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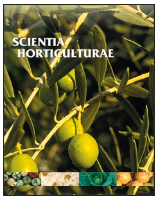

Research Paper

Silk sericin as a biostimulant for lettuce (*Lactuca sativa* L.): Effects of foliar spray and soil drenching under water stress

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ABSTRACT

Sericin solution (SER), derived from silkworm cocoons, is a mixture with promising properties that may enhance plant growth. To evaluate the potential use of this SER preparation as a biostimulant, a study was conducted on lettuce (*Lactuca sativa* L.) plants, assessing both leaf spray and soil drenching methods. The experimental design included the study of plant responses to SER treatment in well-watered and water stress (WS) conditions and compared them with those of two commercially available reference biostimulants (REF1, REF2). Although some beneficial effects were observed in well-watered plants, the most significant ones occurred under WS. In this condition, the fluorescence performance index (PI) increased in plants treated with SER via leaf spray. Additionally, both SER and the commercial references reduced leaf temperature, suggesting a positive impact on leaf transpiration. Moreover, shoot head diameter remained unaffected in plants treated with REF1 and SER via leaf spray. Although SER did not fully counteract the effects caused by WS, several biochemical parameters, such as the phenolic index, and the contents of total sugars, sucrose, proline, and malondialdehyde (MDA), revealed notable differences in metabolic responses, some of which were comparable to those induced by the commercial references. Interestingly, the mode of SER application appeared to elicit distinct physiological responses. Notably, leaf application of both SER and REF1 significantly reduced leaf nitrate content under non-stress condition.

This study supports the potential use of SER as a biostimulant. Being derived from textile industry waste, it aligns well with the principles of the circular economy.

1. Introduction

Given the rapid growth of the global population, agriculture faces an urgent need to adopt new strategies aimed at maximizing crop yield. In this context, the proposed solutions should also be evaluated considering their impact on both human health and the environment (Horrigan et al., 2002).

The main factors that hinder crop production, and are exacerbated by the ongoing climate change, include environmental stressors such as high temperatures, salinity, nutrient deficiencies, and limited water availability (Mariani and Ferrante, 2017; Bulgari et al., 2019; Chaudhry and Sidhu, 2022; Palmgren and Shabala 2024). Among these, water scarcity-induced stress is one of the most significant limitations for crops, especially in regions like the Mediterranean basin (Turner, 2004; del Pozo et al., 2019; Toscano et al., 2023; Palmgren and Shabala, 2024 and references therein). Common symptoms observed in plants subjected to water shortage include a reduction in leaf gas exchanges, with direct consequences on the photosynthetic performance, loss of cell turgor, and an increase in oxidative stress, all of which ultimately affect both the functionality and integrity of plant tissues (Chaves et al., 2009; Tardieu et al., 2018; Nour et al., 2024). Typical responses to these negative conditions are the accumulation of various osmolytes and specific molecules, such as proline, sugars, alcohols, and nitrate (Fang and Xiong, 2015; Singh et al., 2015; Ozturk et al., 2021; Nour et al.,

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2024). Moreover, the activation of the plant antioxidant system plays a crucial role in the response to drought stress (Laxa et al., 2019). In this context, hormones play a central role in mediating responses involved in maintaining adequate plant water content, as well as in reprogramming gene expression associated with plant adaptation to stress (Zheng et al., 2023; Liao et al., 2025).

A promising solution to improve plant resilience to environmental stresses is the use of biostimulants. These comprise a diverse group of biologically derived substances that, when applied to plants, can stimulate growth and development, enhance stress tolerance, and improve crop quality (EU 2019/1009, https://eur-lex.europa.eu/eli/reg/2019/1009/oj). The development of biostimulants relies heavily on waste streams originating from industrial, food, and agricultural sectors. Valuable biostimulants are derived from various waste sources, including extracts from food and plant waste, composts, manures, vermicompost, aquaculture waste streams, and sewage treatment processes (Calvo et al., 2014; Halpern et al., 2015; Du Jardin 2015; Van Oosten et al., 2017; Yakhin et al., 2017; Bulgari et al., 2019; Sharma et al., 2024).

Among the biostimulants that have received particular attention there are protein hydrolysates (PHs), a category of biostimulants consisting of mixtures of polypeptides, oligopeptides, and amino acids derived from partial protein hydrolysis (Colla et al., 2015; Monterisi et al., 2024; Peli et al., 2025). Sericin is a protein derived from silkworm cocoons, typically discarded in the wastewater produced during the degumming process used by the silk manufacturing industry (Zhang, 2002; Wu et al., 2007; Paladini et al., 2025). Given its properties, a wide range of applications for sericin in the cosmetic, medical, and food sectors has been proposed (Zhang, 2002; Kunz et al., 2016; Liu et al., 2022; Seo et al., 2023). Nevertheless, these different uses strongly depend on the extraction and purification procedures, which significantly affect the properties of the obtained sericin solution (SER) (Wu et al., 2007; Aramwit et al., 2010; Seo et al., 2023; Paladini et al., 2025). The industrial degumming process leads to a partial hydrolysis of the protein, generating numerous peptides with varying molecular weights and increasing the amount of free amino acids, such as serine, aspartic acid, glycine and glutamic acid (Aramwit et al., 2010; Seo et al., 2023 and references therein). These features could contribute to give valuable functional properties, considering that many PHs were found to have biostimulant activity in plants (Calvo et al., 2014; Colla et al., 2015; Bulgari et al., 2019). In support of the use of wastewater-derived SER recovered from the textile industry, Orlandi and co-workers highlighted that this preparation retains many of the biological properties of the native sericin (Orlandi et al., 2020).

The aim of the present study was to explore the potential use of SER as a biostimulant, also investigating its effects on plants subjected to water stress. In a glasshouse, two trials were conducted on lettuce (*Lactuca sativa* L.) plants, testing two different application methods: leaf spray and soil drenching. The experimental design also included two commercial references: reference 1 (REF1) and reference 2 (REF2), that were previously used for leaf spray and drench applications, respectively. The selection of these two references was based on literature and evidence obtained from preliminary experiments (Petrozza et al., 2014; Parađiković et al., 2019; Niu et al., 2022). Plant performance was evaluated through *in vivo* analyses, including chlorophyll fluorescence and leaf temperature measurements, as well as through the assessments of biomass, head diameter, and water content. Additionally, several biochemical parameters, such as the contents of chlorophyll, carotenoids, anthocyanins, and phenolic index, as well as the contents of total sugars, reducing sugars, sucrose, nitrate, thiobarbituric acid reactive substances (TBARS), and proline were analyzed.

Table 1

Effect of both water stress and the leaf application of Reference 1 or Sericin on mean **leaf temperature.** The parameter was measured at different times according to Fig. 1. REF1: Reference 1. Data were analyzed within each time point by two-way ANOVA, using Holm-Sidak's test as post-hoc ($** = P \leq 0.01$; $* = P \leq 0.05$). When the interaction Treatment (T) x Condition (C) was significant, data were subjected to one-way ANOVA. Data are expressed as mean ± standard error ($n = 6$). Different letters indicate significant differences.

Time	Condition	Treatment			Two-way ANOVA P value		
		Water	REF1	Sericin	T	C	T x C
T1	Control	18.8 ± 0.43	18.9 ± 0.40	18.6 ± 0.34	**ns**	**ns**	**ns**
	WS	19.23 ± 0.73	18.9 ± 0.46	19.2 ± 0.12			
T2	Control	16.8 ± 0.08 ^{b}	15.3 ± 0.07 ^{b}	15.9 ± 0.09 ^{b}	**	**	*
	WS	19.0 ± 0.65 ^{a}	15.6 ± 0.25 ^{b}	16.9 ± 0.16 ^{b}			
T3	Control	19.8 ± 0.16	19.3 ± 0.08	19.6 ± 0.06	**	**	**ns**
	WS	21.1 ± 0.33	20.0 ± 0.10	20.2 ± 0.08			
T4	Control	16.8 ± 0.08^{c}	17.8 ± 0.18^{bc}	17.1 ± 0.09^{b}	*	**	**
	WS	20.3 ± 0.35^{a}	18.5 ± 0.27^{b}	18.5 ± 0.16^{b}			

2. Material and methods

2.1. Sericin preparation, plant material, growth conditions

A sericin solution, recovered from textile industry wastewater, was provided by Artefil srl (Como, Italy). This solution was produced from a raw silk degumming process conducted at high temperature and by a subsequent ultrafiltration step to obtain a final sericin concentration of 5% (w/v). For long-term storage, the solution was stabilized by adding 0.3% (w/v) sodium benzoate and H_2SO_4 to adjust the pH to 3. Before use, the pH of the stabilized preparation was raised to 7 by adding KOH, and the preparation was diluted with water to a final sericin concentration of 0.25% (w/v), to obtain the sericin solution (SER) used for the experimental treatments.

Head lettuce (*Lactuca sativa* L., var. Capitata) seedlings, purchased from a local plant nursery (Fratelli Ingegnoli, Milan, Italy), were transplanted into pots filled with peat-based substrate and grown in a glasshouse equipped with a supplementary cooling and light system, adopting a photoperiod of 16-h light (PPFD of ~350 μmol of photons m^{-2} s^{-1}) and 8-h darkness. The daily average temperature, which depended on the experimental period, resulted to be 24.8 ± 0.6 °C and 26 ± 1.4 °C, in the first (trial 1, conducted from March to April) and in the second (trial 2, conducted from June to July) experiments, respectively. After 3 days from the transplant, each seedling was fertilized with a slow-release granular fertilizer NPK [NovaTec® Pro 14–7–17(+2+TE), Compo Expert, Cesano Maderno Italy] and grown for further two weeks. During this period, plants were watered regularly to maintain an optimal water availability (80% of the pot capacity). At the end of this growing period, only the plants having a shoot homogeneous in size were selected for further experiments.

2.2. Experimental design

The effects of leaf application (foliar spray) and of substrate drench application were evaluated in trial 1 and trial 2, respectively. To better evaluate the performance of SER, both trials also included the treatment with two different appropriate commercially available reference biostimulants. For both trials that will be described further, a completely randomized design (CRD) has been considered to be the most

Table 2

Effect of both water stress and the substrate drench application of Reference 2 or Sericin on mean leaf temperature. The parameter was measured at different times according to Fig. 1. REF2: Reference 2. Data were analyzed within each time point by two-way ANOVA, using Holm-Sidak's test as post-hoc (($** = P \leq 0.01$; $* = P \leq 0.05$). When the interaction Treatment (T) x Condition (C) was significant, data were subjected to one-way ANOVA. Data are expressed as mean ± standard error ($n = 5$). Different letters indicate significant differences.

Time	Condition	Treatment			Two-way ANOVA P value		
		Water	REF2	Sericin	T	C	T x C
T1	Control	26.1 ± 0.41	25.4 ± 0.16	25.8 ± 0.12	ns	ns	ns
	WS	26.3 ± 0.52	25.6 ± 0.11	26.1 ± 0.20			
T2	Control	24.1 ± 0.06^b	24.9 ± 0.05^b	24.3 ± 0.16^b	*	**	*
	WS	25.5 ± 0.34^a	25.3 ± 0.20^b	24.7 ± 0.15^b			
T3	Control	24.1 ± 0.06	24.9 ± 0.05	24.3 ± 0.16	ns	*	ns
	WS	25.5 ± 0.64	25.3 ± 0.120	24.7 ± 0.08			
T4	Control	25.4 ± 0.28	23.8 ± 0.15	24.2 ± 0.14	**	*	ns
	WS	26.6 ± 0.50	24.0 ± 0.06	24.4 ± 0.10			

appropriate design, as no systematic environmental gradient was expected within the experimental area that would justify blocking.

2.2.1. *Trial 1: leaf application*

A total of 36 plants grown as described in paragraph 2.1 were randomized to obtain three groups. At the beginning of the experiment (14 days after the transplant), plants were sprayed with the same volume of either water, a 0.25% SER solution, or a 0.3% REF1 (Table 1S). Six plants of each group were then watered regularly, maintaining a substrate pot capacity of 80% (Control), while the irrigation of the other 6 plants was stopped to induce water stress (WS). The Control and WS conditions were constantly monitored by tensiometers. A second leaf application of water, SER or REF1 was performed seven days after the first one (21 days after the transplant). Five days after the second treatment, shoots were harvested, weighed, frozen in liquid nitrogen and stored at - 80 °C.

2.2.2. *Trial 2: substrate drench application*

A total of 30 plants grown as described in paragraph 2.1 were randomized to obtain three groups. At the beginning of the experiment (14 days after the transplant), plants were treated with 100 mL of water, or with 100 mL of a 0.25% SER solution or with 100 mL of a 0.2% solution of the REF2 (Table 2S). Five plants of each group were then watered regularly, maintaining a substrate pot capacity of 80% (Control), while the irrigation of the other 5 plants was stopped to induce water stress (WS). The Control and WS conditions were constantly monitored by tensiometers. A second substrate application of water, SER or REF2 was performed seven days after the first application (21 days after the transplant). Two days after the second treatment, shoots were harvested, weighed, frozen and pulverized using a mortar and pestle in liquid nitrogen to produce a fine, homogeneous powder, which was then stored at –80 °C. Portions of each sample were subsequently used for further analyses.

2.3. *In vivo estimation of chlorophyll fluorescence and evaluation of leaf temperature*

Chlorophyll *a* fluorescence was measured using a hand-portable fluorimeter (Handy-PEA, Hansatech Instruments) randomly choosing a leaf for each plant of each condition as previously described by Ali and coworkers (2025). To evaluate the photosynthetic functionality, the maximum quantum efficiency of photosystem II (Fv/Fm) and leaf fluorescent performance index (PI) was considered (Kalaji et al., 2011).

The leaf temperature was estimated by an infrared camera (FLIR C2). The thermal images were taken approximately 120 cm from the plants between 11:00 a.m. and 2:00 pm., considering that during this period the stomatal conductance does not significantly change. The calibration of the instrument and the measurements was performed according to James and Sirault (2012).

2.4. *Determinations of lettuce head diameter, shoot biomass, and shoot water content*

All parameters were estimated at harvest. The head of each lettuce of every treatment and condition was measured manually with a ruler. Total biomass of the shoot was evaluated as fresh weight (FW). The water content, expressed as a percentage (%), was estimated by allowing samples to dry in an oven for 3 days at a temperature of 105 °C. After this period, the dry weight (DW) of the samples was measured, and the water content [WC (%)] was calculated as follows:

$$WC\ (\%) = 100 - \left(\frac{DW}{FW} \cdot 100\right)$$

2.5. *Chlorophyll, carotenoids, anthocyanins contents and phenolic index*

Thirty milligrams of the frozen powdered sample were added to 5 mL of methanol and incubated in the dark at 4 °C for 24 h. After this period, the extract was clarified by centrifugation at 8000 *g* for 10 min, and the absorbance at 665.2, 652.4 and 470 nm was measured. The levels of pigments were calculated using the Lichtenthaler's formula based on the absorbance readings at 665.2 and 652.4 nm for chlorophylls and at 470 nm for total carotenoids. The results were expressed as μg of pigments g^{-1} FW (Lichtenthaler, 1987).

Starting from 30 mg of the sample, total anthocyanins and phenolic index were determined as described by Ali and coworkers (2025).

2.6. *Total sugars, reducing sugars, sucrose and nitrate contents*

One gram of the frozen powdered sample was homogenized with 3 mL of distilled water using a mortar and pestle. The resulting homogenate was centrifuged for 15 min at 4000 *g* at room temperature, and the supernatant was collected for the determination of total sugars, reducing sugars, sucrose, and nitrate.

Total sugars were determined using the anthrone method according to Yemm and Willis (1954). Reducing sugars were quantified using the dinitro salicylic acid (DNS) method, according to Miller (1959). Sucrose was determined as previously described by Rorem and co-workers (1960). Nitrate was measured as described by Ali and coworkers (2025), according to Cataldo's method (Cataldo et al., 1975).

2.7. *Thiobarbituric acid reactive substances (TBARS) and proline contents*

One gram of the frozen powdered sample was homogenized with 5 mL of 0.1% (w/v) trichloroacetic acid (TCA) and centrifuged at 4500 *g* for 10 min at 4 °C. For the TBARS assay, that was conducted as previously described by Heath and Packer (1968), 1 mL of the supernatant was mixed with 4 mL of 20% (w/v) TCA, 25 μL of 0.5% (w/v) thiobarbituric acid, and incubated in a water bath at 95 °C for 30 min. After cooling the samples on ice, they were centrifuged at 4000 *g* for 10 min, and the optical density was determined at 532 and 600 nm. The absorbance at 600 nm was subtracted from the absorbance at 532 nm (to eliminate non-specific turbidity), and the concentration of TBARS was

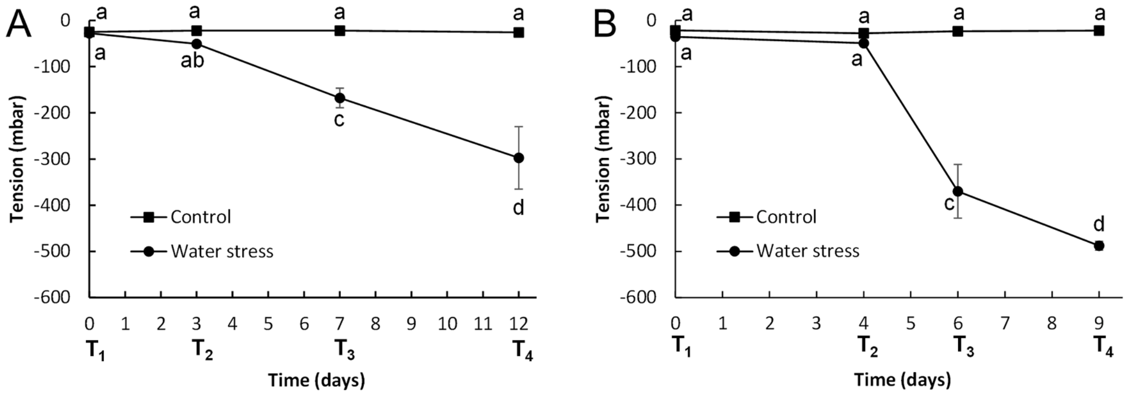

Fig. 1. Trend of the substrate tension. The figure shows the values measured in the two trials, in which sericin or commercial biostimulant were sprayed to the shoot (A) or drenched to the substrate (B), respectively. The stop of irrigation coincided with the first treatment with sericin (T1). Within each treatment, data were analyzed by two-way ANOVA, using Holm-Sidak's test as post-hoc ($P \leq 0.05$). Since the interaction Time x Condition was significant, data were subjected to one-way ANOVA. Data are expressed as mean ± standard error ($n = 6$ in A, $n = 5$ in B). Different letters indicate significant differences.

calculated using the Lambert-Beer law with an extinction coefficient $\varepsilon_{molar} = 155\ mM^{-1}\ cm^{-1}$. The results were expressed as malondialdehyde (MDA) equivalents (nmol g^{-1} FW).

Proline content was determined according to Bates et al. (1973). One gram of the frozen powdered sample was homogenized with 5 mL of 3% (w/v) sulfosalicylic acid, afterwards centrifuged at 4500 *g* for 10 min. One hundred microliters of 3% sulfosalicylic acid, 200 μL of glacial acetic acid, and 200 μL of ninhydrin solution (1.25 g of ninhydrin dissolved in 30 mL of acetic acid + 20 mL of 6 M orthophosphoric acid) were added to 100 μL of supernatant in a 2 mL tube to achieve the formation of the proline-ninhydrin complex. Samples were incubated at 96 °C for 1 h, then the reaction was stopped on ice. One ml of toluene was added to separate the organic phase from water: samples were briefly mixed and then let to rest up to 5 min. The absorbance of the upper phase at 520 nm was finally read. Proline concentration, expressed as μg of proline g^{-1} FW, was referred to a proline calibration curve.

2.8. Statistical analysis

The statistical analysis was performed using SigmaPlot 15.0 (Systat Software Inc., San Jose, *CA*, USA). For each trial, a two-way ANOVA was conducted, followed by a Holm-Sidak's test as post-hoc ($P < 0.05$), considering two factors, the treatment (T: water, commercial references or SER) and the condition (C: Control or WS), as well as their interaction (T x C). For the non-destructive analyses, the two-way ANOVA was carried out within each time sampling (twice a week, from the stop of irrigation to the harvest). When the interaction of the two factors was significant, data were analyzed by one-way ANOVA.

3. Results

3.1. Changes in substrate water availability

Water stress was induced by suspending the irrigation and the changes in the substrate water availability were monitored by tensiometers. Fig. 1 shows the trends of substrate tension measured in the two trials. In both the experiments, the interruption of irrigation induced a progressive decrease in the substrate water tension, which was significantly different compared to the Control on the 7th and 6th day after the last irrigation in trial 1 (Fig. 1A, leaf application) and in trial 2 (Fig. 1B, substrate drench application), respectively. The values measured at these time points are those typically observed under water stress (Eriksen et al., 2016). Also, a similar approach to water stress management has been previously described in detail (Araniti et al., 2024).

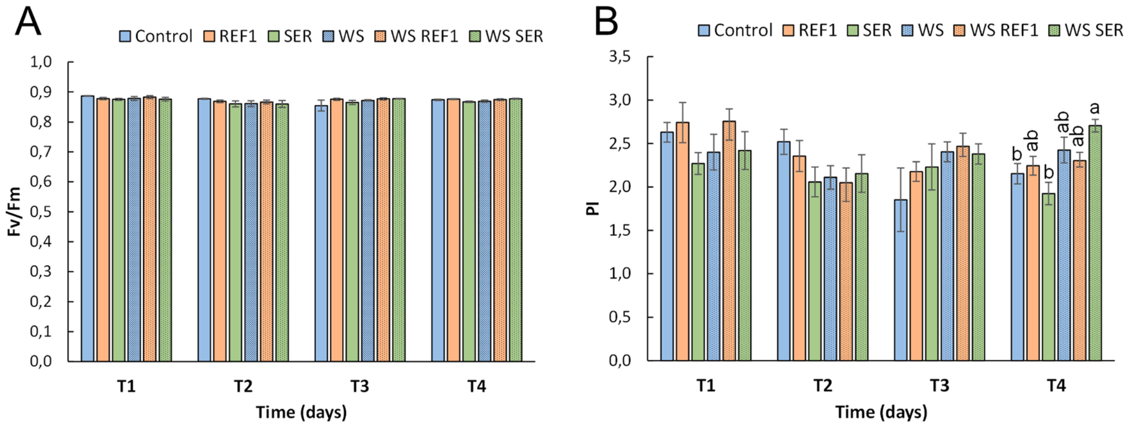

Fig. 2. Effects of different degrees of water stress and the leaf application of Reference 1 or Sericin on maximum quantum efficiency of photosystem II and the performance index. Maximum quantum efficiency of photosystem II (Fv/Fm, A) and leaf fluorescence performance index (PI, B) were measured at different times according to Fig. 1. Control: well-watered plants; REF1: treatment with reference 1; SER: treatment with sericin solution; WS: stressed plants. Data were analyzed within each time point by two-way ANOVA, using Holm-Sidak's test as post-hoc ($P \leq 0.05$). When the interaction Treatment x Condition was significant, data were subjected to one-way ANOVA. Data are expressed as mean ± standard error ($n = 6$). Different letters indicate significant differences.

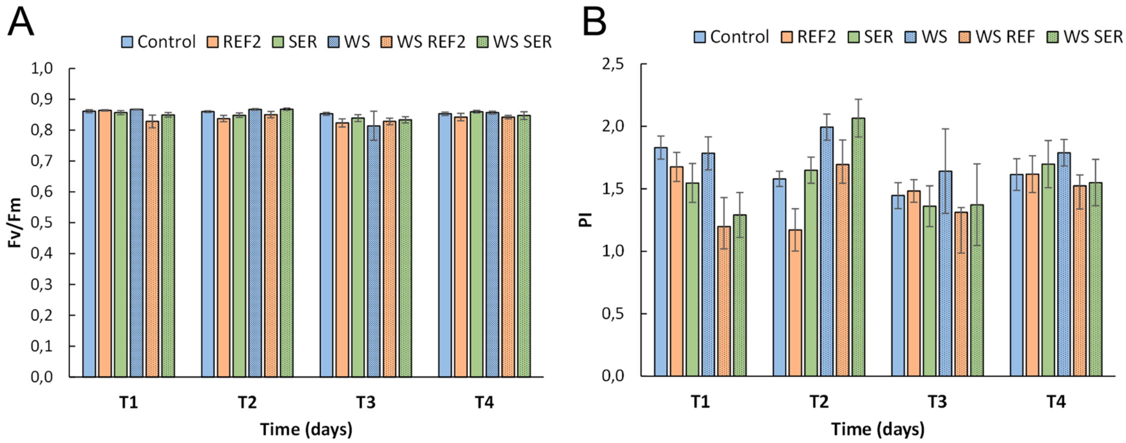

Fig. 3. Effects of both water stress and the leaf application of Reference 1 or Sericin on shoot biomass, shoot head diameter and water content. Shoot biomass (A), shoot head diameter (B), and water content (C) were measured on samples collected at the T4. REF1: treatment with reference 1; SER: treatment with sericin solution. Data were analyzed by two-way ANOVA, using Holm-Sidak's test as post-hoc ($P \leq 0.05$). When the interaction Treatment x Condition was significant, data were subjected to one-way ANOVA. Data are expressed as mean ± standard error ($n = 6$). Different letters indicate significant differences.

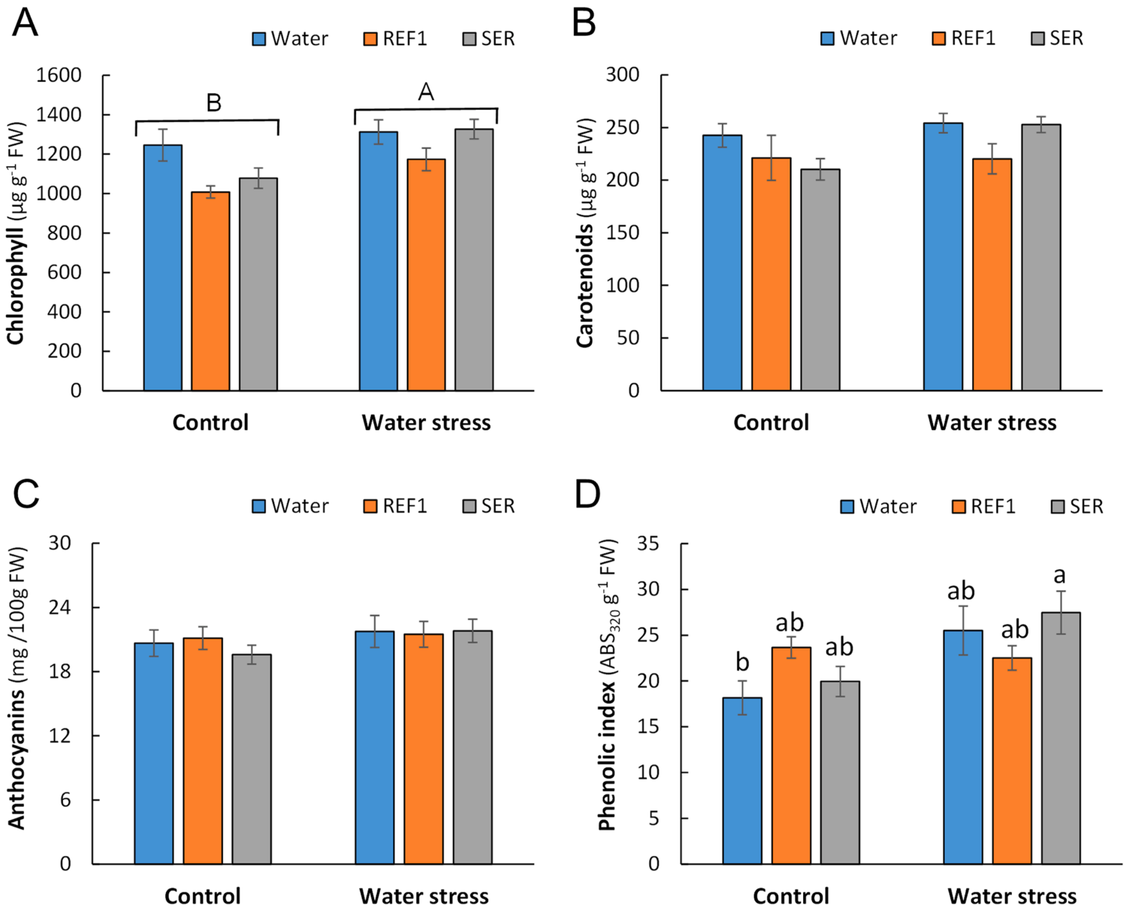

Fig. 4. Effects of both water stress and the leaf application of Reference 1 or Sericin on the levels of total chlorophyll, carotenoids, anthocyanins, and phenolic index. The contents of total chlorophyll (A), carotenoids (B), and anthocyanins (C) and the phenolic index (D), were measured on samples collected at the T4. REF1: treatment with reference 1; SER: treatment with sericin solution. Data were analyzed by two-way ANOVA, using Holm-Sidak's test as post-hoc ($P \leq 0.05$). When the interaction Treatment x Condition was significant, data were subjected to one-way ANOVA. Data are expressed as mean ± standard error ($n = 6$). Different letters indicate significant differences.

3.2. Trial 1: effects induced by leaf application

3.2.1. Photosynthetic functionality, mean leaf temperature, biomass, shoot head diameter and water content

Considering the possible influences of environmental conditions, even if the experiments were performed in glasshouse, the statistical analysis of the data was carried out within each date. The values of maximum quantum efficiency of photosystem II (Fv/Fm) were very similar in all the experimental conditions as well as at all the times considered (Fig. 2A). The measured values were *ca.* 0.88, highlighting a

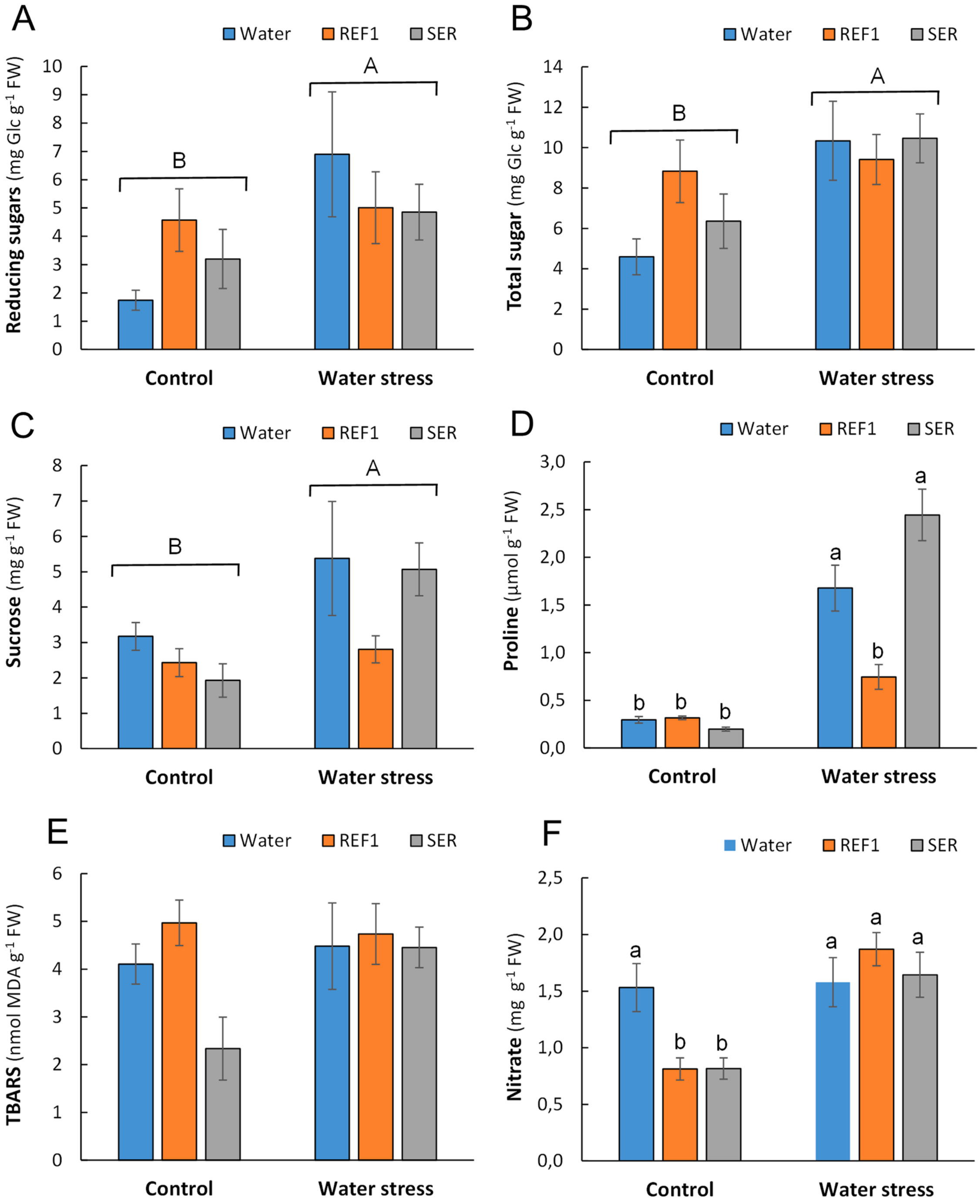

Fig. 5. Effects of both water stress and the leaf application of Reference 1 or Sericin on the levels of reducing sugars, total sugars, sucrose, proline, malondialdehyde and nitrate. Reducing sugars (A), total sugar (B), sucrose (C), proline (D), malondialdehyde (E) and nitrate (F) were measured on samples collected at the T4. Glc: glucose; TBARS: thiobarbituric acid reactive substances; MDA: malondialdehyde. Data were analyzed by two-way ANOVA, using Holm-Sidak's test as post-hoc ($P \leq 0.05$). When the interaction Treatment x Condition was significant, data were subjected to one-way ANOVA. Data are expressed as mean ± standard error ($n = 6$). Different letters indicate significant differences.

good status of photosystem II (Maxwell and Johnson, 2000).

Regarding leaf fluorescence performance index (PI), a sensitive indicator of plant stress status (Kalkaji et al., 2011; Ceusters et al., 2019), the two-way ANOVA revealed a significant interaction between Treatment (T) x Condition (C) at the 12th day (T4, Fig. 2B). At this experimental time point, the treatment with sericin (SER) induced a significant increase in PI in stressed plants, while no change was observed under the other experimental conditions.

Mean leaf temperature was measured to evaluate stomatal conductance (James and Sirault, 2012; Iseki and Olaleye, 2020). At all the time points, the mean leaf temperature of plants treated only with water increased under WS, consistent with a reduction in stomatal conductance (Table 1). Conversely, plants treated with reference 1 (REF1) or SER exhibited leaf temperature values comparable to those of the unstressed plants. The positive effect became evident as early as T2, with the interaction between time and treatment (T × C) reaching statistical significance at both T2 and T4.

Water stress induced an evident decrease in the shoot biomass of lettuce, that was of similar extent in all the treatments (Fig. 3A). A similar trend was also observed in the water content, with an average

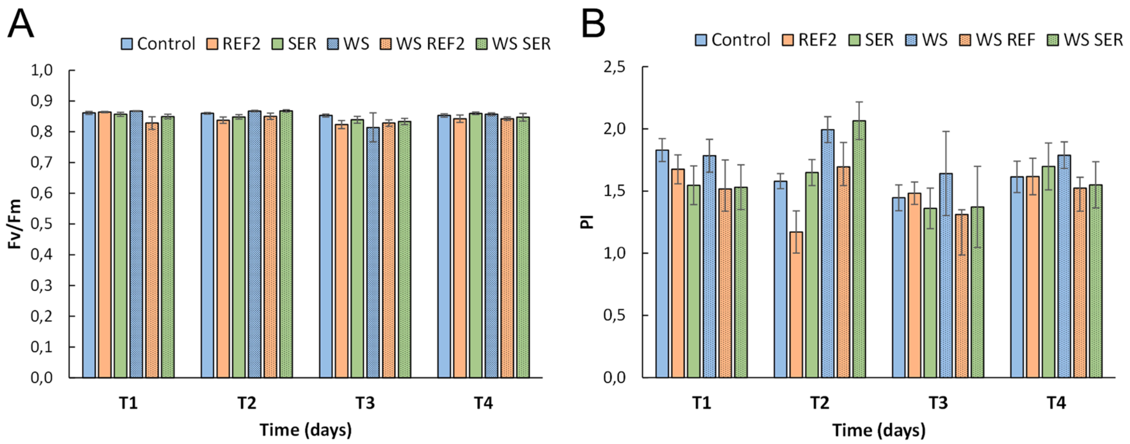

Fig. 6. Effects of different degrees of water stress and the substrate drench application of Reference 2 or Sericin on maximum quantum efficiency of photosystem II and performance index. Maximum quantum efficiency of photosystem II (Fv/Fm, A) and leaf fluorescence performance index (PI, B) were measured at different times according to Fig. 1. Control: well-watered plants; REF2: treatment with reference 2; SER: treatment with sericin solution; WS: stressed plants. Data were analyzed within each time point by two-way ANOVA, using Holm-Sidak's test as post-hoc ($P \leq 0.05$). Data are expressed as mean ± standard error ($n = 5$).

reduction of 1.8% (Fig. 3C).

In contrast, the reduction in shoot head diameter induced by WS was reversed by both REF1 and SER treatments. However, under the control condition, the shoot head diameter values of the REF1 and SER-treated plants were lower than those observed in plants treated with water (Fig. 3B).

3.2.2. *Total chlorophyll, carotenoids, phenolic index, and anthocyanins*

Overall, water stress induced a slight increase in chlorophyll content across all the treatments (Fig. 4A), while carotenoid and anthocyanin contents were not affected (Figs. 4B and 4 C).

The phenolic index, used to assess the content of total phenols, revealed that under WS, SER treatment led to significantly higher values compared to the untreated control (Fig. 4D).

3.2.3. *Reducing sugars, total sugars, sucrose, nitrate, MDA and proline*

The contents of reducing sugars, total sugars, and sucrose increased under WS (Figs. 5A, 5 B, and 5 C), independently of the treatments. Interestingly, the treatment with both REF1 and SER led to a consistent decrease in nitrate content in the unstressed plants. However, under WS, the levels of this anion were similar to those observed in the untreated control plants (Fig. 5F).

The Two-way ANOVA did not reveal significant changes in MDA levels due to either treatment or condition (Fig. 5E).

Finally, under WS proline content increased in both water- and SER-treated plants, whereas no significant change was observed in REF1-treated plants (Fig. 5D).

3.3. *Trial 2: effects induced by soil application*

3.3.1. *Photosynthetic functionality, mean leaf temperature, biomass, shoot head diameter and water content*

No significant differences were observed in either the maximum quantum efficiency of photosystem II (Fv/Fm) or the leaf fluorescence performance index (PI) across all the experimental conditions and time points (Fig. 6). Generally, mean leaf temperature increased under WS conditions while the treatment with REF2 or SER reduced this parameter (Table 2). The treatment affected this parameter at time points T2, and T4, while the condition induced significant differences at T2, T3, and T4. At T2, where the interaction between treatment and condition (T × C) was significant, both REF2 and SER treatments significantly reduced the increase in mean leaf temperature induced by WS. Shoot biomass, shoot head diameter, and water content were all affected by WS, while no changes were induced by both SER and REF2 treatments (Fig. 7).

3.3.2. *Total chlorophyll, carotenoids, phenolic index, and anthocyanins*

Overall, WS induced an increase in both chlorophyll and carotenoid contents across all the treatments (Figs. 8A and 8 B). Under control condition, REF2 treatment led to a marked increase in phenolic index (Fig. 8C). Under WS, this parameter remained comparable to the control in the REF2-treated plants, while it significantly increased in both untreated and SER-treated plants, reaching similar levels.

Anthocyanin content was significantly higher in plants treated with REF2 compared to those subjected to the other treatments (Fig. 8D). However, under WS none of the treatments resulted in significant changes in this parameter.

3.3.3. *Reducing sugars, total sugars, sucrose, proline, MDA and nitrate*

Overall, reducing sugars, total sugars, and sucrose, proline, MDA, and nitrate levels increased under WS, while the various treatments did not induce any further changes (Fig. 9A, 9 B, 9 C, 9 D, 9 E, and 9 F).

4. Discussion

The use of biomolecules that support crop productivity, especially under stress conditions, represents a promising strategy in modern agriculture (Calvo et al., 2014; Colla et al., 2015, 2017; Bulgari et al., 2019; Loconsole et al., 2023). In this context, waste products appear to be of interest for such purposes, especially considering that this approach is also crucial for reducing the environmental impact closely associated with industrial and agricultural activities, as promoted by the circular economy model (Xu and Geelen, 2018; Corsi et al., 2022; Afsah-Hejri etal., 2025).

Numerous studies have highlighted the interesting properties of the protein named sericin, discarded by the textile industry, suggesting various applications in the cosmetic and medical fields, as well as in the food industry (Orlandi et al., 2020; Seo et al., 2023 and references therein). In this context, it must also be considered that the degumming process used to remove sericin from fibroin influences the final chemical characteristics of this protein (Wu et al., 2007; Orlandi et al., 2020; Seo et al., 2023; Paladini et al., 2025). This procedure, in fact, causes partial hydrolysis of the protein, leading to the release of both peptides and free amino acids. The electrophoretic separation of such samples results in broad bands, consistent with a very wide molecular-weight distribution (Wu et al., 2007; Aramwit et al., 2010; Orlandi et al., 2020). Amino acid analysis of the sericin solution derived from degumming process

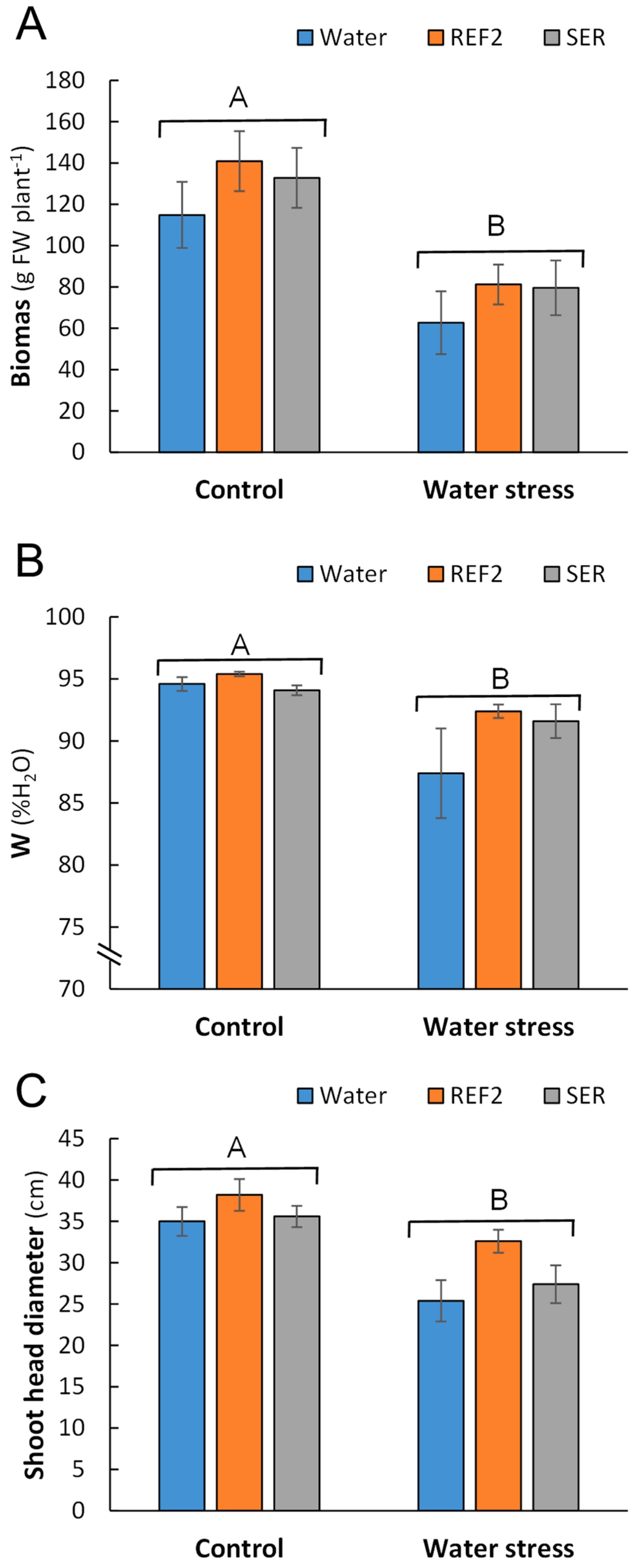

Fig. 7. Effects of both water stress and the substrate drench application of Reference 2 or Sericin on shoot biomass, shoot head diameter and water content. Shoot biomass (A), shoot head diameter (B), and water content (C) were measured on samples collected at the T4. Data were analyzed by two-way ANOVA, using Holm-Sidak's test as post-hoc ($P \leq 0.05$). Data are expressed as means ± standard error (n = 5). Different letters indicate significant differences.

revealed that the most abundant free amino acids are aspartic acid, glycine, and serine (Vaithanomsat and Kitpreechavanich, 2008; Bungthong et al., 2021; Seo et al., 2023). These findings highlight that the sericin solution produced by the textile industry is characterized by components typically found in protein-hydrolysate biostimulants (Colla et al., 2015; du Jardin, 2015, 2017; Bulgari et al., 2019). To investigate on the potential use of sericin in agriculture, the present study, performed on lettuce, evaluated the effects of a sericin solution (SER), currently largely discarded as wastewater. The experimental design included two different SER application methods (*i.e.*, leaf spray and soil drench), a parallel evaluation of two commercially available reference biostimulants (*i.e.*, REF1 and REF2, Tables 1S and 2S), and the assessment of plant responses to water stress (WS).

Although some interesting effects were also observed in well-watered plants, most changes occurred under WS. The decrease in soil water availability, as well as the substrate tension values reached at the later experimental stages, were of the same order of magnitude in both experiments, characterized by leaf spray (Trial 1) and soil drench (Trial 2), respectively (Fig. 1). This similar trend suggested a comparable stress intensity, thereby allowing for a confident attribution of the differences to the application method used.

In both trials, the photosynthetic functionality, evaluated by Fv/Fm ratio and fluorescent performance index (PI), did not reveal negative effects induced by WS (Figs. 2 and 6). Interestingly, at the last experimental stage (T4), PI significantly increased in WS-stressed plants treated with SER via the leaf spray method (Fig. 2B), suggesting that SER may enhance the photosystem's ability to cope with WS (Živčák et al., 2008; Kalaji et al., 2011; Ceusters et al., 2019). This effect did not occur when SER was applied to the substrate, nor was it influenced by treatment with REF1 or REF2.

As expected, the mean leaf temperature decreased under WS (Tables 1 and 2), consistent with a reduction in stomatal conductance aimed at maintaining adequate water content (Leinonen et al., 2006; Buckley, 2019; Asargew et al., 2024). The treatment with REF1, REF2, or SER significantly reduced leaf temperature, confirming that both the commercial references positively influence plant adaptation to WS and demonstrating a comparable effectiveness of SER (Petrozza et al., 2014).

Biomass, shoot head diameter, and water content (WC) of lettuce plants were affected by WS in both trials (Fig. 3 and 7). No counteracting effects on biomass and WC were observed in plants treated with REF1, REF2, or SER (Fig. 3A, 3 C, 7 A, and 7C). In contrast, shoot head diameter was not affected in WS plants treated with REF1 or SER via leaf spray (Fig. 3B).

Taken together, these results highlight that neither the commercial references nor SER were able to reverse the growth reduction in lettuce plants caused by WS. However, they were effective in counteracting some typical WS symptoms, such as the reduction in stomatal conductance and shoot head diameter. In this regard, SER demonstrated an action comparable to that of REF1 and other biostimulants (Parađiković et al., 2019; Bulgari et al., 2019).

Chlorophyll content increased under WS in all the treatments evaluated (Figs. 4A and 8 A). A similar effect was observed for carotenoid levels in substrate-drenched plants, while the levels of these metabolites in leaf-sprayed plants were not affected by either water availability or treatments (Figs. 4B and 8 B). According to these results, Shin et al. (2021) reported that lettuce seedlings subjected to drought initially show an increase in chlorophyll content, followed by a decline at later stages, while Paim and co-workers (2020) reported that carotenoid levels increase in lettuce plants grown under mild drought stress.

Using leaf spray application, only the treatment with SER in WS plants induced a significant increase in phenolic index (Fig. 4C), highlighting SER interesting potential to enhance the content of these functional compounds (Shi et al., 2022). Differently, with substrate drench application, REF2 induced an increase in this parameter in control condition, while both water and SER treatments led to a similar significant increase in WS plants (Fig. 8C). These results, in addition to

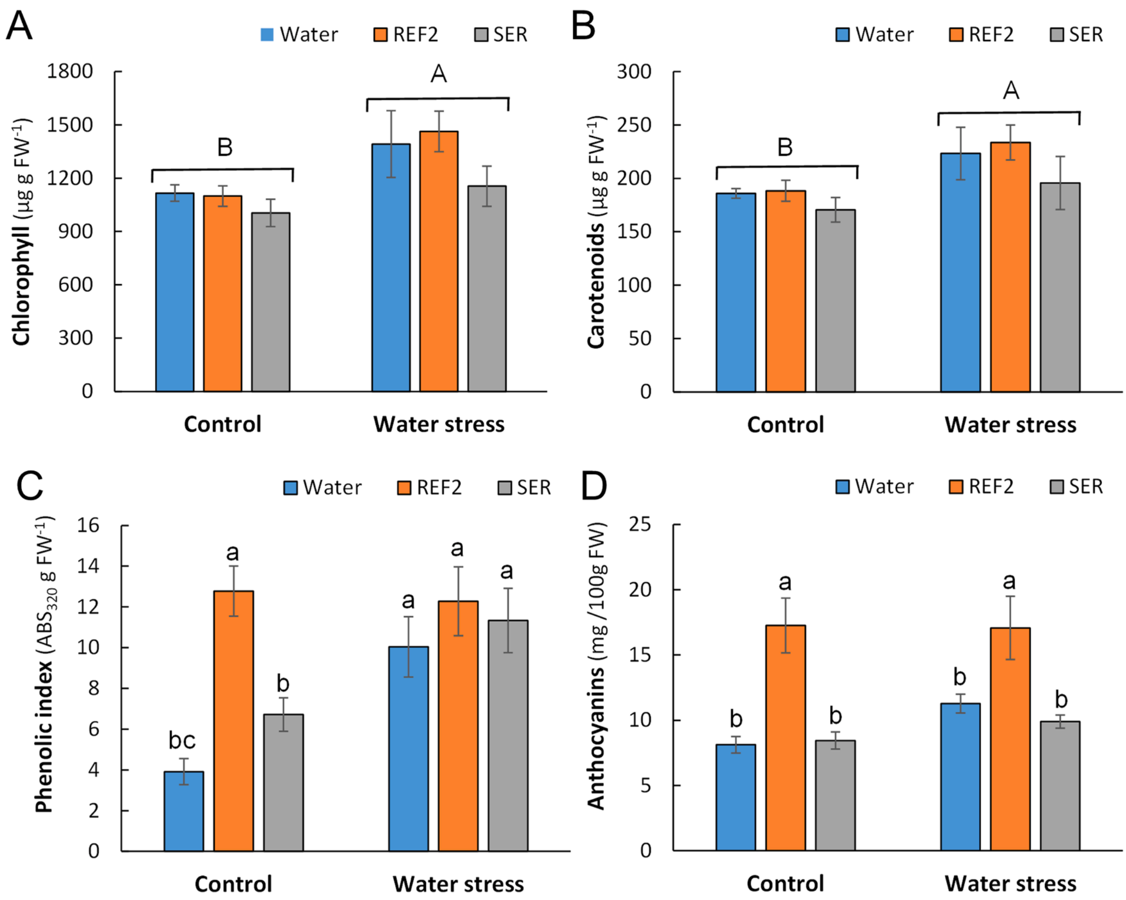

Fig. 8. Effects of both water stress and the substrate drench application of Reference 2 or Sericin on the levels of total chlorophyll, carotenoids, phenolic index, and anthocyanins. The contents of total chlorophyll (A) carotenoids (B), phenolic index (C), and anthocyanins (D) were measured on samples collected at the T4. REF2: treatment with reference 2; SER: treatment with sericin solution. Data were analyzed by two-way ANOVA, using Holm-Sidak's test as post-hoc ($P \leq 0.05$). When the interaction Treatment x Condition was significant, data were subjected to one-way ANOVA. Data are expressed as mean ± standard error ($n = 5$). Different letters indicate significant differences.

highlight an interesting property of REF2, suggest that the accumulation of phenolic compounds helps plants to cope with WS. (Oh et al., 2009; Santander et al., 2022). Excluding the effect of REF2 in substrate drenched plants, no changes in anthocyanin contents were observed (Figs. 4D and 8 D). Further studies aimed at identifying which classes of phenolic compounds are positively influenced by SER treatment could help clarify the specific traits involved in the induction of their metabolism evoked by this textile industry waste.

The increase in the contents of osmolytes, such as sugars and amino acids, as well as the activation of plant antioxidant systems, are typical responses to stress conditions (Fang and Xiong, 2015; Singh et al., 2015; Laxa et al., 2019; Ozturk et al., 2021; Nour et al., 2024). In the present study, WS induced an increase in reducing sugars, total sugars, and sucrose (Figs. 5A, 5 B, 5 C, 9 A, 9 B, and 9 C). Excluding the plants treated with REF1 via leaf spray, WS also induced an increase in proline content (Figs. 5D and 9 D). Moreover, in drenched plants occurred an increase in MDA under WS (Fig. 9E). These metabolic responses also suggest a beneficial effect of REF1, REF2, and SER in alleviating stress conditions. Hence, it is interesting to note that further studies would be useful to better define the specific modes of action of these formulations, which evidently also depend on the method of application. In this regard, investigating the relationship between proline content and the activation state of the other antioxidant systems may help clarify the mechanisms through which the different treatments influence plant responses to WS (de Campos et al., 2011; Liang et al., 2013; Kaur and Asthir, 2015; Laxa et al., 2019; Nour et al., 2024). In this view, the change in MDA content also appeared to depend on the type of application, considering that significant differences were observed only in drenched plants. MDA is not only a final product of lipid peroxidation and therefore an indicator of membrane damage, but its increase can also activate regulatory genes involved in plant defense and acclimation (Morales and Munné-Bosch, 2019). Further studies are required to investigate the intriguing hypothesis that SER may affect MDA metabolism, thereby positively modulating plant stress responses.

According to a previous study conducted by Franzoni and co-workers (2021), WS induced an increase in leaf nitrate content (Figs. 5F and 9 F), which contributes to cell osmotic adjustment (Burns et al., 2010). Interestingly, foliar application of REF1 or SER significantly reduced nitrate content in the control condition (Fig. 3A). According to European regulations, the accumulation of this anion represents a commercial issue in leafy vegetables when its concentration exceeds 4000 mg kg^{-1} fresh weight. In other words, SER shows a very promising properties for improving the quality and safety of leafy vegetables by reducing nitrate content without negatively affecting leaf biomass.

5. Conclusion

This study highlights some interesting properties of SER that may improve the resilience of lettuce under WS, in some cases comparable to those observed for the two reference biostimulants. The effects of SER, which appeared to depend on the method of application, were found to positively influence specific traits such as leaf transpiration and fluorescence and performance index, shoot head diameter, and phenolic index under WS. Moreover, the ability of SER to reduce nitrate content

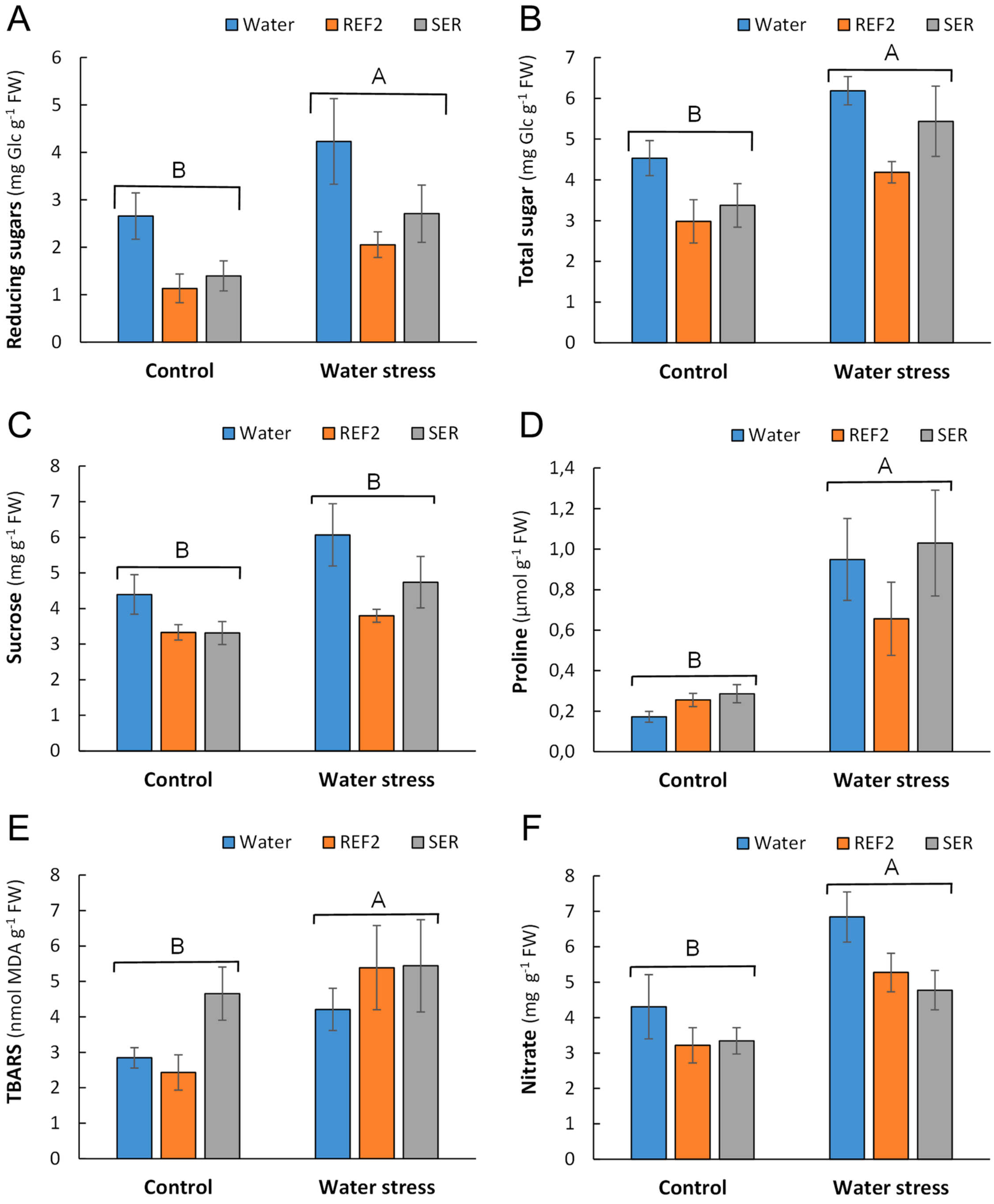

Fig. 9. Effects of both water stress and the substrate drench application of Reference 2 or Sericin on the levels of reducing sugars, total sugars, sucrose, proline, malondialdehyde and nitrate. Reducing sugars (A), total sugars (B), sucrose (C), proline (D), malondialdehyde (E) and nitrate (F) were measured on samples collected at the T4. Glc: glucose; TBARS: thiobarbituric acid reactive substances; MDA: malondialdehyde. Data were analyzed by two-way ANOVA, using Holm-Sidak's test as post-hoc ($P \leq 0.05$). Data are expressed as mean ± standard error ($n = 5$). Different letters indicate significant differences.

in well-watered plants broadens its potential use. In conclusion, although further research is needed to validate the properties of SER, the present study supports its potential use as a biostimulant. Being derived from textile industry waste, it aligns well with the principles of the circular economy.

Founding

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CRediT authorship contribution statement

Viviana Cavallaro: Writing – review & editing, Writing – original draft, Investigation, Data curation, Conceptualization. **Alice Petrini:** Investigation. **Carla Colombani:** Investigation. **Bhakti Prinsi:** Writing – review & editing, Funding acquisition, Conceptualization. **Chiara Muratore:** Writing – review & editing, Investigation. **Antonio Ferrante:** Writing – review & editing. **Luca Espen:** Writing – review &

editing, Formal analysis, Conceptualization. **Giacomo Cocetta:** Writing – review & editing, Supervision, Conceptualization.

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.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.scienta.2026.114844.

Data availability

Data will be made available on request.

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