Italian Journal of Agronomy 20 (2025) 100027

Contents lists available at ScienceDirect

Italian Journal of Agronomy

journal homepage: www.sciencedirect.com/journal/italian-journal-of-agronomy

Full Length Article

Combining nitrogen fertilization and biostimulant application in durum wheat: Effects on morphophysiological traits, grain production, and quality

Angelo Rossini, Roberto Ruggeri*, Francesco Rossini

Department of Agriculture and Forest Sciences, University of Tuscia, Via S. Camillo de Lellis, Viterbo 01100, Italy

ARTICLE INFO

Keywords:
Glycine betaine
PGPB
Seaweed extracts
Triticum turgidum L. subsp. *durum*
Vaterite

ABSTRACT

New crop nutrition strategies are needed to accomplish the goal of "producing more while consuming less". A field experiment was conducted under rainfed conditions in Viterbo (central Italy), during two growing seasons (2022–23 and 2023–24), to explore the effect of different combinations of biostimulants and N fertilization on the agronomic performances of durum wheat. A split-plot design with three replicates was used. Nitrogen fertilization level was tested in the whole plots, while biostimulant application in the subplots. Durum wheat cv. 'Iride' was used, and three topdressing N fertilizer rates were tested: 50, 100 and 150 kg ha^{-1}. In sub-plots, three experimental products containing different biostimulants (e.g., seaweed extracts, glycine betaine, micronized vaterite and plant growth-promoting bacteria) were compared. For each plot, the following traits were recorded at different growth stages: length and dry weight of roots, chlorophyll content, total yield, yield components, grain protein content and test weight. Wheat plants treated with biostimulant products outperformed the control plants for both root development (more than doubled in length and dry weight) and chlorophyll content (ranging from +75 % to +82 %). Regarding grain yield, the application of biostimulants was most effective at low and medium nitrogen doses. Specifically, a mixture of seaweed extracts and microorganisms at medium N dose (100 kg ha^{-1}) allowed wheat to reach a grain yield similar to that of the control treatment at the standard N dose (150 kg ha^{-1}), thus saving 33 % N. In terms of grain quality, the foliar treatments with biostimulants increased grain protein content by 4 %, and increased the test weight by 1 to 2 %, as compared to the control.

1. Introduction

Due to the relevant increase in the global fertilizer prices and awareness of the environmental impact of their excessive use, the application of synthetic fertilizers, especially nitrogen (N), to boost crop yield and quality is becoming increasingly questionable. Therefore, overcoming the excessive use of synthetic fertilizer without reducing crop yield and quality is one of the main goals of future agriculture (Mathlouthi et al., 2022a). Moreover, the higher frequency of drought and thermal stress events during crops cycle often makes traditional N fertilization practice (i.e., based on synthetic granular fertilizer) ineffective or even damaging for plants (Van Herwaarden et al., 1998). However, many crops are dependant from N to achieve high yields and quality standards. Among these crops, are bread and durum wheat, two staple crops securing food for 40 % of the world's population, with a current annual production of 790.6 million tons (USDA, 2023). Since durum wheat (*Triticum turgidum* L. subsp. *durum* (Desf.) Husn.) cultivation zones are mainly located in a relatively restricted geographic area (the Mediterranean Basin), global supply for this commodity is particularly vulnerable to the effects of climate change, and as a consequence, is highly sensitive to the agronomic strategies adopted for the mitigation of those effects (Rossini et al., 2024a).

Wheat production and N fertilization are intrinsically linked and, currently, a major challenge is to maintain (or increase) the former while decreasing the latter. As such, exploring alternative and sustainable products and their mode and timing of application may potentially allow a rethinking of crop nutrition in the coming years. The use of biostimulants as foliar treatment has been suggested as a valid option to increase plant growth, yield, quality, nitrogen use efficiency and tolerance to biotic and abiotic stresses (Calvo et al., 2014), while reducing the amount of chemicals used (Abbas et al., 2022; Li et al., 2023). Recently, other studies have shown that foliar fertilization can limit the use of nitrogen fertilizer applied to the soil, while improving grain yield and grain protein content in bread wheat under

* Corresponding author.
E-mail address: r.ruggeri@unitus.it (R. Ruggeri).

https://doi.org/10.1016/j.ijagro.2025.100027
Received 4 November 2024; Received in revised form 9 January 2025; Accepted 14 January 2025
Available online 19 January 2025

Mediterranean climatic conditions (Mathlouthi et al., 2022b). Many studies reported that PGPB (Plant Growth-Promoting Bacteria), seaweed extract and glycine betaine applied as foliar spraying enhanced the performance of wheat and other crops under stress conditions (Bakaeva et al., 2022; Díaz-Zorita et al., 2001; Singh, 2018). The application of a consortium of rhizobacteria could guarantee enough nutrients and increase the development of the crop under different environmental conditions (Dal Cortivo et al., 2020; Zia et al., 2021). The use of glycine betaine and vaterite ($CaCO_3$) was found to enhance the response of different crops to abiotic stresses such as drought, heat and salinity (Khalid et al., 2022; Sadak and Talaat, 2021) while seaweed extracts and *Pseudomonas* spp. were reported to promote plant growth and root development thanks to their phytohormones and micronutrient content (Najafi Vafa et al., 2022; Noreen et al., 2012). Since foliar application of biostimulants can alleviate the adverse effects of abiotic stresses on crop yield and quality, this method may probably be the best way forward for the management of crop nutrition in the Mediterranean environment. In most of the experiments that were conducted to explore the potential of biostimulants, the effect of just one product has been explored. At the same time, several studies reported that the use of a single biostimulant could not reduce the amount of nitrogen fertilizer without negatively affecting the yield of cereals like corn and wheat (Kapela et al., 2020; Valente et al., 2024). We posit, instead, that a combination of different biostimulants could really improve the crop performance and produce a significant reduction in the use of synthetic N fertilizers. The interaction between biostimulants and N fertilization has not been studied extensively, especially for durum wheat. Moreover, while many authors studied the effect of biostimulants on plant roots in an artificial environment (rhizotrons, pots, Petri dishes, etc.), very few experiments have sampled roots directly from the field. This is both a time-consuming and difficult task to accomplish but can provide precious information on root development under real field conditions.

The objective of this study was to assess the agronomic performance of durum wheat treated with different combinations of biostimulants under three different levels of N fertilization (low, medium and standard). In particular, the following hypotheses were tested: (i) biostimulant application improves morpho-physiological traits of durum wheat compared to the control; (ii) biostimulant application enhances grain yield and quality compared to the control; (iii) biostimulant application allows durum wheat to reach similar agronomic performance as compared to the control treatment, but at a reduced N dose.

2. Material and methods

2.1. Location and experimental design

A rainfed field trial was conducted in Viterbo, central Italy (latitude 42°43′N, longitude 12°07′E, altitude 310 m), during 2022–2023 and 2023–2024 seasons (hereafter referred to as 2023 and 2024, respectively). Weather conditions during the crop cycles are reported in Fig. 1. Physical and chemical properties of soil are reported in Table 1. A split plot design with three replicates was used. Individual plots were 6 m^2.

Three levels of N topdressing were tested in the whole plots: 1) a low input rate with 50 kg N ha^{-1} (hereafter referred to as N50), 2) a medium rate with 100 kg N ha^{-1} (hereafter referred to as N100), and 3) the standard fertilization for durum wheat in this area, with 150 kg N ha^{-1} (hereafter referred to as N150). The N fertilization was applied broadcasting granular urea (46 % N) and splitting the total dose in two equal distributions: the first at the late tillering stage (BBCH stage 25), and the second at flag leaf emergence (BBCH stage 37). All the plots were subjected to a pre-sowing fertilization using diammonium phosphate (18 % N – 46 % P) at a rate of 150 kg ha^{-1}.

Three different experimental products were tested in the split plots in comparison with the control (T0). The formulations and dose of each treatment are reported in Table 2. All of the biostimulants were

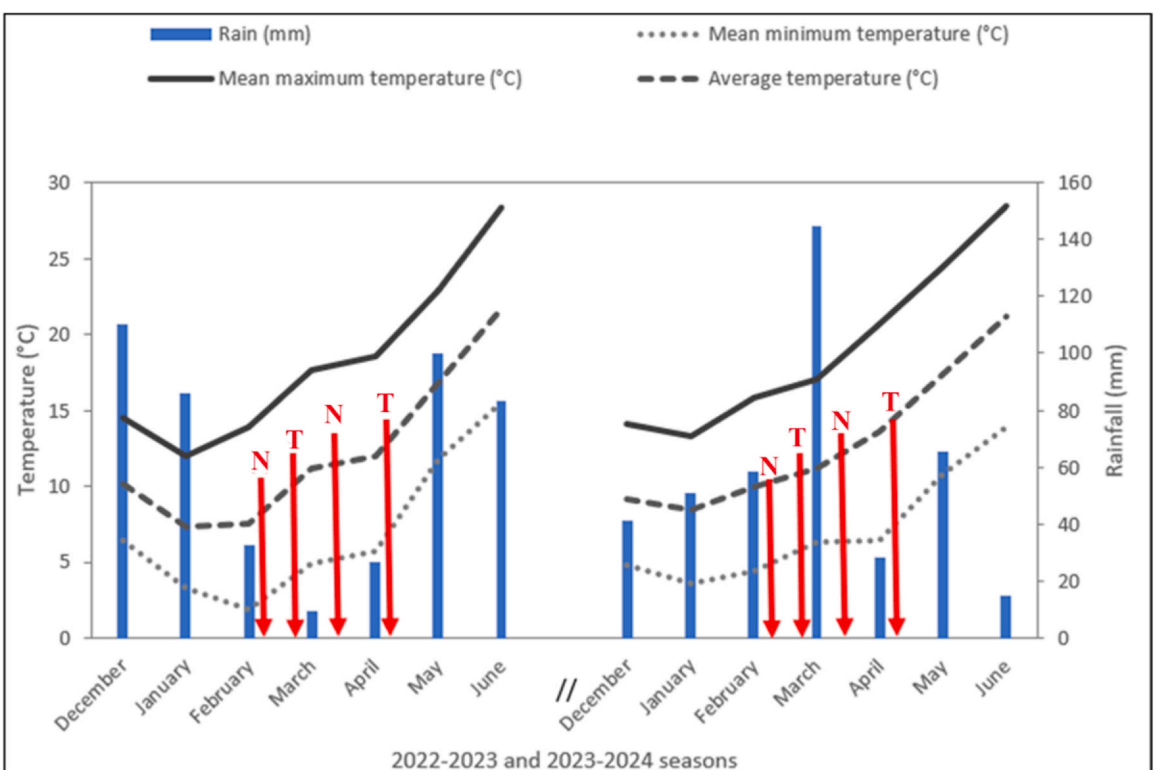

Fig. 1. Climatic patterns of the two growing seasons. The red arrows indicate the time of application of foliar treatment with biostimulants (T) and topdressing with nitrogen fertilizer (N).

Table 1
Physical and chemical properties of soil.

Soil characteristic	Value
Clay (%)	16.30
Silt (%)	14.60
Sand (%)	69.10
pH	7.34
Organic Matter (% w/w)	1.18
Total N (‰ w/w)	1.01
C/N ratio	6.78

Clay (Ø < 2 μm); Silt (2 μm < Ø < 20 μm); Sand (2 mm > Ø > 0.02 mm)

distributed as foliar treatments and applied two times during the crop cycle, the first during the end of tillering (BBCH stage 25–26) and the second at heading (BBCH stage 59).

Soil was ploughed at the end of summer (30 cm depth) and then arrowed twice before seeding. Plots were sown on 14 December in 2022 and 26 November in 2023, respectively. The durum wheat cultivar Iride was used for its excellent environmental adaptability and yield stability (Graziano et al., 2020). Weeds were chemically controlled.

2.2. Sampling and measurements

During the experiment, the following traits were recorded: chlorophyll content (Chl content), length and dry weight of roots, grain yield (13 % moisture content), nitrogen use efficiency (NUE), number of spikes per square meter (NSM), number of kernels per spike (NKS), thousand kernel weight (TKW), grain protein content (GPC) and test weight (TW).

Chlorophyll content was measured during heading (BBCH stage 59), anthesis (BBCH stage 65) and late grain filling (BBCH stage 87) using a hand-held SPAD 502 m (Konica-Minolta Inc., Hino-shi Tokyo, Japan). The root analysis was performed on 3 wheat plants for each replica, during the late grain filling stage (BBCH stage 87). To minimize the risk of root damage, we performed a form of shovelomics methodology for plant excavation. A 50 cm × 50 cm x 30 cm (depth) portion of soil was dug in each subplot, using a standard shovel (Trachsel et al., 2011). Clods were extracted from the ground and were gently shaken to remove as much soil as possible. Three plants with intact roots were chosen from the central part of the clods for the subsequent analysis. The roots were carefully washed, and manually separated into single roots, before analysis with WinRHIZO™ scanning and software (Regent Instruments, 2016). Root images were scanned at 600 dpi (945 pixels) resolution. Root length was calculated by the number of pixels in the root skeleton multiplied by the pixel size (Boudiar et al., 2021; Hobson et al., 2023). Later, to determine root dry weight, the roots were oven dried at 60 °C until a constant weight was achieved.

Grain yield was determined by harvesting the entire plot (excluding the external rows) with a plot harvester and weighing the resultant production. Subsequently, a subsample of grains (60 g) for each plot was oven dried (70 °C, until constant weight) to determine grain moisture percentage and then the yield was expressed at 13 % standard moisture content.

To determine the NUE, grain yield (expressed as kg ha^{-1}) was divided by the total dose (pre-sowing + topdressing) of N fertilizer (expressed as kg ha^{-1}) (Goñi et al., 2021).

As for grain quality, grain samples (250 g) from each plot were ground through a PerkinElmer LM-3610 grinder (PerkinElmer Health Sciences Canada Inc., Winnipeg, MB, Canada) using a 1 mm sieve. Grain protein content (%) was determined by scanning the samples with a benchtop NIR (Near Infrared Reflectance) spectrometer DS2500 flour analyzer (FOSS-DS2500; FOSS Electric A/S, Hillerød, Denmark), while test weight was measured by the Schopper chondrometer, reporting the results as kg hL^{-1}.

2.3. Statistical analysis

Analysis of variance (ANOVA) was performed using R software, version 3.5.2 (R Core Team, 2021). The library *aomisc* was used to test the effect of nitrogen, biostimulants, and their interactions. Normality and homoscedasticity were verified visually via the 'plot' function in R before conducting the ANOVA. The experimental year was considered as a random factor. Means were separated using Tukey test at 95 % probability level.

3. Results

3.1. Weather pattern

The two experimental years were characterized by similar climatic conditions (Fig. 1), particularly when considering total rainfall (mm). Taking the entire crop cycle into account (from sowing to harvest), rainfalls showed a slightly higher value in 2022–2023 than 2023–2024 (448 mm vs. 404 mm). March 2023 was very dry (with just 9.6 mm), while March 2024 was the rainiest month of the second growing season (144.8 mm). When compared to the historical weather data for this location, May and June 2023 were unusually rainy (+ 100 % rains for both months). As for the average and maximum temperatures, there was not a clear difference between the two cropping seasons. In terms of minimum temperature, 2023 showed lower temperatures than 2024, especially in February (1.9 °C vs. 4.4 °C).

3.2. Root system

Both nitrogen and biostimulants significantly affected the measured root traits (Table 3). As for root dry weight, the interaction between factors was found to be significant.

No significant differences in root length were detected between N150 and N100 (respectively 352 cm and 338 cm), while the lowest N dose significantly reduced root length by more than 20 % (275 cm, Fig. 2A). Fig. 2B shows that all biostimulants strongly enhanced root length when compared to the control treatment (T0), with a mean increase ranging from 75 % (T1 and T2) to 160 % (T3).

As shown in Fig. 3, foliar application of biostimulants compensated the reduction of nitrogen in terms of root dry weight. All treatments at the lowest N dose showed a root dry weight that was significantly higher than that of control treatment at the highest N levels. T3 treatment showed the best results without any significant difference along the N dose gradient.

Table 2
Formulations and doses of the different foliar treatments.

Treatment	Glycine Betaine (35 %)	Micronized vaterite (Ca 29 %)	Extracts of *Codium fragile* and *Opuntia ficus-barbarica*	*Pseudomonas protegens (10^9 FCU g^{-1})*	PGPB *($5 * 10^9$ FCU g^{-1})*
T0	-	-	-	-	-
T1	3 kg ha^{-1}	2 kg ha^{-1}	1 kg ha^{-1}	-	-
T2	3 kg ha^{-1}	2 kg ha^{-1}	1 kg ha^{-1}	1 kg ha^{-1}	-
T3	3 kg ha^{-1}	2 kg ha^{-1}	1 kg ha^{-1}		0.5 kg ha^{-1}

PGPB (plant growth-promoting bacteria): *Pseudomonas protegens*, *Azospirillum brasilense*, *Bacillus subtilis*, *Bacillus megaterium*, *Bacillus amyloliquefaciens*.

Table 3
Results from the analysis of variance for the root system traits of durum wheat grown under three N top-dressing rates factorially combined with three different biostimulants according to a split-plot design.

	Root length	Root dry weight
Nitrogen	***	*
Biostimulant	***	***
Nitrogen × Biostimulant	ns	*

Levels of significance: *** < 0.001; ** < 0.01; * < 0.05; ns: not significant.

3.3. Chlorophyll content

The foliar Chl content was significantly affected by biostimulants at all growing stages. During grain filling, the interaction between factors was found to be significant (Table 4).

In comparison to the control treatment, biostimulant application increased chlorophyll content by 9.6 % and 11.9 % during heading and anthesis, respectively (data not shown). During late grain filling stage (BBCH stage 87), biostimulants induced the stay-green phenotype in wheat plants, roughly doubling the Chl content. The T3 treatment showed the best results even at the lowest N dose, while all treatments performed equally at the highest N dose (Fig. 4).

3.4. Grain yield and NUE

All yield components were significantly affected by biostimulant application. The interaction between factors was significant for grain yield, NUE and the number of spikes m^{-2} (Table 5).

As shown in Fig. 5, T1 and T2 treatment did not significantly improve grain yield at the lowest N dose, compared to the control, while T3 resulted in an 18 % increase. A similar outcome was observed at N100, although, in this case, T2 also significantly increased grain yield compared to T0. Interestingly, wheat plants treated with T3 at N100 showed a grain yield that was not statistically different to that of T0 at N150 (5.48 and 5.64 t ha^{-1}, respectively). Overall, no differences among treatments were detected at the highest N dose.

As shown in Fig. 6, the reduction of N dose significantly reduced the spike number per unit area. Overall, wheat plants produced 249, 229 and 202 spikes m^{-2} at N150, N100 and N50, respectively. Consequently, the reduction of N input from N150 to N50 caused a 23 % decrease in spike number. As observed in grain yield, the T3 treatment compensated the reduction of nitrogen, maintaining a spike number similar or even higher than that reached by the control treatment at the superior N level (e.g., the number of spikes observed in the combination T3-N50 was not significantly different from T0-N100; spike number of T3-N100 was significantly higher than T0-N150). As compared to T0, the application of the T3 treatment increased the spike number per unit area by 13 %, 19 % and 6 % at N50, N100 and N150, respectively.

All biostimulant treatments, except for T1, resulted in a NKS significantly higher than that of T0 (Table 6). In particular, when compared to control (43.1 grains per spike), the T2 and T3 treatment showed a 10 % and 13 % increase, respectively. As for TKW, T3 showed a value significantly higher than that of the control (Table 6), raising the grain weight by 7 % (55.5 mg vs. 51.9 mg).

The highest NUE was obtained at the lowest N dose, while at N100 and N150 NUE dropped by 22 % and 37 %, respectively (Fig. 7). T1 and T2 did not significantly enhance the NUE compared to T0, while the T3 treatment produced a significant rise in the NUE at both N50 and N100 (30 % and 16 %, respectively). At N150 no biostimulant significantly affected the NUE in comparison to the control.

3.5. Grain quality

Grain protein content (GPC) was significantly affected by both nitrogen and biostimulants, while test weight was significantly influenced by the interaction between the two factors (Table 7).

As expected, the N level had a strong effect on GPC (Table 8). This trait decreased by 23 % between N150 and N50 (14 % vs. 11.2 %). All the biostimulant treatments caused a slight (4 %) but significant increase in the GPC when compared to T0, with no significant differences among the biostimulant treatments.

The control treatment (T0) showed the lowest values, averaging 77.8 kg hL^{-1}. At N50 and N100, all biostimulant treatments allowed grains to reach a test weight (TW) significantly higher than that of T0 (Fig. 8). At the highest N dose, only T3 showed a result significantly higher than T0. T2 and T3 treatment compensated for the reduction of nitrogen, allowing grains to reach a TW higher than that reached by the control treatment at the superior N level (e.g., the combinations T2-N50 or T3-N50 evidenced a TW significantly higher than that of T0-N100; the same for T2-N100 and T3-N100 compared to T0-N150). Surprisingly, the TW obtained with T2 and T3 treatments at the lowest N dose were already at the highest level (78.9 and 78.8 kg hl^{-1}, respectively).

4. Discussion

The use of biostimulants significantly increased the performance of durum wheat in comparison to the traditional use of synthetic fertilizers. Interestingly, foliar treatments (especially T3 treatment) often compensated the reduction of nitrogen, maintaining or even exceeding the results obtained by the control treatment at superior N levels. A similar result was obtained using a combination of bacteria and arbuscular mycorrhizae on tomato, which led to a 30 % reduction in synthetic fertilizer (Adesemoye et al., 2009). In another study, the application of *Azospirillum* and *Bacillus* spp. increased the grain yield of wheat and barley (Ozturk et al., 2003). As

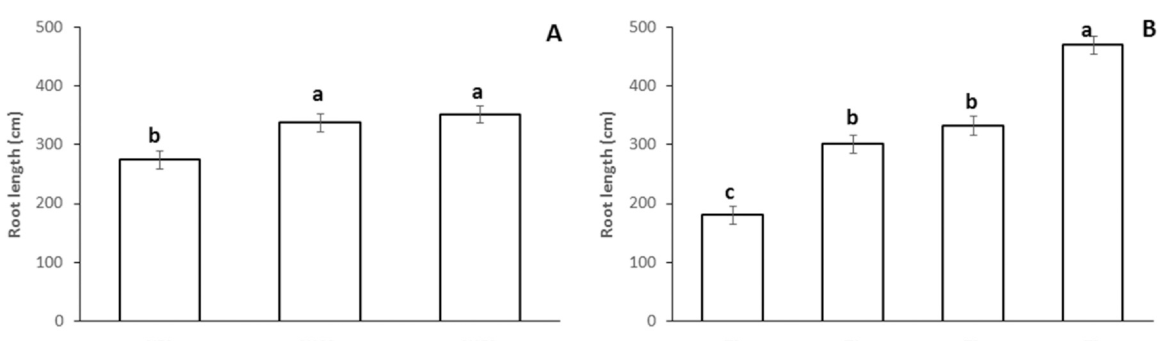

Fig. 2. Root length of durum wheat plants (BBCH stage 87) as affected by nitrogen (A) and biostimulant (B) application. The vertical bars represent the standard error of the mean. Letters above the histograms correspond to the ranking of the Tukey's test at $p < 0.05$. N50, N100, N150: N topdressing rates, 50, 100 and 150 kg ha^{-1}, respectively; T0: no biostimulant treatment; T1: plants treated with a mixture of glycine betaine, micronized vaterite and extracts of *Codium fragile* and *Opuntia ficus-barbarica*; T2: plants treated combining T1 and *Pseudomonas protegens*; T3: plants treated combining T2 and PGPB.

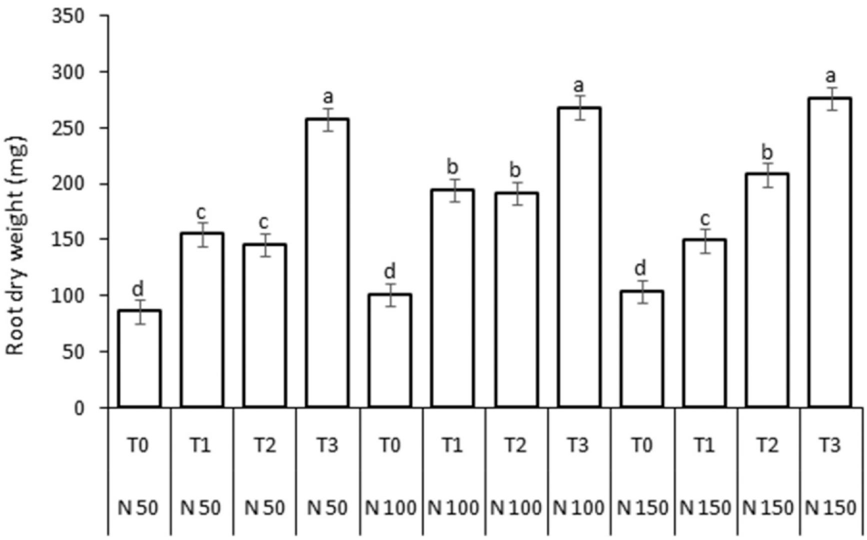

Fig. 3. Root dry weight of durum wheat plants (BBCH stage 87) as affected by the nitrogen × treatment interaction. The vertical bars represent the standard error of the mean. Letters above the histograms correspond to the ranking of the Tukey's test at $p < 0.05$. N50, N100, N150: N topdressing rates, 50, 100 and 150 kg ha^{-1}, respectively; T0: no biostimulant treatment; T1: plants treated with a mixture of glycine betaine, micronized vaterite and extracts of *Codium fragile* and *Opuntia ficus-barbarica*; T2: plants treated combining T1 and *Pseudomonas protegens*; T3: plants treated combining T2 and PGPB.

Table 4
Results from the ANOVA for Chl content recorded in different growing stages of durum wheat grown under three N top-dressing rates factorially combined with three different biostimulants according to a split-plot design.

	Heading	Anthesis	Grain filling
Chl content	(BBCH stage 59)	(BBCH stage 65)	(BBCH stage 87)
Nitrogen	ns	ns	ns
Biostimulant	***	***	***
Nitrogen × Biostimulant	ns	ns	*

Levels of significance: *** < 0.001; ** < 0.01; * < 0.05; ns: not significant.

Table 5
Results from the ANOVA for grain yield, yield components and NUE of durum wheat grown under three N top-dressing rates factorially combined with three different biostimulants according to a split-plot design.

	Grain yield	NSM	NKS	TKW	NUE
Nitrogen	**	ns	ns	ns	***
Biostimulant	***	***	**	**	***
Nitrogen × Biostimulant	*	*	ns	ns	**

Levels of significance: *** < 0.001; ** < 0.01; * < 0.05; ns: not significant. NSM: number of spikes m^{-2}; NKS: number of kernels per spike; TKW: thousand kernel weight; NUE: nitrogen use efficiency.

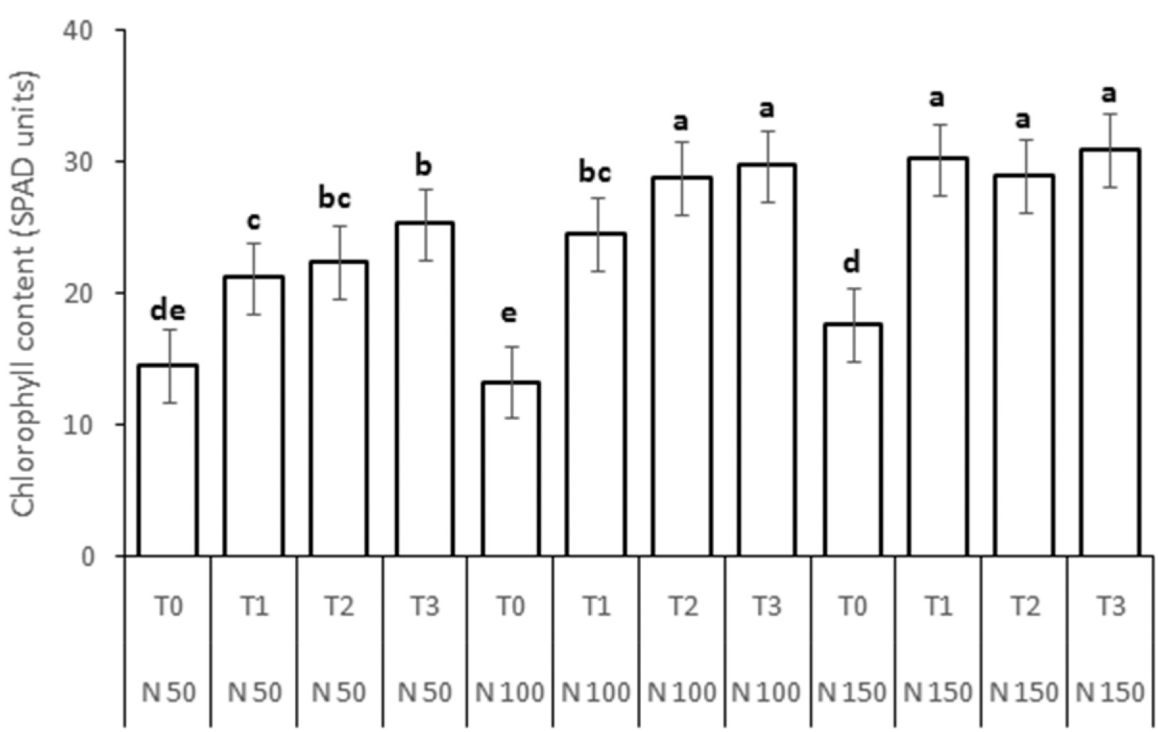

Fig. 4. Chlorophyll content of durum wheat during late grain filling stage (BBCH stage 87) as affected by the nitrogen × treatment interaction. The vertical bars represent the standard error of the mean. Letters above the histograms correspond to the ranking of the Tukey's test at $p < 0.05$. N50, N100, N150: N topdressing rates, 50, 100 and 150 kg ha^{-1}, respectively; T0: no biostimulant treatment; T1: plants treated with a mixture of glycine betaine, micronized vaterite and extracts of *Codium fragile* and *Opuntia ficus-barbarica*; T2: plants treated combining T1 and *Pseudomonas protegens*; T3: plants treated combining T2 and PGPB.

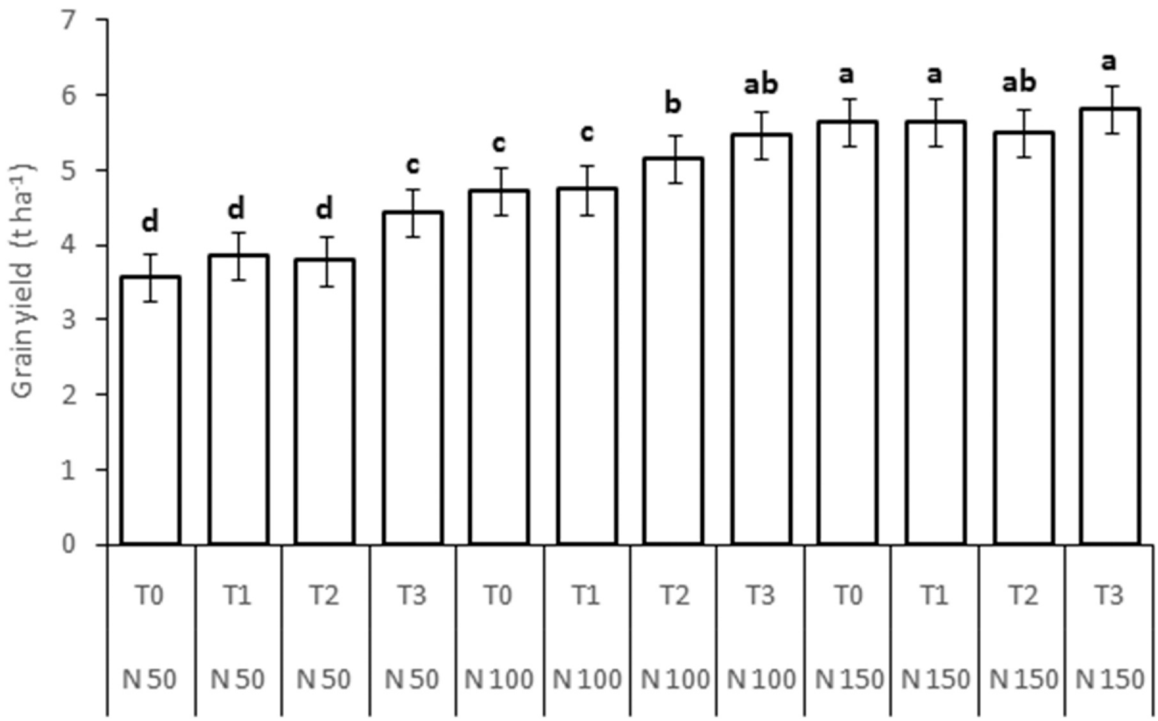

Fig. 5. Grain yield of durum wheat as affected by the nitrogen × treatment interaction. The vertical bars represent the standard error of the mean. Letters above the histograms correspond to the ranking of the Tukey's test at $p < 0.05$. N50, N100, N150: N topdressing rates, 50, 100 and 150 kg ha^{-1}, respectively; T0: no biostimulant treatment; T1: plants treated with a mixture of glycine betaine, micronized vaterite and extracts of *Codium fragile* and *Opuntia ficus-barbarica*; T2: plants treated combining T1 and *Pseudomonas protegens*; T3: plants treated combining T2 and PGPB.

with our study, the PGPR were reported to perform better under low nitrogen and stress conditions, in which they enhanced grain yield in comparison to the use of chemical fertilizers (Artyszak and Gozdowski, 2021; Sedri et al., 2022; Tounsi-Hammami et al., 2022).

This was probably due also to the significantly higher NUE we found in plants treated with T3 as compared to the control ones. This result is consistent with other works reporting the efficacy of biostimulants. Particularly, seaweed extracts and PGPR were reported to improve NUE

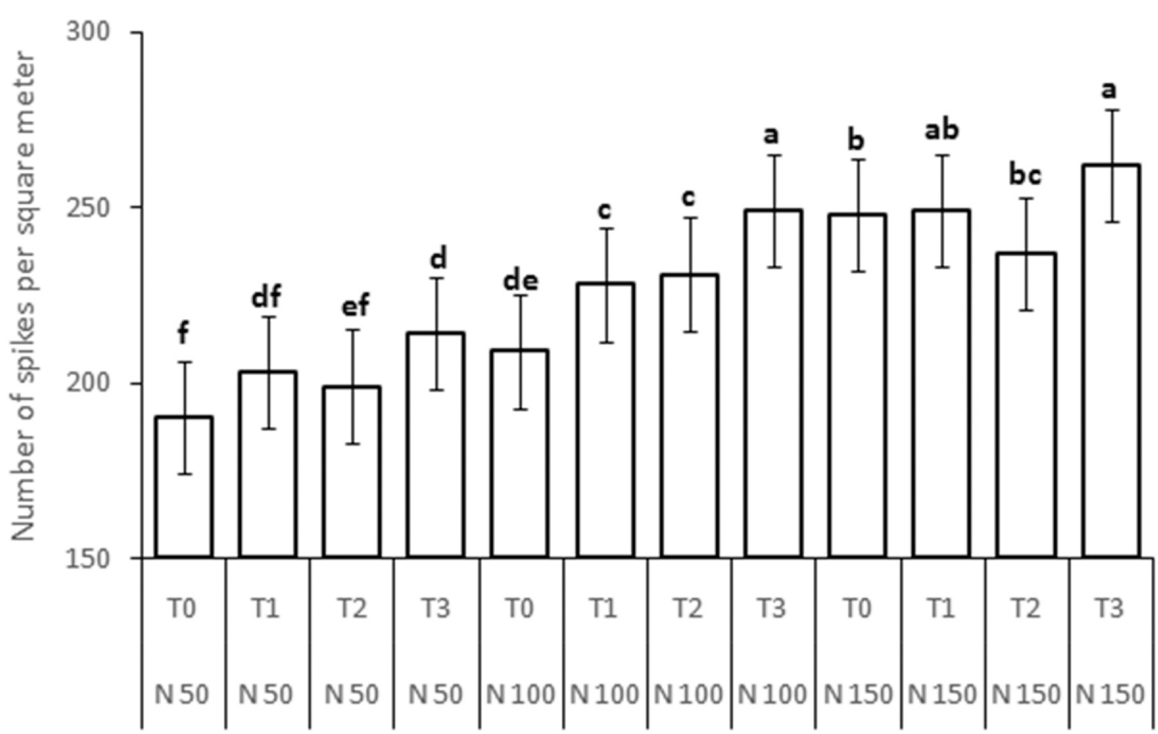

Fig. 6. Number of spikes per square meter of durum wheat as affected by nitrogen × treatment interaction. The vertical bars represent the standard error of the mean. Letters above the histograms correspond to the ranking of the Tukey's test at $p < 0.05$. N50, N100, N150: N topdressing rates, 50, 100 and 150 kg ha^{-1}, respectively; T0: no biostimulant treatment; T1: plants treated with a mixture of glycine betaine, micronized vaterite and extracts of *Codium fragile* and *Opuntia ficus-barbarica*; T2: plants treated combining T1 and *Pseudomonas protegens*; T3: plants treated combining T2 and PGPB.

Table 6
Number of kernels per spike (NKS) and thousand kernel weight (TKW) of durum wheat as affected by the biostimulant treatment (means ± standard error).

	NKS (n)	TKW (g)
T0	43.1 ± 2.36 b	51.9 ± 0.99 b
T1	47.3 ± 2.36 ab	54.0 ± 0.99 ab
T2	47.4 ± 2.36 a	54.3 ± 0.99 ab
T3	48.8 ± 2.36 a	55.5 ± 0.99 a

The letters correspond to the ranking of the Tukey's test at $p < 0.05$. T0: no biostimulant treatment; T1: plants treated with a mixture of glycine betaine, micronized vaterite and extracts of *Codium fragile* and *Opuntia ficus-barbarica*; T2: plants treated combining T1 and *Pseudomonas protegens*; T3: plants treated combining T2 and PGPB.

in different crops such as spinach, sunflower (Arif et al., 2017; La Bella et al., 2021) and wheat (Alaskar and AL-Shwaiman, 2023). The rhizobacteria appear to be involved in both ammonium and nitrite oxidation, leading to a faster and more efficient nutrient uptake (Di Benedetto et al., 2017). Moreover, root length is one of the most influential traits to improve NUE, because it allows crops to explore a wider portion of soil (Meena et al., 2016). Accordingly, in our study, biostimulant treatment doubled the root length of wheat plants compared to untreated plants. Seaweed extract was previously found to boost the root system and especially the development of new tillers due to the presence of phytohormones and micronutrients (Bakhoum et al., 2023). In particular, *Codium fragile* extracts showed this effect on wheat seedlings in other studies (Rossini et al., 2024c). The same effect was reported for PGPR, due to the capabilities of different microorganisms to synthetize hormones (such as cytokinins and auxins), and increase nutrient bioavailability (Vafa et al., 2021). Generally, biostimulant treatments showed the best results at low and medium level of nitrogen, in accordance with previous studies (Dalla Santa et al., 2004).

The positive effect that T3 application had on grain yield at low N doses was the consequence of the strong influence this treatment had in increasing all yield components. Similar effects were reported in other studies conducted on wheat plants (Kumar and Sahoo, 2011; Shah et al., 2013).

Biostimulant treatments strongly enhanced the Chl content of wheat plants, especially during the grain filling stage, thus inducing a stay-green phenotype. This was probably due to the presence of i) calcium included in the vaterite that was able to increase the photosynthetic rate and the chlorophyll content in the leaves (Dolatabadian et al., 2013), and ii) glycine betaine, that delayed the natural senescence of the flag leaf by enhancing the net photosynthetic rate (Islam et al., 2021; Wang et al., 2014). Since wheat yield is not only determined by the carbohydrates stored during the early growth stages, but also by

Table 7
Results from the ANOVA for grain quality traits of durum wheat grown under three N top-dressing rates factorially combined with three different biostimulants according to a split-plot design.

	GPC	TW
Nitrogen	*	ns
Biostimulant	***	***
Nitrogen × Biostimulant	ns	*

Levels of significance: *** < 0.001; ** < 0.01; * < 0.05; ns: not significant. GPC: grain protein content; TW: test weight

Table 8
Grain protein content (GPC) of durum wheat as affected by the N top-dressing rates and biostimulant treatments (means ± standard error).

Treatment	GPC (%)
Nitrogen	
N50	11.2 ± 0.6 b
N100	13.1 ± 0.6 ab
N150	14.0 ± 0.6 a
Biostimulant	
T0	12.4 ± 0.35 b
T1	12.8 ± 0.35 a
T2	12.9 ± 0.35 a
T3	12.9 ± 0.35 a

The letters correspond to the ranking of the Tukey's test at $p < 0.05$. N50, N100, N150: N topdressing rates, 50, 100 and 150 kg ha^{-1}, respectively; T0: no biostimulant treatment; T1: plants treated with a mixture of glycine betaine, micronized vaterite and extracts of *Codium fragile* and *Opuntia ficus-barbarica*; T2: plants treated combining T1 and *Pseudomonas protegens*; T3: plants treated combining T2 and PGPB.

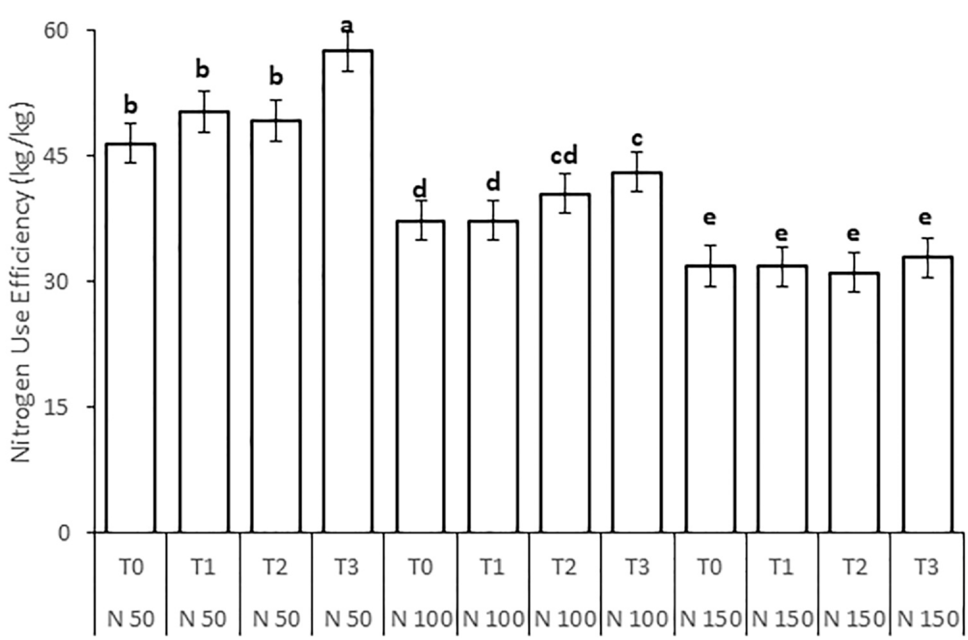

Fig. 7. Nitrogen use efficiency of durum wheat as affected by the nitrogen × treatment interaction (kg of grains at 13 % moisture per kg of total N applied). The vertical bars represent the standard error of the mean. Letters above the histograms correspond to the ranking of the Tukey's test at $p < 0.05$. N50, N100, N150: N topdressing rates, 50, 100 and 150 kg ha^{-1}, respectively; T0: no biostimulant treatment; T1: plants treated with a mixture of glycine betaine, micronized vaterite and extracts of *Codium fragile* and *Opuntia ficus-barbarica*; T2: plants treated combining T1 and *Pseudomonas protegens*; T3: plants treated combining T2 and PGPB.

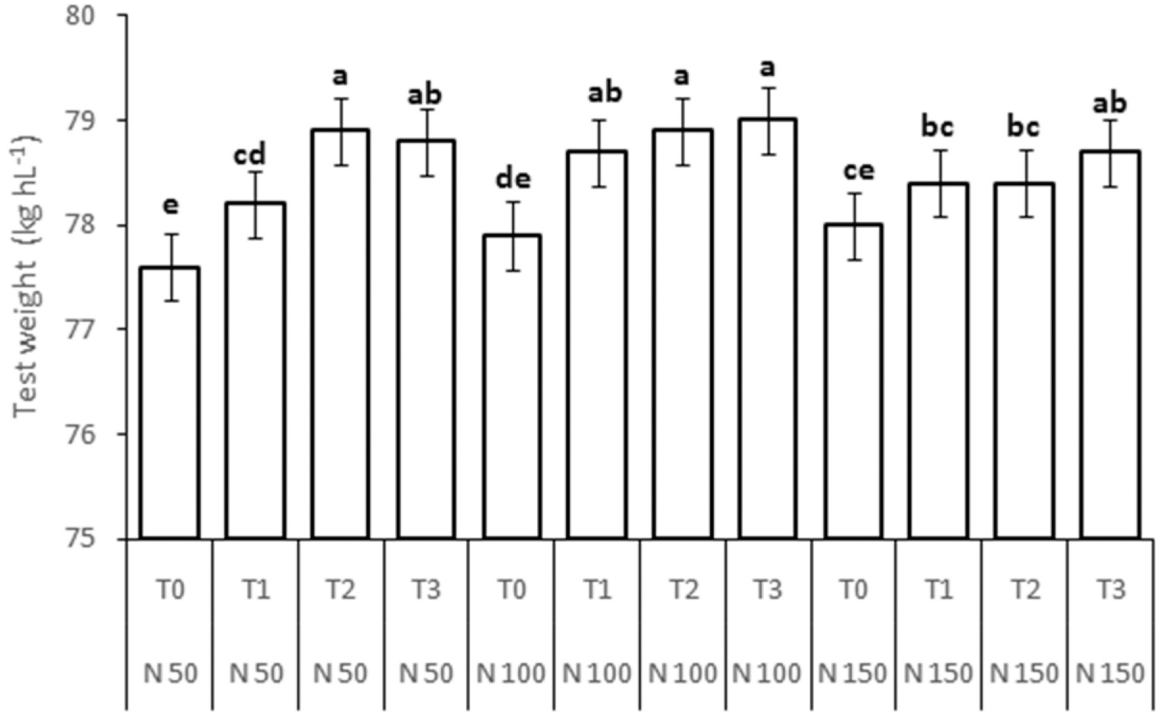

Fig. 8. Test weight of durum wheat as affected by the nitrogen × treatment interaction. The vertical bars represent the standard error of the mean. Letters above the histograms correspond to the ranking of the Tukey's test at $p < 0.05$. N50, N100, N150: N topdressing rates, 50, 100 and 150 kg ha^{-1}, respectively; T0: no biostimulant treatment; T1: plants treated with a mixture of glycine betaine, micronized vaterite and extracts of *Codium fragile* and *Opuntia ficus-barbarica*; T2: plants treated combining T1 and *Pseudomonas protegens*; T3: plants treated combining T2 and PGPB.

photosynthetic production after heading, inducing a stay-green phenotype during the grain-filling stage translates to an increase in accumulation of photoassimilates and to greater grain yield (Roychowdhury et al., 2023; Zhou and Yang, 2023).

In our study, N level did not significantly affect the Chl content during heading and anthesis. A similar result was obtained in wheat in Mexico and China (Ibarra-Villarreal et al., 2023; Noor et al., 2023). Noor et al. (2023) showed that, after booting, Chl content did not vary with N rate over a range of 0 to 210 kg N ha^{-1}, while, before the booting stage, a significant difference in Chl content existed only between N treatments and control treatment (no N application). This led us to speculate that Chl content in wheat is responsive to N application depending on growth stage more than on N rate. Additionally, considering the total N availability (soil N + pre-sowing fertilization + topdressing), the plots treated with the lowest dose (N50) could benefit from more than 80–90 kg N ha^{-1} during the crop cycle. This availability could have been sufficient to reach good Chl values, thus eliminating any statistical differences among N treatments.

Nitrogen applications, especially late applications, are fundamental to obtain durum grain with high quality attributes (Blandino et al., 2015; Tedone et al., 2014). In the present study, biostimulant application resulted in a higher protein content and test weight compared to control treatment, thus influencing its nutritional and economic value as well as its final use (Veraverbeke and Delcour, 2002). The grain quality of wheat appeared to be positively influenced by the foliar applications of micronutrients, like those contained in the micronized vaterite (Mathlouthi et al., 2022b; Saquee et al., 2023). The same effect has also been found in wheat treated with PGPR able to mitigate abiotic stress, especially during grain filling stage (Pagnani et al., 2020).

The limits of the conventional fertilization strategy were highlighted by other studies, confirming that high chemical inputs cannot always sustain high yields and grain quality, especially when severe abiotic stresses occur during the growing season (Mitura et al., 2023; Yaghoubi Khanghahi et al., 2022).

We demonstrated that a new sustainable crop nutrition approach could be a valid practice to reduce the amount of nitrogen fertilizer in durum wheat cultivation (third hypothesis of our study). Biostimulant treatment enhanced chlorophyll content, root development, and grain yield and quality of durum wheat, thus verifying the first and second hypothesis of the present experiment. It is worth noting that the study was conducted during two optimal growing seasons, thus allowing wheat plants to avoid severe abiotic stress (e.g., heat stress and terminal drought). On one hand, these growing conditions have strongly enhanced the yield performance of durum wheat, but on the other, they probably reduced the effect of the biostimulant application. Indeed, different authors reported a better performance of biostimulant products under limiting environmental conditions when compared to optimal growing conditions (Rossini et al., 2024b; Spada et al., 2024).

5. Conclusions

The application of an alternative crop nutrition protocol, based on the use of sustainable products in combination with reduced synthetic N fertilisation, clearly enhanced grain yield and quality as well as morphophysiological traits of durum wheat in central Italy. Biostimulant application dramatically developed the root system of wheat plants and induced a beneficial stay-green phenotype during late growth stages.

Our study suggests that the use of foliar biostimulants can help farmers to reduce the amount of synthetic nitrogen fertilizers up to 33 %, without reducing durum yield and grain protein content. This result is a breakthrough in the development of sustainable wheat cultivation in line with the "Farm to Fork" European strategy, which aims to reduce the use of synthetic fertilizers by 20 % before 2030.

Additional studies are required to better understand the mechanisms of action of the single foliar biostimulants, and to verify whether this alternative fertilization program will be suitable for other major crops of the Mediterranean region (e.g., processing tomato, barley and maize) and also under abiotic stress conditions.

Funding

This research was conducted as activity of the PhD course in “Plant and Animal Production Sciences (XXXVIII cycle)” of the University of Tuscia and was co-funded by PNRR, Missione 4, componente 2 “Dalla Ricerca all’Impresa” — Investimento 3.3 and by ISLA srl.

CRediT authorship contribution statement

Francesco Rossini: Writing – review & editing, Visualization, Funding acquisition, Conceptualization. **Roberto Ruggeri:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Conceptualization. **Angelo Rossini:** Writing – original draft, Investigation, Formal analysis, Data curation.

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.

References

Abbas, M., Abdel-Lattif, H., Badawy, R., El-Wahab, M.A., Shahba, M., 2022. Compost and biostimulants versus mineral nitrogen on productivity and grain quality of two wheat cultivars. Agriculture 12. https://doi.org/10.3390/agriculture12050699

Adesemoye, A.O., Torbert, H.A., Kloepper, J.W., 2009. Plant growth-promoting Rhizobacteria allow reduced application rates of chemical fertilizers. Micro Ecol. 58, 921–929. https://doi.org/10.1007/s00248-009-9531-y

Alaskar, A.A., AL-Shwaiman, H.A., 2023. The effect of plant growth-promoting rhizobacteria on soil properties and the physiological and anatomical characteristics of wheat under water-deficit stress conditions. Agriculture 13, 2042. https://doi.org/10.3390/agriculture13112042

Arif, M.S., Shahzad, S.M., Riaz, M., Yasmeen, T., Shahzad, T., Akhtar, M.J., Bragazza, L., Buttler, A., 2017. Nitrogen-enriched compost application combined with plant growth-promoting rhizobacteria (PGPR) improves seed quality and nutrient use efficiency of sunflower. J. Plant Nutr. Soil Sci. 180, 464–473. https://doi.org/10.1002/jpln.201600615

Artyszak, A., Gozdowski, D., 2021. Is it possible to maintain the quantity and quality of winter wheat grain by replacing part of the mineral nitrogen dose by growth activators and plant growth-promoting rhizobacteria (Pgpr)? Sustainability 13. https://doi.org/10.3390/su13115834

Bakaeva, M., Chetverikov, S., Timergalin, M., Feoktistova, A., Rameev, T., Chetverikova, D., Kenjieva, A., Starikov, S., Sharipov, D., Hkudaygulov, G., 2022. PGP-bacterium pseudomonas protegens improves bread wheat growth and mitigates herbicide and drought stress. Plants 11. https://doi.org/10.3390/plants11233289

Bakhoum, G.S., Tawfik, M.M., Kabesh, M.O., Sadak, M.S., 2023. Potential role of algae extract as a natural stimulating for wheat production under reduced nitrogen fertilizer rates and water deficit. Biocatal. Agric. Biotechnol. 51. https://doi.org/10.1016/j.bcab.2023.102794

Blandino, M., Vaccino, P., Reyneri, A., 2015. Late-season nitrogen increases improver common and durum wheat quality. Agron. J. 107, 680–690. https://doi.org/10.2134/agronj14.0405

Boudiar, R., Cabeza, A., Fernández-Calleja, M., Pérez-Torres, A., Casas, A.M., González, J.M., Mekhlouf, A., Igartua, E., 2021. Root trait diversity in field grown durum wheat and comparison with seedlings. Agronomy 11. https://doi.org/10.3390/agronomy11122545

Calvo, P., Nelson, L., Kloepper, J.W., 2014. Agricultural uses of plant biostimulants. Plant Soil. https://doi.org/10.1007/s11104-014-2131-8

Dal Cortivo, C., Ferrari, M., Visioli, G., Lauro, M., Fornasier, F., Barion, G., Panozzo, A., Vamerali, T., 2020. Effects of seed-applied biofertilizers on rhizosphere biodiversity and growth of common wheat (Triticum aestivum L.) in the field. Front. Plant Sci. 11. https://doi.org/10.3389/fpls.2020.00072

Dalla Santa, O.R., Hernández, R.F., Alvarez, G.L.M., Ronzelli Junior, P., Soccol, C.R., 2004. Azospirillum sp. inoculation in wheat, barley and oats seeds greenhouse experiments. Braz. Arch. Biol. Technol. 47, 843–850. https://doi.org/10.1590/S1516-89132004000600002

Di Benedetto, N., Corbo, M.R., Campaniello, D., Pia Cataldi, M., Bevilacqua, A., Sinigaglia, M., Flagella, Z., 2017. The role of plant growth promoting bacteria in improving nitrogen use efficiency for sustainable crop production: a focus on wheat. AIMS Microbiol. 3, 413–434. https://doi.org/10.3934/microbiol.2017.3.413

Díaz-Zorita, M., Fernández-Canigia, M.V., Grosso, G.A., 2001. Applications of foliar fertilizers containing glycinebetaine improve wheat yields. J. Agron. Crop Sci. 186, 209–215. https://doi.org/10.1046/j.1439-037X.2001.00469.x

Dolatabadian, A., Sanavy, S.A.M.M., Gholamhoseini, M., Joghan, A.K., Majdi, M., Kashkooli, A.B., 2013. The role of calcium in improving photosynthesis and related physiological and biochemical attributes of spring wheat subjected to simulated acid rain. Physiol. Mol. Biol. Plants 19, 189–198. https://doi.org/10.1007/s12298-013-0165-7

Goñi, O., Łangowski, Ł., Feeney, E., Quille, P., O'Connell, S., 2021. Reducing nitrogen input in barley crops while maintaining yields using an engineered biostimulant derived from Ascophyllum Nodosum to enhance nitrogen use efficiency. Front. Plant Sci. 12. https://doi.org/10.3389/fpls.2021.664682

Graziano, S., Marmiroli, N., Visioli, G., Gullì, M., 2020. Proteins and metabolites as indicators of flours quality and nutritional properties of two durum wheat varieties grown in different Italian locations. Foods 9, 315. https://doi.org/10.3390/foods9030315

Hobson, D.J., Harty, M.A., Langton, D., McDonnell, K., Tracy, S.R., 2023. The establishment of winter wheat root system architecture in field soils: The effect of soil type on root development in a temperate climate. Soil Use Manag 39, 198–208. https://doi.org/10.1111/sum.12795

Ibarra-Villarreal, A.L., Villarreal-Delgado, M.F., Parra-Cota, F.I., Yepez, E.A., Guzmán, C., Gutierrez-Coronado, M.A., Valdez, L.C., Saint-Pierre, C., Santos-Villalobos, S. de L., 2023. Effect of a native bacterial consortium on growth, yield, and grain quality of durum wheat (*Triticum turgidum* L. subsp. *durum*) under different nitrogen rates in the Yaqui Valley, Mexico. Plant Signal Behav. 18. https://doi.org/10.1080/15592324.2023.2219837

Islam, S., Parrey, Z.A., Shah, S.H., Mohammad, F., 2021. Glycine betaine mediated changes in growth, photosynthetic efficiency, antioxidant system, yield and quality of mustard. Sci. Hortic. 285. https://doi.org/10.1016/j.scienta.2021.110170

Kapela, K., Sikorska, A., Niewęgłowski, M., Krasnodębska, E., Zarzecka, K., Gugała, M., 2020. The impact of nitrogen fertilization and the use of biostimulants on the yield of two maize varieties (Zea mays L.) cultivated for grain. Agronomy 10, 1408. https://doi.org/10.3390/agronomy10091408

Khalid, M., Rehman, H.M., Ahmed, N., Nawaz, S., Saleem, F., Ahmad, S., Uzair, M., Rana, I.A., Atif, R.M., Zaman, Q.U., Lam, H.M., 2022. Using exogenous melatonin, glutathione, proline, and glycine betaine treatments to combat abiotic stresses in crops. Int. J. Mol. Sci. https://doi.org/10.3390/ijms232112913

Kumar, G., Sahoo, D., 2011. Effect of seaweed liquid extract on growth and yield of Triticum aestivum var. Pusa Gold. J. Appl. Phycol. 23, 251–255. https://doi.org/10.1007/s10811-011-9660-9

La Bella, S., Consentino, B.B., Rouphael, Y., Ntatsi, G., De Pasquale, C., Iapichino, G., Sabatino, L., 2021. Impact of Ecklonia maxima seaweed extract and mo foliar treatments on biofortification, spinach yield, quality and NUE. Plants 10, 1139. https://doi.org/10.3390/plants10061139

Li, J., Ma, Haiyan, Ma, Hongliang, Lei, F., He, D., Huang, X., Yang, H., Fan, G., 2023. Comprehensive effects of N reduction combined with biostimulants on N use efficiency and yield of the winter wheat–summer maize rotation system. Agronomy 13, 2319. https://doi.org/10.3390/agronomy13092319

Mathlouthi, F., Ruggeri, R., Rossini, F., 2022a. Alternative solution to synthetic fertilizers for the starter fertilization of bread wheat under mediterranean climatic conditions. Agronomy 12, 511. https://doi.org/10.3390/agronomy12020511

Mathlouthi, F., Ruggeri, R., Rossini, A., Rossini, F., 2022b. A new fertilization approach for bread wheat in the Mediterranean environment: effects on yield and grain protein content. Agronomy 12, 2152. https://doi.org/10.3390/agronomy12092152

Meena, S.K., Rakshit, A., Meena, V.S., 2016. Effect of seed bio-priming and N doses under varied soil type on nitrogen use efficiency (NUE) of wheat (Triticum aestivum L.) under greenhouse conditions. Biocatal. Agric. Biotechnol. 6, 68–75. https://doi.org/10.1016/j.bcab.2016.02.010

Mitura, K., Cacak-Pietrzak, G., Feledyn-Szewczyk, B., Szablewski, T., Studnicki, M., 2023. Yield and grain quality of common wheat (Triticum aestivum L.) depending on the different farming systems (organic vs. integrated vs. conventional). Plants 12. https://doi.org/10.3390/plants12051022

Najafi Vafa, Z., Sohrabi, Y., Mirzaghaderi, G., Heidari, G., 2022. Soil microorganisms and seaweed application with supplementary irrigation improved physiological traits and yield of two dryland wheat cultivars. Front. Plant Sci. 13. https://doi.org/10.3389/fpls.2022.855090

Noor, H., Yan, Z., Sun, P., Zhang, L., Ding, P., Li, L., Ren, A., Sun, M., Gao, Z., 2023. Effects of nitrogen on photosynthetic productivity and yield quality of wheat (Triticum aestivum L.). Agronomy 13, 1448. https://doi.org/10.3390/agronomy13061448

Noreen, S., Ali, B., Hasnain, S., 2012. Growth promotion of Vigna mungo (L.) by Pseudomonas spp. exhibiting auxin production and ACC-deaminase activity. Ann. Microbiol. 62, 411–417. https://doi.org/10.1007/s13213-011-0277-7

Ozturk, A., Caglar, O., Sahin, F., 2003. Yield response of wheat and barley to inoculation of plant growth promoting rhizobacteria at various levels of nitrogen fertilization. J. Plant Nutr. Soil Sci. 166, 262–266. https://doi.org/10.1002/jpln.200390038

Pagnani, G., Galieni, A., Stagnari, F., Pellegrini, M., Del Gallo, M., Pisante, M., 2020. Open field inoculation with PGPR as a strategy to manage fertilization of ancient Triticum genotypes. Biol. Fertil. Soils 56, 111–124. https://doi.org/10.1007/s00374-019-01407-1

R Core Team, 2021. A Language and Environment for Statistical Computing 2021 [WWW Document]. URL ⟨https://www.R-project.org/⟩ (accessed 9.17.23).

Regent Instruments, 2016. WinRHIZO.

Rossini, A., Ruggeri, R., Belocchi, A., Rossini, F., 2024a. Response of durum wheat cultivars to climate change in a Mediterranean environment: trends of weather and crop variables at the turn of 21st century. J Agron Crop 210, e12786. https://doi.org/10.1111/jac.12786

Rossini, A., Ruggeri, R., Mzid, N., Rossini, F., Di Miceli, G., 2024b. Codium fragile (Suringar) Hariot as biostimulant agent to alleviate salt stress in durum wheat: preliminary results from germination trials. Plants 13, 283. https://doi.org/10.3390/plants13020283

Rossini, A., Ruggeri, R., Rossini, F., 2024c. Discriminating among alternative dressing solutions for cereal seed treatment: effect on germination and seedling vigor of durum wheat. Int. J. Plant Biol. 15, 230–241. https://doi.org/10.3390/ijpb15020019

Roychowdhury, R., Zilberman, O., Chandrasekhar, K., Curzon, A.Y., Nashef, K., Abbo, S., Slafer, G.A., Bonfil, D.J., Ben-David, R., 2023. Pre-anthesis spike growth dynamics and its association to yield components among elite bread wheat cultivars (Triticum aestivum L. spp.) under Mediterranean climate. Field Crops Res. 298. https://doi.org/10.1016/j.fcr.2023.108948

Sadak, M.Sh, Talaat, I.M., 2021. Attenuation of negative effects of saline stress in wheat plant by chitosan and calcium carbonate. Bull. Natl. Res. Cent. 45. https://doi.org/10.1186/s42269-021-00596-w

Saquee, F.S., Diakite, S., Kavhiza, N.J., Pakina, E., Zargar, M., 2023. The efficacy of micronutrient fertilizers on the yield formulation and quality of wheat grains. Agronomy. https://doi.org/10.3390/agronomy13020566

Sedri, M.H., Niedbała, G., Roohi, E., Niazian, M., Szulc, P., Rahmani, H.A., Fieziasl, V., 2022. Comparative analysis of plant growth-promoting rhizobacteria (PGPR) and chemical fertilizers on quantitative and qualitative characteristics of rainfed wheat. Agronomy 12. https://doi.org/10.3390/agronomy12071524

Shah, M.T., Zodape, S.T., Chaudhary, D.R., Eswaran, K., Chikara, J., 2013. Seaweed SAP as an alternative liquid fertilizer for yiled and quality improvement on wheat. J. Plant Nutr. 36, 192–200. https://doi.org/10.1080/01904167.2012.737886

Singh, I., 2018. Plant growth promoting rhizobacteria (PGPR) and their various mechanisms for plant growth enhancement in stressful conditions: a review. Eur. J. Biol. Res. 8, 191–213. https://doi.org/10.5281/zenodo.1455995

Spada, M., Marín-Sanz, M., Bigini, V., Quagliata, G., Coppa, E., Barro, F., Savatin, D., Ruggeri, R., Sestili, F., Rossini, F., Astolfi, S., 2024. Use of biostimulants for water stress mitigation in two durum wheat (Triticum durum Desf.) genotypes with different drought tolerance. Plant Stress 14, 100566. https://doi.org/10.1016/j.stress.2024.100566

Tedone, L., Verdini, L., Grassano, N., Tarraf, W., De Mastro, G., 2014. Optimising nitrogen in order to improve the efficiency, eco-physiology, yield and quality on one cultivar of durum wheat. Ital. J. Agron. 9, 49. https://doi.org/10.4081/ija.2014.536

Tounsi-Hammami, S., Hammami, Z., Dhane-Fitouri, S., Le Roux, C., Jeddi, F.Ben, 2022. A mix of agrobacterium strains reduces nitrogen fertilization while enhancing economic returns in field trials with durum wheat in contrasting agroclimatic regions. J. Soil Sci. Plant Nutr. 22, 4816–4833. https://doi.org/10.1007/s42729-022-00962-1

Trachsel, S., Kaeppler, S.M., Brown, K.M., Lynch, J.P., 2011. Shovelomics: high throughput phenotyping of maize (Zea mays L.) root architecture in the field. Plant Soil 341, 75–87. https://doi.org/10.1007/s11104-010-0623-8

USDA, 2023. World Agricultural Production [WWW Document]. URL 〈https://www.fas.usda.gov/data/world-agricultural-production〉 (accessed 9.13.23).

Vafa, Z.N., Sohrabi, Y., Sayyed, R.Z., Suriani, N.L., Datta, R., 2021. Effects of the combinations of rhizobacteria, mycorrhizae, and seaweed, and supplementary irrigation on growth and yield in wheat cultivars. Plants 10. https://doi.org/10.3390/plants10040811

Valente, F., Panozzo, A., Bozzolin, F., Barion, G., Bolla, P.K., Bertin, V., Potestio, S., Visioli, G., Wang, Y., Vamerali, T., 2024. Growth, photosynthesis and yield responses of common wheat to foliar application of methylobacterium symbioticum under decreasing chemical nitrogen fertilization. Agriculture 14, 1670. https://doi.org/10.3390/agriculture14101670

Van Herwaarden, A.F., Farquhar, G.D., Angus, J.F., Richards, R.A., Howe, G.N., 1998. Haying-off ', the negative grain yield response of dryland wheat to nitrogen fertiliser I. Biomass, grain yield, and water use. Aust. J. Agric. Res. 49, 1067–1081. https://doi.org/10.1071/AR97039

Veraverbeke, W.S., Delcour, J.A., 2002. Wheat protein composition and properties of wheat glutenin in relation to breadmaking functionality. Crit. Rev. Food Sci. Nutr. 42, 179–208. https://doi.org/10.1080/10408690290825510

Wang, Y., Liu, S., Zhang, H., Zhao, Y., Zhao, H., Liu, H., 2014. Glycine betaine application in grain filling wheat plants alleviates heat and high light-induced photoinhibition by enhancing the psbA transcription and stomatal conductance. Acta Physiol. Plant 36, 2195–2202. https://doi.org/10.1007/s11738-014-1596-7

Yaghoubi Khanghahi, M., AbdElgawad, H., Verbruggen, E., Korany, S.M., Alsherif, E.A., Beemster, G.T.S., Crecchio, C., 2022. Biofertilisation with a consortium of growth-promoting bacterial strains improves the nutritional status of wheat grain under control, drought, and salinity stress conditions. Physiol. Plant 174. https://doi.org/10.1111/ppl.13800

Zhou, M., Yang, J., 2023. Delaying or promoting? Manipulation of leaf senescence to improve crop yield and quality. Planta 258, 48. https://doi.org/10.1007/s00425-023-04204-1

Zia, R., Nawaz, M.S., Yousaf, S., Amin, I., Hakim, S., Mirza, M.S., Imran, A., 2021. Seed inoculation of desert-plant growth-promoting rhizobacteria induce biochemical alterations and develop resistance against water stress in wheat. Physiol. Plant 172, 990–1006. https://doi.org/10.1111/ppl.13362