels. Figure 1 illustrates the experimental setup.

Figure 1. Experimental design illustrating the evaluation of *Triticum durum* and *Triticum aestivum* growth under saline soil conditions amended with bentonite. (Top left) Germination of *Triticum durum* and *Triticum aestivum* seeds on moistened paper under controlled conditions prior to transplanting

2.3. Measured parameters

2.3.1. Morphological traits

After 30 days of growth, the number of leaves per plant was counted. Plants were carefully removed from the pots, and roots were washed with distilled water to remove soil residues. Shoot and root lengths (cm) were measured using a graduated ruler. Fresh biomass was immediately recorded, and samples were then oven-dried at 80 °C for 48 h to determine dry weights of both shoot and root tissues.

2.3.2. Relative water content (RWC)

RWC was determined following the method of Scippa et al. (2004). One fully expanded leaf of similar developmental stage was collected from each plant to determine its fresh weight (FW). The leaf was then immersed in distilled water at 4 °C in darkness for 24 h to obtain the turgid weight (TW), and subsequently dried at 80 °C for 48 h to measure the dry weight (DW). RWC was calculated using Equation 1.

$$RWC = \left(\frac{FW-DW}{TW-DW}\right) * 100 \quad [1]$$

Where FW: Fresh weight; DW : Dry weight; and TW: Weight in full turgidity.

2.4. Biochemical parameters

2.4.1. Photosynthetic pigments (chlorophylls and carotenoids)

The concentrations of chlorophyll a (Eq. 2), chlorophyll b (Eq. 3), and total carotenoids (Eq. 4) were estimated using the acetone extraction method described by Omrani et al. (2022). Approximately 0.1 g of fresh leaf tissue was homogenized in 5 mL of 80% acetone and centrifuged at 4000 rpm for 15 min. The supernatant was stored in darkness at 4 °C for 48 h. Absorbance was measured at 470, 646, and 663 nm using a UV–Vis spectrophotometer. Pigment concentrations ($mg \cdot g^{-1}$ FW) were calculated according to the following equations:

$$Chlorophyll\ a = \frac{(13.36*A663)-(5.19*A646)*8.81}{FW} \quad [2]$$

$$Chlorophyll\ b = \frac{(27.43*A646)-(8.12-A663)*8.81}{FW} \quad [3]$$

$$Carotenoids = \frac{(4.785*A470)+(3.657*A663)-(12.76*A646)*8.1}{FW} \quad [4]$$

2.4.2. Soluble sugars

Total soluble sugars were quantified according to the phenol–sulphuric acid method described by Dubois et al. (1956). Dried plant material (100 mg) was extracted with ethanol, and the resulting extract was treated with phenol and concentrated sulphuric acid. Absorbance was read at 485 nm. A calibration curve was prepared using glucose standards ranging from 10 to 100 $\mu g \cdot mL^{-1}$.

2.5. Statistical analysis

All collected data were subjected to statistical analysis based on a completely randomized design. Differences among treatment means were evaluated using Fisher's least significant difference (LSD) test at $p < 0.05$. Pearson's correlation coefficients were calculated to examine relationships among morphological, physiological, and biochemical parameters. Analyses were performed using STATISTICA version 10.1 (StatSoft Inc., USA). Principal Component Analysis (PCA) was conducted using the R programming environment to identify patterns of variation and association among traits across treatments and species.

3. RESULTS

3.1. Growth parameters

After one month of growth, both *Triticum durum* and *Triticum aestivum* exhibited clear sensitivity to soil salinity, as reflected in reduced plant height, biomass accumulation, and leaf number in control conditions. The mean shoot height under saline control soil reached 9.05 cm for bread wheat and 9.7 cm for durum wheat. The incorporation of bentonite into the growth substrate significantly improved shoot elongation, particularly at the 5% inclusion rate, regardless of the bentonite type. Similarly, root length increased markedly at moderate bentonite doses, with *T. durum* consistently showing longer roots and shoots than *T. aestivum* across all treatments. However, excessive bentonite addition (10% Na-bentonite) produced the shortest organs in both species, indicating that high amendment levels may hinder growth under saline conditions (Fig. 2).

The number of leaves also varied significantly among treatments ($p < 0.005$) (Fig. 3). Under control conditions, durum wheat plants developed slightly more leaves than bread wheat. The application of 5% Na-bentonite (B-Na) resulted in a significant increase in leaf number for both species, reaching the highest values observed in the experiment. Conversely, 10% B-Na markedly reduced leaf production, particularly in *T. aestivum*. In contrast, 5% Ca-bentonite (B-Ca) had no significant effect on the number of leaves in *T. durum*, whereas in *T. aestivum* it produced a noticeable increase compared to the saline control. At 10% B-Ca, the mean numbers of leaves were 4.8 and 5.1 for *T. durum* and *T. aestivum*, respectively, still higher than those observed under 10% Na-bentonite.

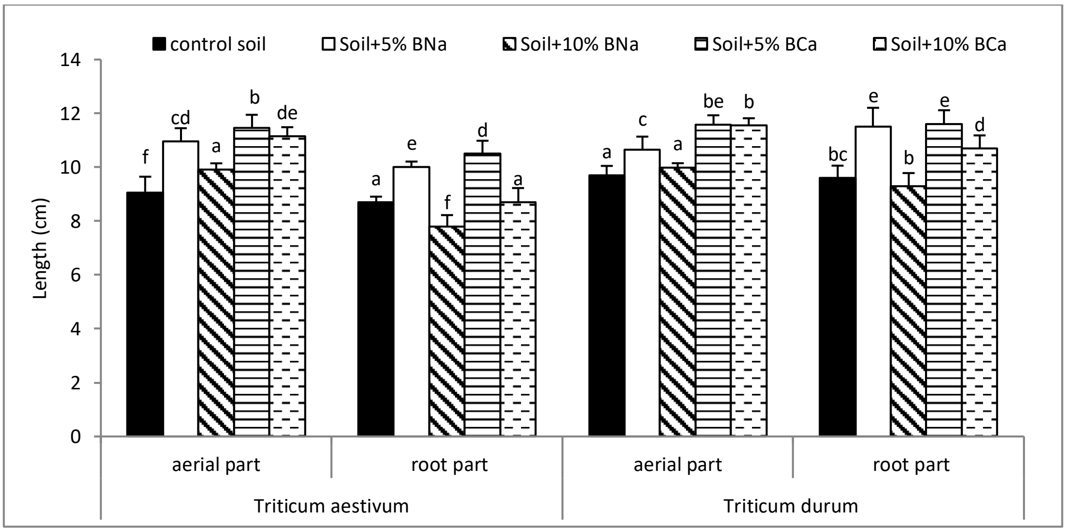

Figure 2. Effect of sodium and calcium bentonite amendments on shoot and root length of *Triticum durum* and *Triticum aestivum* grown in saline soil. Values represent means ± SE (n = 10). Means followed by the same letter are not significantly different at $p < 0.05$ according to Fisher's LSD test

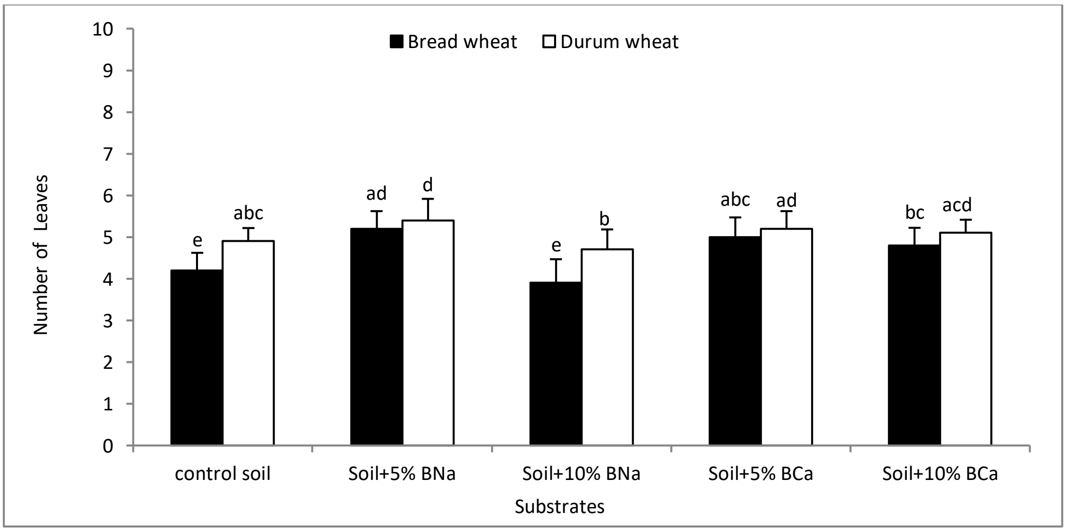

Figure 3. Effect of sodium (B-Na) and calcium (B-Ca) bentonite amendments on the number of leaves of *Triticum durum* and *Triticum aestivum* grown in saline soil. Values represent means ± SE (n = 10). Means followed by the same letter are not significantly different at $p < 0.05$ according to Fisher's LSD test

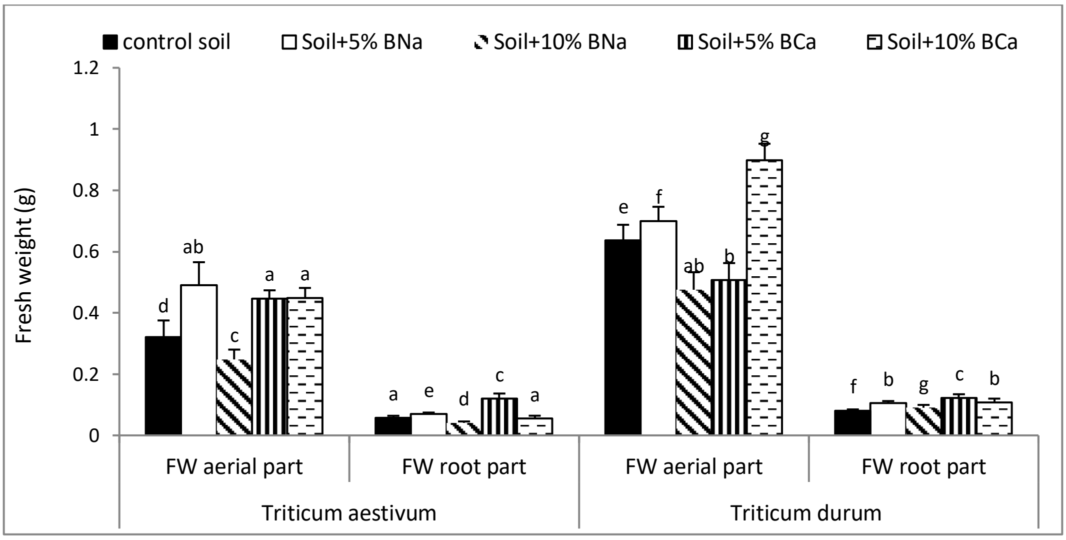

Figure 4. Effect of sodium (B-Na) and calcium (B-Ca) bentonite amendments on the fresh weight of *Triticum durum* and *Triticum aestivum* grown in saline soil. Values represent means ± SE (n = 10). Means followed by the same letter are not significantly different at $p < 0.05$ according to Fisher's LSD test

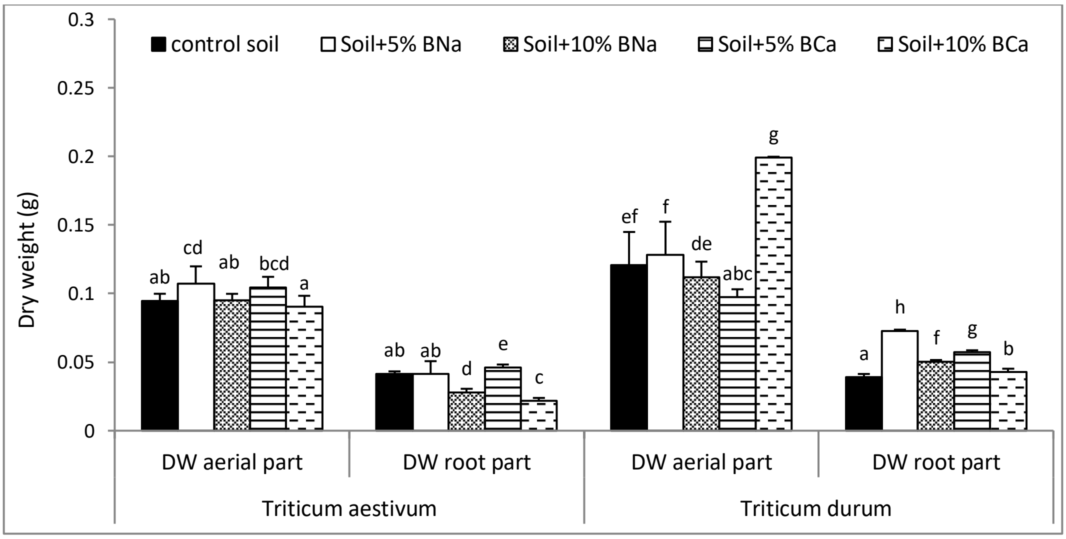

Figure 5. Effect of sodium (B-Na) and calcium (B-Ca) bentonite amendments on the dry weight of *Triticum durum* and *Triticum aestivum* grown in saline soil. Values represent means ± SE (n = 10). Means followed by the same letter are not significantly different at $p < 0.05$ according to Fisher's LSD test

Fresh biomass of the aerial part also responded positively to moderate bentonite application (Fig. 4). For *T. aestivum*, the 5% B-Na treatment significantly enhanced shoot fresh weight, whereas a 10% B-Na dose caused a pronounced decline ($p < 0.05$). In *T. durum*, the 10% B-Ca treatment led to the highest fresh biomass values. Root fresh weight responded most favorably to 5% B-Ca, reaching 0.11 g for bread wheat and 0.12 g for durum wheat. The lowest root biomass was recorded in T. *aestivum* wheat grown with 10% B-Na.

The trend for dry biomass followed a similar pattern (Fig. 5). The highest dry shoot biomass (0.199 g) was observed in *T. durum* treated with 10% B-Ca, differing highly significantly from all other treatments. In contrast, *T. aestivum* showed limited improvement in dry shoot weight under any treatment. For root dry weight, *T. durum* again exhibited a more favorable response, with 5% B-Na and 5% B-Ca yielding the highest values, representing approximately a 50% increase compared to the saline control. Bread wheat roots showed only a slight but statistically significant improvement under 5% B-Ca, while other treatments produced no notable effects.

3.2. Relative water content

Relative water content (RWC) reflected the physiological capacity of the plants to maintain hydration under saline conditions (Fig. 6). Under control soil, *T. durum* leaves exhibited a higher RWC (79.2%) than *T. aestivum* (76.7%). The incorporation of 5% bentonite improved leaf water status in both species. In 5% B-Na, RWC increased to 82.4% and 83.5% for *T. aestivum* and *T. durum*, respectively. Similarly, 5% B-Ca resulted in the highest values, with T. durum reaching 84.1%. In contrast, the 10% B-Na treatment drastically reduced RWC, particularly in *T. aestivum*, where water potential losses approached 60%. Even at a 10% application rate, Ca-bentonite maintained a higher RWC than Na-bentonite, showing statistically significant differences ($p < 0.05$).

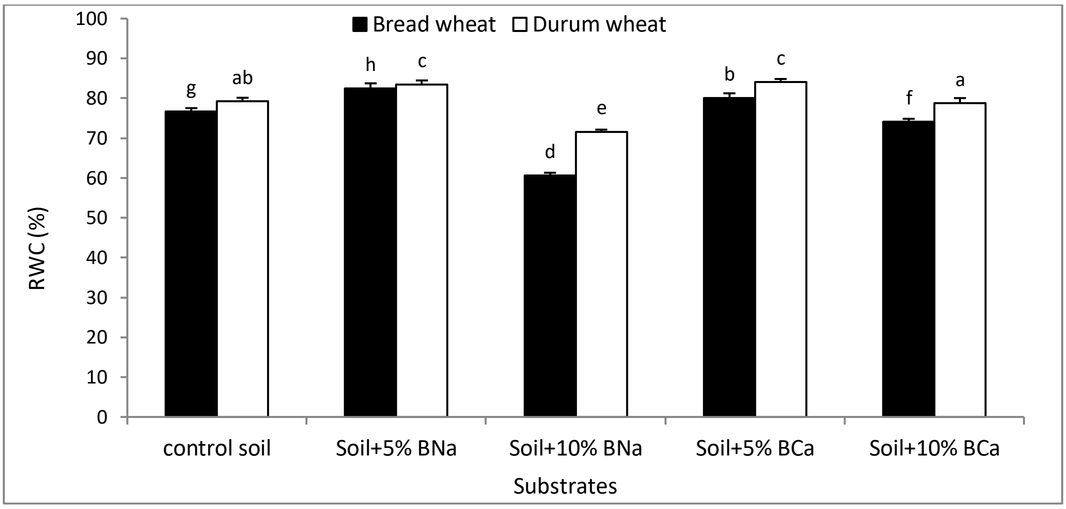

Figure 6. Effect of sodium (B-Na) and calcium (B-Ca) bentonite amendments on the relative water content (RWC) of *Triticum durum* and *Triticum aestivum* grown in saline soil. Values represent means ± SE (n = 10). Means followed by the same letter are not significantly different at $p < 0.05$ according to Fisher's LSD test

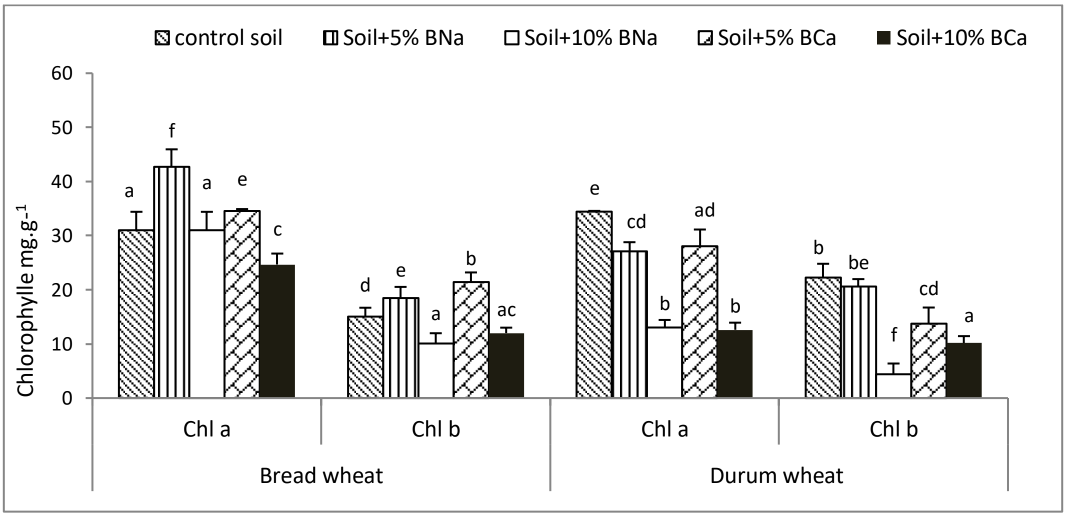

Figure 7. Effect of sodium (B-Na) and calcium (B-Ca) bentonite amendments on the chlorophyll (a + b) content of *Triticum durum* and *Triticum aestivum* grown in saline soil. Values represent means ± SE (n = 10). Means followed by the same letter are not significantly different at $p < 0.05$ according to Fisher's LSD test

3.3. Chlorophyll content

Chlorophyll content varied significantly under the combined influence of salinity and bentonite application (Fig. 7). For *T. aestivum*, the 5% bentonite treatments resulted in the highest pigment concentrations. Chlorophyll a reached 42.73 mg·g^{-1} in plants treated with 5% B-Na and 34.67 mg·g^{-1} with 5% B-Ca. A similar trend was observed for chlorophyll b, which increased to 21.41 mg·g^{-1} under 5% B-Ca and 18.51 mg·g^{-1} under 5% B-Na, compared to 15.03 mg·g^{-1} in the control. Increasing the bentonite concentration to 10% led to a substantial decline in pigment content, particularly with Ca-bentonite, where Chl a and Chl b dropped to 10.09 and 12.03 mg·g^{-1}, respectively. In *T. durum*, chlorophyll levels were consistently lower than in *T. aestivum*, and all bentonite treatments, regardless of type or dose, resulted in reductions relative to the control (Fig. 7).

3.4. Carotenoid content

Carotenoid levels followed a pattern similar to that of chlorophylls (Fig. 8). In *T. durum*, bentonite application led to an overall decline compared to the control (0.18 mg·g^{-1}). The highest carotenoid content (0.18 mg·g^{-1}) was found in *T. aestivum* grown in 5% B-Na, indicating that moderate doses may enhance pigment stability under salinity. However, all other treatments (5% B-Ca, 10% B-Ca, and 10% B-Na) significantly reduced carotenoid content in both species.

3.5. Soluble sugar content

The soluble sugar content was markedly influenced by the addition of bentonite (Fig. 9). In *T. aestivum*, the highest concentrations were recorded in the control and 5% B-Na treatments, reaching 234.1 and 233.8 µg·100 mg^{-1}, respectively. All other treatments significantly reduced sugar accumulation. A similar pattern was observed in *T. durum*, where calcium bentonite caused a notable decrease in soluble sugar content. The most pronounced reductions occurred under 10% B-Na, where sugar levels dropped to 80.46 µg·100 mg^{-1} in bread wheat and 114.59 µg·100 mg^{-1} in durum wheat, suggesting metabolic impairment under excessive bentonite amendment.

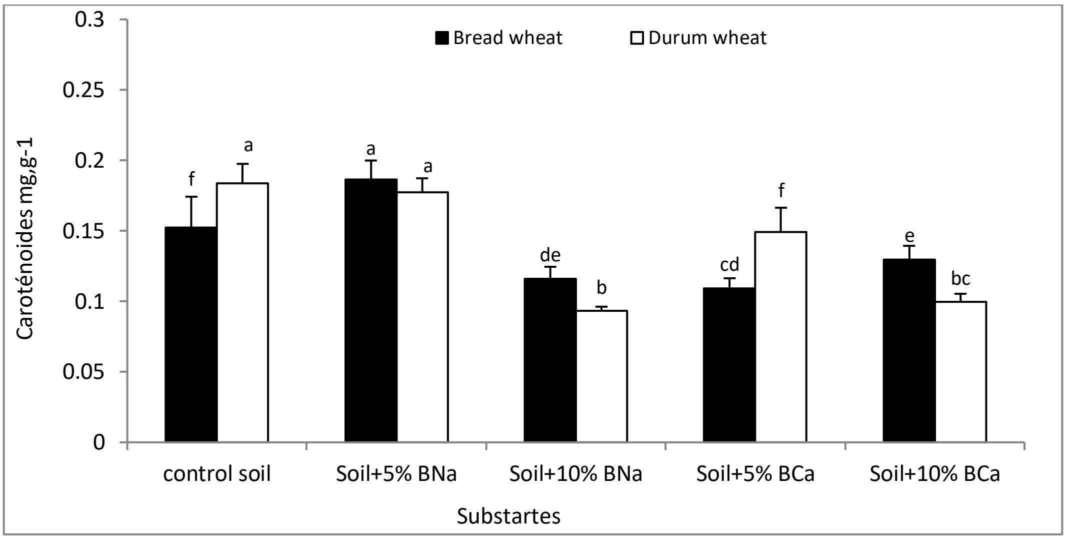

Figure 8. Effect of sodium (B-Na) and calcium (B-Ca) bentonite amendments on the carotenoid content of *Triticum durum* and *Triticum aestivum* grown in saline soil. Values represent means ± SE (n = 10). Means followed by the same letter are not significantly different at $p < 0.05$ according to Fisher's LSD test

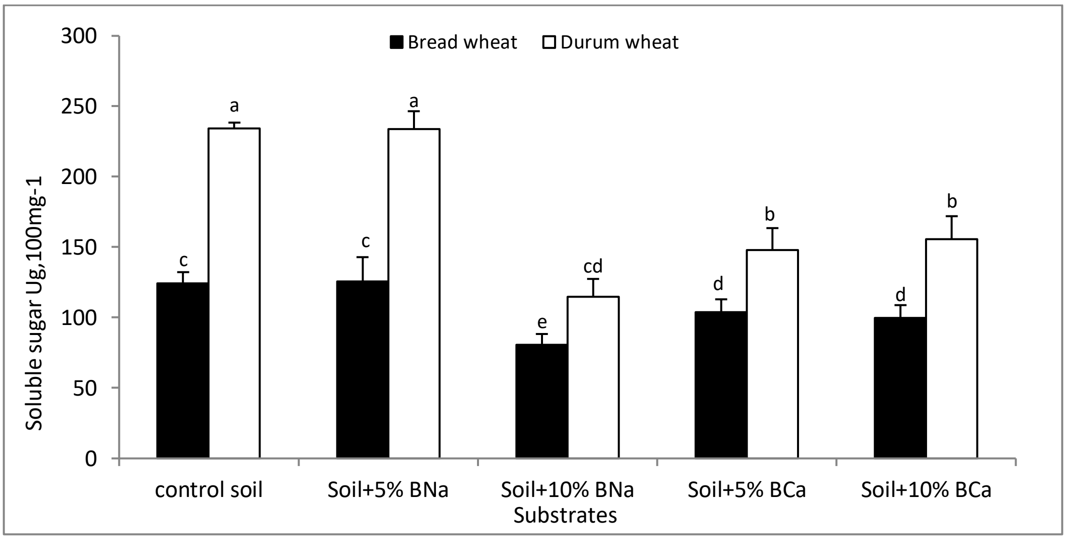

Figure 9. Effect of sodium (B-Na) and calcium (B-Ca) bentonite amendments on the soluble sugar content of *Triticum durum* and *Triticum aestivum* grown in saline soil. Values represent means ± SE (n = 10). Means followed by the same letter are not significantly different at $p < 0.05$ according to Fisher's LSD test

3.6. Correlation analysis

Pearson correlation coefficients among morphological, physiological, and biochemical parameters are presented in Table 2. Relative water content showed a strong positive correlation with root length (r = 0.788*), indicating that enhanced root development promotes water retention capacity under saline stress. Soluble sugar concentration also correlated positively with dry biomass (r = 0.806*), suggesting a potential osmoprotective role in growth maintenance. Conversely, chlorophyll a displayed a weak negative correlation with fresh biomass (r = –0.401 for shoots; r = –0.229 for roots), implying that pigment concentration alone may not directly reflect overall plant vigor under saline conditions.

4. DISCUSSION

Soil salinity is one of the most detrimental abiotic stresses affecting agricultural productivity, and cereal cultivation is no exception. Excess soluble salts interfere with nutrient uptake, osmotic balance, and photosynthetic activity, ultimately restricting plant growth and yield (Shalaby, 2025). In this context, the search for natural, ecological, and sustainable strategies to mitigate salinity damage has become a global priority. Among these, mineral soil amendments such as bentonite offer a promising and environmentally safe alternative for improving soil structure and plant performance under saline conditions.

In the present study, salinity caused a pronounced reduction in the morphological traits of both *Triticum durum* and *Triticum aestivum*. These findings align with numerous studies reporting that high salt concentrations severely constrain plant growth (Al-Huqail et al., 2024; Alhudhaibi et al., 2024; Shahzadi et al., 2024). The observed growth inhibition is likely associated with ionic imbalance, specifically reduced K^+ uptake and increased Na^+ accumulation (Saeedi et al., 2025; Souana et al., 2020), which disrupts cellular homeostasis and promotes the formation of reactive oxygen species (ROS), leading to oxidative stress and metabolic dysfunction (Vasilik et al., 2024). Despite these constraints, the incorporation of bentonite significantly improved plant growth. Both wheat species exhibited greater stem and root elongation compared with the saline control, especially at moderate application levels (5%). Calcium bentonite enhanced stem length, whereas 10% sodium bentonite reduced growth, suggesting that excessive amendment may alter soil aeration or ion balance. Root development responded positively to 5% bentonite, with *T. durum* consistently outperforming *T. aestivum*, confirming its higher innate tolerance to salinity (Table 1).

Table 1. General linear model (ANOVA) results for physiological, biochemical, and agronomic traits of *Triticum durum* and *Triticum aestivum* grown in saline soil under different bentonite treatments. Significant (p < 0.05). ns: non-significant

Response: Carotenoid					
	Sum Sq	Df	F value	Pr(>F)	
Species	0.000315	1	3.0511	0.09028	ns
Substrate	0.035564	3	114.7199	< 2.2e-16	***
Species:Substrate	0.00754	3	24.3208	2.17E-08	***
Response: chlorophyll a					
Species	1696.53	1 3	26.872	< 2.2e-16	***
Substrate	1756.69	3 1	12.821	< 2.2e-16	***
Species:Substrate	187.54	3	12.0440	0.00001933	***
Response: chlorophyll b					
Species	108.48	1 3	0.384 4.	4.75E-04	***
Substrate	980.86	3 9	1.574 8.	6.40E-14	***
Species:Substrate	143.33	3 1	3.381 7.	9.13E-04	***
Response: Length of the aerial part					
Species	0.361	1 1	0.6742	0.205	ns
Substrate	13.88	3 21	.4570 0.	00000008362	***
Species:Substrate	0.555	3 0	0.858	0.4728	ns
Response: Length of the root					
Species	23.716	1 13	6.6916 4	3.41E-14	***
Substrate	48.608	3 9	3.3871 6	5.28E-17	***
Species:Substrate	1.228	3	2.3593	0.09001	ns
Response: Number of leaves					
Species	2.025	1 9	.0000 0.	0051947	**
Substrate	4.875	3 7	.2222 0.	0007806	***
Species:Substrate	0.875	3 1	.2963 0.	2925875	ns
Response: Fresh weight of the aerial parts					
Species	0.54943	1 1	87.590 6	.232e-15	***
Substrate	0.60962	3	69.379 4	.270e-14	***
Species:Substrate	0.17422	3	19.828 1	.900e-07	***
Response: Fresh weight of the root					
Species	0.013727	0 1	95.4591	3.990e-11	***
Substrate	0.014681	5 3	34.0322	4.497e-10	***
Species:Substrate	0.003499	9 3	8.1128	0.0003696	***
Response: Dry weight of the aerial part					
Species	0.012355	2 1	48.273	7.20E-08	***
Substrate	0.012072	9 3	15.723	1.84E-06	***
Species:Substrate	0.019713	9 3	25.675	1.19E-08	***
Response: Dry root weight					
Species	0.005382	4 1	473.178	< 2.2e-16	***
Substrate	0.004079	4 3	119.543	< 2.2e-16	***
Species:Substrate	0.000615	8 3	18.045	0.0000004903	***
Response: relative water content					
Species	313.6	1 29	1.721 <	2.2e-16	***
Substrate	1866.4	3 57	8.729 <	2.2e-16	***
Species:Substrate	117.6	3 3	6.465 1	.94e-10	***
Response: soluble sugar					
Species	36606	1 21	8.536 7.	487e-16	***
Substrate	35310	3 7	0.266 3.	583e-14	***
Species:Substrate	8101	3 1	6.120 1.	460e-06	***

Table 2. Pearson correlation matrix for morphological and biochemical parameters of *Triticum durum* and *Triticum aestivum*

	LAP	LRP	NL	FWAP	FWRP	DWAP	DWRP	RWC	CHLa	CHLb	CAR	SS
LAP	1.000											
LRP	0.567	1.000										
NL	0.372	0.642	1.000									
FWAP	0.479	0.620	0.528	1.000								
FWRP	0.410	0.803	0.546	0.565	1.000							
DWAP	0.264	0.289	0.190	0.787	0.382	1.000						
DWRP	0.150	0.790	0.581	0.514	0.727	0.243	1.000					
RWC	0.614	0.878	0.664	0.631	0.693	0.212	0.685	1.000				
CHLa	0.007	0.039	-0.019	-0.401	-0.229	-0.496	-0.100	0.163	1.000			
CHLb	0.332	0.512	0.391	0.162	0.240	-0.102	0.350	0.574	0.676	1.000		
CAR	0.136	0.400	0.287	0.116	-0.040	-0.242	0.331	0.503	0.638	0.626	1.000	
SS	0.196	0.718	0.526	0.689	0.503	0.420	0.806	0.644	-0.151	0.358	0.464	1.000

Notes: LAP: Length of the aerial part; LRP: Length of the root part; FWAP: Fresh weight of the aerial parts; FWRP: Fresh weight of the root parts; NL: number of leaves; DWAP: Dry weight of the aerial part; DWRP: Dry root weight; RWC: Relative water content; CHLa: Chlorophyll a; CHLb: Chlorophyll b; CAR: Carotenoids; SS: Soluble sugars

The enhancement of shoot and root biomass in the presence of bentonite confirms the beneficial role of this mineral in mitigating salt stress. *T. durum* plants showed higher biomass accumulation under both Na- and Ca-bentonite treatments, indicating an improved capacity for water and nutrient utilization. Similar positive effects of bentonite on biomass production have been reported in other crops (Abdeen, 2020; Meena et al., 2023; Ntanos et al., 2021). However, as emphasized by Bandian et al. (2019), the dose of bentonite is critical, as moderate application levels often provide optimal benefits. Excessive amendment may increase soil density and reduce oxygen diffusion to roots, counteracting its positive effects (Mi et al., 2018; Shahad & Hamid, 2025). The improvement in plant growth can be attributed to the physicochemical properties of bentonite, its high surface area and cation exchange capacity enable retention of essential nutrients and water, thus stabilizing soil structure and reducing salinity-induced osmotic stress (El-Etr & Hassan, 2017; Garbowski et al., 2023). The superior performance of *T. durum* compared to *T. aestivum* may also reflect species-specific physiological adaptations and genetic potential of local varieties for salt tolerance (Bellatreche et al., 2019; Bellil et al., 2019; Turki et al., 2024).

Relative water content (RWC) serves as a key indicator of plant water status under saline conditions. The decline in RWC under high bentonite doses observed in this study is consistent with reports that excessive amendment can impair root water uptake due to reduced soil porosity. In contrast, the addition of 5% bentonite significantly improved RWC in both wheat species compared to the saline control, with *T. aestivum* responding better to Na-bentonite and *T. durum* showing similar improvements with both types. These findings agree with earlier reports indicating that moderate bentonite levels enhance soil moisture retention and reduce evaporation (Beig et al., 2015; Ntanos et al., 2021; Zulqurnain Haider et al., 2019). Bentonite rich in montmorillonite exhibits a high swelling index (Abulimiti et al., 2023; Idiart et al., 2020), which enhances water-holding capacity (Abdelfattah & Mostafa, 2024) and improves soil aggregation and hydraulic conductivity (Weijing et al., 2025). Several studies have demonstrated that 3–5% bentonite addition optimizes plant water balance, while higher levels can adversely affect RWC, particularly in sodium-based bentonites ((El Amine et al., 2020; Fernandes et al., 2023). Sodium bentonite, with its strong swelling potential, can retain excess moisture and limit oxygen availability, whereas calcium bentonite tends to stabilize soil structure without excessive compaction (Daniels et al., 2024; Tadesse, 2022).

Photosynthetic pigments are reliable indicators of plant physiological health under stress (Shalaby, 2024). The increase in chlorophyll a and b observed at 5% bentonite supports previous findings that moderate clay amendments can enhance pigment stability and photosynthetic performance (Beig et al., 2015; Younas et al., 2022). However, higher doses, especially of Ca-bentonite, caused pigment reductions, indicating a threshold beyond which the benefits of bentonite are offset by ionic or structural stress. The lack of improvement in *T. durum* chlorophyll levels may suggest species-specific differences in pigment regulation or antioxidant capacity under salt stress. Similarly, the moderate increase in carotenoids under 5% Na-bentonite suggests a role of bentonite in stabilizing the photosynthetic apparatus and scavenging ROS (El-Gabiery & Ata Allah, 2017; Shahkolaie et al., 2020). Conversely, excessive application reduced carotenoid levels, corroborating the observations of Mohammadifard et al. (2022) that high clay concentrations can interfere with chloroplast integrity.

Soluble sugar accumulation is another crucial adaptive mechanism under saline stress (Shalaby, 2025). In our study, both wheat species accumulated soluble sugars as osmoprotectants to counteract osmotic imbalance, which is consistent with the results (Shahzadi et al., 2024). The addition of 5% Na-bentonite maintained high sugar concentrations comparable to control plants, suggesting that moderate amendment levels did not disrupt carbohydrate metabolism. However, higher doses significantly reduced sugar content, indicating metabolic inhibition under excessive amendment. Similar findings were reported by Kenawy et al.

(2022), who demonstrated that bentonite at optimal doses enhances sugar synthesis by improving root nutrient absorption and water availability.

Overall, the results confirm the positive influence of bentonite on morphological, physiological, and biochemical attributes of wheat grown in saline soils. The high cation exchange capacity ensures greater nutrient availability, including essential elements such as K^+ (Weijing et al., 2025), Ca^{2+} (El-Gabiery & Ata Allah, 2017), Mg^{2+} (Rahmani et al., 2020), P (Souri & Sayadi, 2021), and N (Zhou et al., 2022). These nutrients play a central role in maintaining ionic balance and osmotic adjustment under saline stress (Horchani et al., 2025). Nevertheless, careful management of application rates is essential, as excessive quantities may induce ionic stress or physical soil compaction (Abulimiti et al., 2023). The findings are consistent with recent evidence highlighting bentonite as a promising tool for sustainable soil management in saline and arid regions (Zhang et al., 2024).

5. Limitations and perspectives

Despite the clear benefits demonstrated in this controlled experiment, several limitations should be acknowledged. The study was conducted under greenhouse conditions, which may not fully replicate field variability in temperature, rainfall, and soil heterogeneity. Only two bentonite doses and two wheat cultivars were tested; intermediate concentrations or other local varieties might reveal more nuanced responses. Additionally, no measurements of soil ion dynamics (Na^+, K^+, Ca^{2+} exchange) or microbial activity were performed, which could help clarify the mechanistic basis of bentonite-plant interactions. Future research should therefore include field-scale trials, multi-season assessments, and economic analyses of bentonite application, along with molecular and physiological investigations to elucidate the pathways through which bentonite modulates plant responses to salinity.

6. CONCLUSION

The incorporation of natural bentonites into saline soils can partially alleviate the negative effects of salinity on wheat growth. Both *Triticum durum* and *Triticum aestivum* responded positively to moderate bentonite application, with the 5% dose of either sodium or calcium bentonite significantly improving morphological parameters such as shoot and root development. Physiological and biochemical responses also reflected these trends. In contrast, excessive amendment (10%) reduced growth and pigment levels, emphasizing that dose optimization is critical to avoid secondary stress effects. These findings highlight the importance of selecting both the appropriate bentonite type and application rate according to soil characteristics and plant species sensitivity. The study provides one of the first comparative assessments of sodium versus calcium bentonite in two economically important wheat species under controlled saline conditions, contributing valuable insights into the sustainable management of degraded soils. Future field-based evaluations will be essential to validate these results and to determine the long-term agronomic and economic feasibility of bentonite amendments.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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