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Article

Intensification of Pea (*Pisum sativum* L.) Production in Organic Farming: Effects of Biological Treatments on Plant Growth, Seed Yield, and Protein Content

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Abstract

The adoption of biological control strategies plays a crucial role in ensuring the sustainability of organic agricultural practices. A field experiment was conducted in 2023 and 2024 to evaluate the impact of biological treatments using lactic acid bacteria (LAB) *Lactiplantibacillus plantarum* and mycoparasitic fungus (MPF) *Trichoderma virens* applied through seed treatment and foliar application separately and in combination on agronomic characteristics and pea yield in organic cultivation. Seed treatment with LAB and MPF resulted in a notable improvement in shoot length and root dry weight, while an increase in root nodule number was observed exclusively with LAB. The combined application of MPF as a seed treatment and LAB as a foliar application at the flowering stage significantly enhanced pod weight per plant, seed number per pod and per plant, and seed weight compared to treatments with LAB applied as either a foliar or seed treatment separately, as well as the untreated control. However, the yield responses to individual and combined treatments under field conditions demonstrated variability and inconsistency. Protein content ranged from 21.24% to 21.61%, and no significant differences observed between treatments. This is the first field report directly comparing the effectiveness of treatments on organic pea production. The findings offer promising avenues for assessing the long-term impacts of these treatments on the sustainable intensification of pea cultivation.

Keywords: agrotechnology; biological treatment; lactic acid bacteria; mycoparasitic fungi; pea; organic farming; yield



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1. Introduction

Legumes are of particular significance in organic farming systems, serving as one of the key tools that contribute to ecosystem resilience, support biodiversity, and ensure

long-term sustainability in agriculture [1,2]. They have a great role in maintaining soil fertility through biological nitrogen fixation, supporting the growth of subsequent crops, as well as providing a source of plant proteins for animal feed and human food [3]. The integration of legumes into crop rotation decreased fertilizer N requirements by 38% for subsequent crops [4].

In the Czech Republic, organic farming covered 595,190 hectares, accounting 16.8% of the total agricultural area, with a 3.4% increase in 2023 [5]. Recent trends indicate expansion in both the number of organic farms and organically grown areas within the country [5]. Pea represents the predominant legume species cultivated in the Czech Republic, comprising approximately 90% of the total area of legumes grown for grain [5]. The crop has recently gained new interest due to its biological nitrogen fixation potential, increasing organic farming, and demand for plant-based products. Biological nitrogen fixation (BNF) is a crucial process in pea cultivation, enabling the plant to convert atmospheric nitrogen into a usable form through symbiosis with *Rhizobium* bacteria in root nodules [6]. However, the efficiency of BNF in organic farming systems is often compromised by several limitations, including the lack of suitable symbiotic rhizobial strains, micronutrient deficiencies, and particularly low levels of available phosphorus, one of the most limiting nutrients for peas, which are not always sufficiently supplied under organic conditions where the use of synthetic fertilizers is prohibited [7]. These limitations negatively affect effective nodulation and ultimately impact pea growth and yield. In addition, organic pea production faces challenges like high susceptibility to pests, diseases, and weeds; unstable yields and is often lower than in conventional farming [8–10]. Biological control is integral to organic legume cultivation. In recent years, the study of the beneficial role of plant-associated microbiomes, particularly in enhancing plant growth and defense against diseases of many crops, has gained significant attention as an environmentally friendly alternative [11]. Biocontrol agents, notably *Trichoderma* and *Lactobacillus*, have been utilized in crop protection strategies through different mechanisms [11]. *Trichoderma* genera were reported not only as plant growth promoters but also as antagonists to pathogens, and *Lactobacillus* acts as a biocontrol and plant growth biostimulant [12,13]. *Trichoderma* spp. are widely used as seed treatments to manage seed- and soil-borne pathogens, stimulate host defense mechanisms, and enhance plant growth and yield [14–17]. *Trichoderma virens* has been demonstrated to enhance plant growth and contribute to improved crop yield [18]. After sowing, beneficial fungi are introduced into the soil via seed surface application, where they offer direct antagonism against harmful organisms and improve soil suppressiveness, ensuring sustained preventive protection for plants [19,20]. According to scientific evidence, lactic acid bacteria (LAB) are utilized in agricultural systems to control diseases, promote growth and seed germination, alleviate various abiotic stressors, and improve soils [21]. *Lactobacillus* sp. has been demonstrated to produce gibberellin and indole-3-acetic acid, which contribute to the elongation of roots, enhance stress resistance, and stimulate flowering in plants [22]. *Lactiplantibacillus plantarum* (formerly *Lactobacillus plantarum* [23]) is the most prevalent LAB species associated with antifungal activity and its key role in inhibiting the growth of a variety of fungi [24,25].

These biocontrol efforts, however, have predominantly been limited to controlled laboratory and greenhouse environments; the effectiveness of current treatments under field conditions is unclear [26,27]. Most biological control strategies depend on the use of a single biocontrol agent to resist different plant pathogens [28]. Many researchers have found that a combination of biological control agents (BCAs) can enhance their antagonistic efficacy against a wide range of plant pathogens [29]. Although *L. plantarum* and *T. virens* are widely recognized for their plant growth-promoting and biocontrol capabilities, these beneficial traits can sometimes be limited by genetic burden. The expression of these traits

may vary under field conditions, which can reduce their efficacy compared to controlled settings. In this context, studying the interactions among beneficial microorganisms is of paramount importance because such interactions might either inhibit or enhance the beneficial effects of the individual contributions of each species [28]. Hence, a study of the individual and combined effects of lactic acid bacteria (LAB) and mycoparasitic fungi (MPF) on pea production has substantial potential value for advancing organic agriculture.

The efficacy of seed and foliar treatments can be measured by assessing visual signs of shoot and root growth parameters, nodulation as an indicator of nitrogen fixation potential, yield components, and yield. The objectives of this study were to (1) assess whether the different methods of application of seed treatment, foliar application during vegetation, and their combination are effective on seedling emergence, plant growth, and yield of peas under field conditions; and (2) evaluate if there is any effect of the interaction of lactic acid bacteria and mycoparasitic fungi on pea if they are applied independently or in combination.

2. Materials and Methods

2.1. Experimental Site and Weather Conditions

Field experiments were assessed in organic field trial plots at organic farm in Zvíkov, České Budějovice, for the 2 consecutive growing seasons in 2023–2024. The soil texture of the experimental field is silt loam following USDA with 25% sand, 57% silt, and 18% clay; pH (H₂O): 5.6, total organic carbon (TOC): 1.98%, NO₃[−]: 52 mg kg^{−1}, NH₄⁺: 18.4 mg kg^{−1}, P: 59 mg kg^{−1}, K: 285 mg kg^{−1}. Field management practices of pea cultivation in each growing season are presented in Table 1.

Table 1. Field management practices in site experiments over two years.

Activity	Growth Stage	2023	2024
Plots seeded	BBCH 0	23 March	27 March
Emergence	BBCH 09	24 April	13 April
Plant sample collection 1	BBCH 13–15	11 May	23 April
Plant sample collection 2	BBCH 14–16	18 May	30 April
Plant sample collection 3	BBCH 15–17	25 May	7 May
Foliar application	BBCH 61–62	20 June	14 June
Plot harvest	BBCH 99	28 July	26 July
Day of mature (days)	-	127	121

Plant growth stages were identified according to a phenological growth stage key (BBCH) [30,31].

The weather conditions during the two-year experiment and long term are given in Figure 1. The average air temperature during our research period was higher compared to the multi-year period (2023 season, 12.3 °C; 2024 season, 13.4 °C; and the multi-year period, 10.6 °C; Figure 1). The total rainfall from March to July in the 2023 season was lower than in 2024 and multi-year periods (279 mm, 360 mm, and 343 mm, respectively) (Figure 1). In the 2023 season, the temperature after sowing was lower compared to 2024 and the multi-year period. Rainfall in April 2024 was lower than in April 2023, whereas in May, June, and July 2024, it was higher than in the same months in 2023.

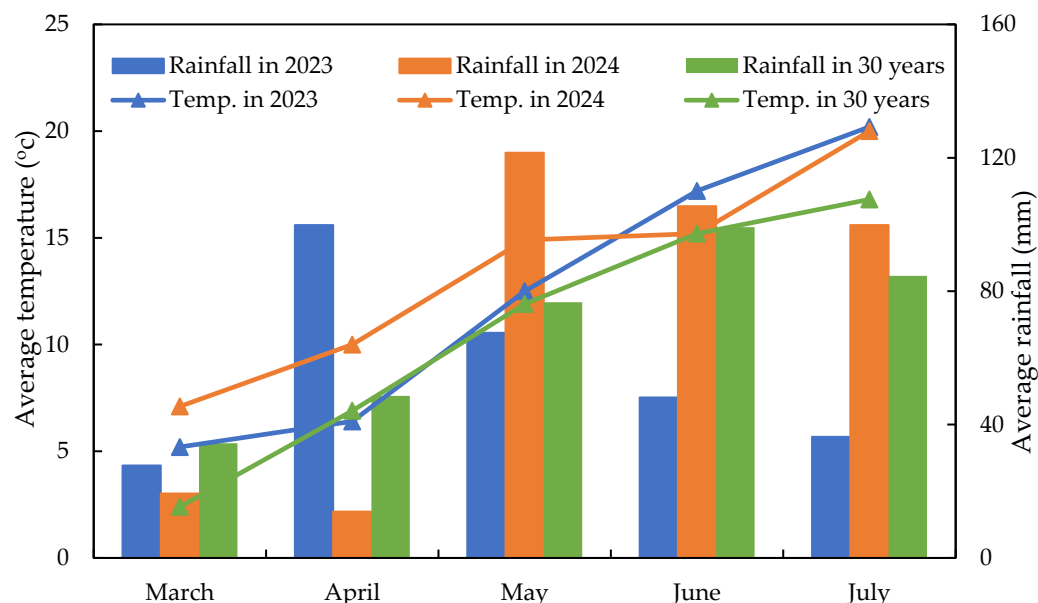


Figure 1. Monthly average temperatures and total precipitation during the pea growing seasons over two years.

2.2. Mycoparasitic Fungus and Lactic Acid Bacteria

The mycoparasitic fungus *Trichoderma virens*, strain TVI 113, was re-isolated from the locality Jiretice, Czech Republic and stored as alginate prills in the collection (Department of Plant Production, Faculty of Agriculture and Technology, USB). The strain was activated and grown on potato dextrose agar (PDA; Sigma-Aldrich, Taufkirchen, Germany) and incubated for 5 days at 25 ± 1 °C in an incubator (Q-Cell 180/40, Pol Lab Ltd., Wilkowice, Poland) under a 0 h:24 h light/dark photoperiod.

The strain *Lactiplantibacillus plantarum* with the acronym CCDM 1110 from the CCDM[®] collection (Milcom Ltd., Tábor, Czech Republic) was used for seed coating and foliar application. The lyophilised strain was activated in reconstituted milk (10%) and then cultivated at 30 °C in De Man, Rogosa and Sharpe (MRS) broth (Merck KGaA, Darmstadt, Germany) enriched with FGGM (fructose, glucose, sodium glutamate and maltose) according to the M638 recipe (DSMZ, German Collection of Microorganisms and Cell Cultures GmbH, Braunschweig, Germany) to increase the production of functional metabolites [32,33].

2.3. Preparation of Spore Suspension and Seed Coating

Carboxymethyl cellulose (CMC; Sigma-Aldrich, Germany) was used as an adhesive to attach fungal spores to the seed surface.

A spore suspension of *T. virens* strain TVI 113 was prepared by flooding the surface of the sporulated culture with a sterile 0.05% Tween[®] 80 solution (Sigma-Aldrich, Taufkirchen, Germany) and spores were scratched with an inoculating loop. The obtained suspension was filtered through sterile gauze and spore concentration was determined using a Neubauer improved counting chamber (Bright-Line[™]), and the suspension was adjusted to a final concentration of 2×10^6 spores.mL⁻¹. A 1.0% CMC solution was first prepared and then mixed in a 1:1 (v/v) ratio with the *T. virens* spore suspension (2×10^6 spores.mL⁻¹). This final solution contains 0.5% CMC and 1×10^6 spores/mL.

The suspension of *L. plantarum* CCDM 1110 was based on hydroxymethyl cellulose (2% v/v) and Tween T81 (1% v/v) in distilled water supplemented by bacterial culture (20% v/v) containing 1×10^7 – 10^8 CFU.mL⁻¹ bacterial cells. The suspension of *L. plantarum* CCDM 1110 suspension (1×10^7 – 10^8 spores.mL⁻¹) was mixed with 1.0% CMC solution in a 1:1 (v/v) ratio. This final solution contains 0.5% CMC and 5×10^6 – 10^7 spores.mL⁻¹.

The seeds were coated and dried under sterile conditions of laminar flow (Telstar, Biostar Plus 4, Terrassa, Spain). Coated seeds were stored in the refrigerator (4 ± 2 °C) to ensure the viability of the fungal spores and to prevent premature germination or rapid inactivation of spores due to high temperatures.

2.4. Experimental Design and Foliar Treatments

The small plot experiment was conducted with the randomized complete block design in three replicates. Plots were 1.2 by 10.0 m with 10 rows. The Avatar pea variety was used in the experiment with field sowing pea density of 400 g plot⁻¹ (33.33 g seed m⁻²). The soil was fertilized with composted sheep manure of 4 t ha⁻¹ before plowing. Pesticides and herbicides were not used for the experiment. Standard small-plot mechanization was used to establish, manage, and harvest the experiment. Treatment combinations (Table 2), including untreated control, seed treatments, and application utilizing lactic acid bacteria by lactic acid bacteria (*L. plantarum*) and the mycoparasitic fungus (*T. virens*) were applied separately or in combination. The foliar applications were applied at the flowering stage, BBCH 61–62. For foliar applications, fermentation of *L. plantarum* CCDM 1110 was produced under static growth conditions (pH 5.7, 34 °C, 24 h). The final concentration of the solution was 1×10^7 – 10^8 CFU.mL⁻¹). Fungus *T. virens* TVI 113 was produced using solid-state fermentation on natural substrate. The spores of *T. virens* were harvested by scraping off spores from the surface of natural substrates. The concentration of spores per 1 g was 1×10^9 spores in 1 g.

Table 2. Experimental treatments to assess the effect of seed and foliar treatments on pea under field conditions.

	Treatment Name	Timing	Abbreviation
1	Control		Control
2	Lactic Acid Bacteria (<i>L. plantarum</i>)	Seed treatment	LABse
3	Mycoparasitic Fungus (<i>T. virens</i>)	Seed treatment	MPFse
4	Lactic Acid Bacteria (<i>L. plantarum</i>)	Foliar application	LABfo
5	Mycoparasitic Fungus (<i>T. virens</i>)	Foliar application	MPFfo
6	Lactic Acid Bacteria (<i>L. plantarum</i>)	Seed treatment + Foliar application	LABse × fo
7	Mycoparasitic Fungus (<i>T. virens</i>)	Seed treatment + Foliar application	MPFse × fo
8	Lactic Acid Bacteria (<i>L. plantarum</i>) + Mycoparasitic Fungus (<i>T. virens</i>)	Seed treatment + Foliar application (combination)	LABse × MPFfo
9	Mycoparasitic Fungus (<i>T. virens</i>) + Lactic Acid Bacteria (<i>L. plantarum</i>)	Seed treatment + Foliar application (combination)	MPFse × LABfo

The *T. virens* strain TVI 113 and *L. plantarum* CCDM 1110 were applied to the stands. For each strain, a 10-L application dose was prepared containing spores of the microorganisms, a wetting agent based on rapeseed oil methyl ester and maltodextrin sugar, which was the nutrient for both microorganisms. The spray thus prepared was applied with a backpack sprayer to the designated variants. Final concentration of application dose of *T. virens* was 5×10^5 spores.mL⁻¹ and *L. plantarum* 5×10^4 – 10^5 CFU.mL⁻¹.

2.5. Plant Measurements and Quality Evaluations

In the initial growth phases from BBCH 13–17, plant sampling was realized at three weekly intervals. Every week whole plants including root systems were collected to evaluate the effect of seed treatment on shoot and root parameters. The field emergence was evaluated before sampling.

For each sampling, 5 plants were randomly excavated from each variant and replication. The root system and canopy of each plant were rinsed under running water and consequently, the number of nodules per plant, shoot length, and root length were recorded.

Nodulation position and plant vigour efficiency were also assessed [34]. The dry matter of the plant and root were recorded after drying at a constant temperature (plant samples are dried at 105 °C for at least 4 h, depending on the size of plants and root systems).

Leaf chlorophyll content was assessed by SPAD 502-plus chlorophyll meter (Mi-nolta Corporation, Ltd., Osaka, Japan) and was measured 1 and 2 weeks after MEF and LAB foliar application with each treatment. The mean SPAD reading was calculated by the instrument from three readings taken from ten plants per plot, on fully expanded stipules, using the second or third node counting down from the tip of a main stem [35].

During the flowering and pod development stages, the number of flowers, number of pods, and plant height were recorded from five randomly selected plants. Before the final harvest, five plants per plot were evaluated for the number of nodes, number of pods and seeds, and the weight of pods and seeds. After the plot harvest, the seeds were cleaned and the mean samples were taken from each replication, and the pea yield was recorded and calculated. A thousand kernel weight was also determined, and the hectoliter weight (kg hL^{-1}) was measured using the Dickey-john GAC500XT. The PSY 20 (Mezos, Hradec Kralove, Czech Republic) and Quadrumat Junior machines (Brabender, Duisburg, Germany) were used to mill the pea flour samples (1 kg). The Kjeldahl technique (Kjeltec 1002 System, Tecator AB, Hoganas, Sweden) was used to calculate the protein content (PC) (in dry matter).

2.6. Statistical Analysis

Statistical analysis was performed to determine the effect of treatments applied independently or in combination on pea. The data were statistically evaluated by an analysis of variance (ANOVA), and Tukey's HSD (Honest Significant Difference) were subsequently used to test for differences in mean values with a significance level of $p < 0.05$. Dunnett's test was used to analyze the effect of treatments relative to the untreated control for shoot length, dry weight of root, crown nodules, lateral nodules, and SPAD value in each year. Principal component analysis (PCA) and correlation analysis were used to determine the correlations among shoot and root growth parameters, as well as the correlation between seed yield, yield components, and protein content. All statistical analyses were performed using the STATISTICA program (version 13.2, StatSoft, Inc., Tulsa, OK, USA).

3. Results

3.1. Effects of Seed Treatment on Emergence, Growth Parameters, and Chlorophyll Content

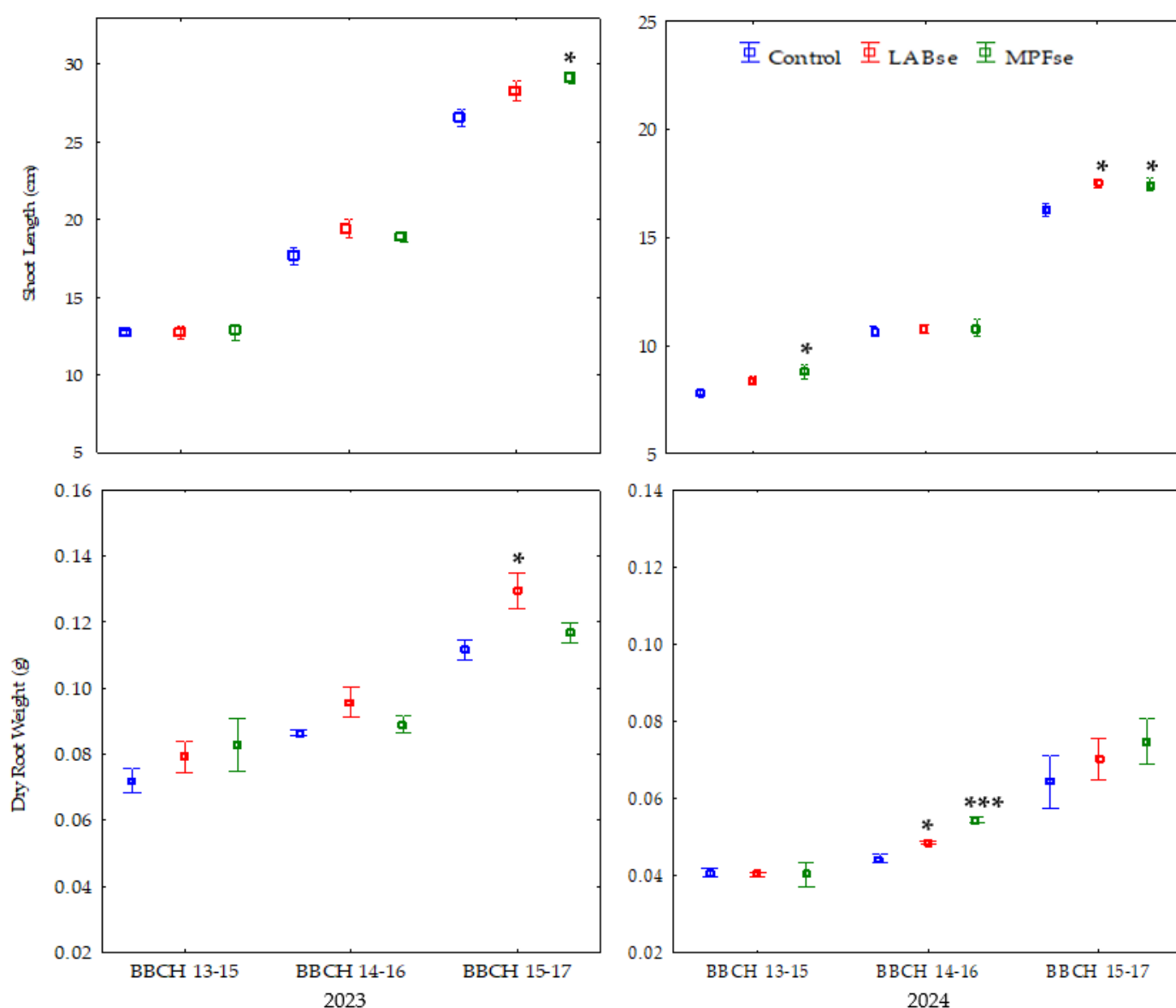
The effect of seed treatment on field emergence, growth parameters, and nodules of roots during both years of experimentation is presented in Table 3, Figures 2 and 3. The results showed that shoot and root length, dry weight of shoot and root, root nodules, and nodule position were higher in the 2023 season than in 2024, except field emergence in 2023 was lower than in 2024, and plant vigour was similar in both years. Detailed results for individual years and growth stages are presented as Supplementary Material in Table S1.

The treatment of pea seeds with LAB and MPF resulted in a significant increase in shoot length ($p < 0.001$) and dry root ($p = 0.007$) (Table 3). Specifically, during the BBCH15–17 growth stage in the 2023 season, significant differences were observed in root length for the MPF seed treatment and in root dry weight for the LAB seed treatment compared to the untreated control. (Dunnett's test, $p < 0.05$). The root length recorded was statistically similar at the BBCH13–15, BBCH15–17 stages, and the root dry weight at the BBCH14–16 stage in the 2024 season (Figure 2).

Table 3. Effect of seed treatment on growth parameters and root nodules of pea under field conditions in 2023 and 2024.

Variation	Field Emergence (%)	Shoot Length (cm)	Root Length (cm)	Dry Shoot Weight (g)	Dry Root Weight (g)	Root Nodules (No. Plant ⁻¹)	Nodule Position	Plant Vigour
Season								
2023	66.19	19.80 ^a	13.35 ^a	0.42 ^a	0.096 ^a	38.95 ^a	2.22 ^a	4.84
2024	67.82	12.06 ^b	10.70 ^b	0.18 ^b	0.053 ^b	19.64 ^b	2.01 ^b	4.84
<i>p</i> -Value	ns	***	***	***	***	***	**	ns
Treatment								
Control	64.64	15.30 ^b	11.86	0.29	0.070 ^b	27.24 ^b	1.99 ^b	4.79
LABse	69.95	16.19 ^a	12.13	0.30	0.077 ^a	31.59 ^a	2.14 ^{ab}	4.84
MPFse	66.43	16.31 ^a	12.09	0.30	0.076 ^a	29.06 ^b	2.22 ^a	4.88
<i>p</i> -Value	ns	***	ns	ns	**	***	*	ns

Different letters within the column show a statistical difference at *p*-Value < 0.05, Tukey HSD test. ns (non-significant); * *p* < 0.05; ** *p* < 0.01; *** *p* < 0.001. The abbreviations are indicated in Table 2.

**Figure 2.** Effect of seed treatment on shoot length and dry root weight of pea at three samplings times in 2023 and 2024. Error bar indicates standard error (*n* = 3). Symbols indicate a significant difference from untreated control at *** *p* < 0.001, * *p* < 0.05 by Dunnett's test. The abbreviations are indicated in Table 2.

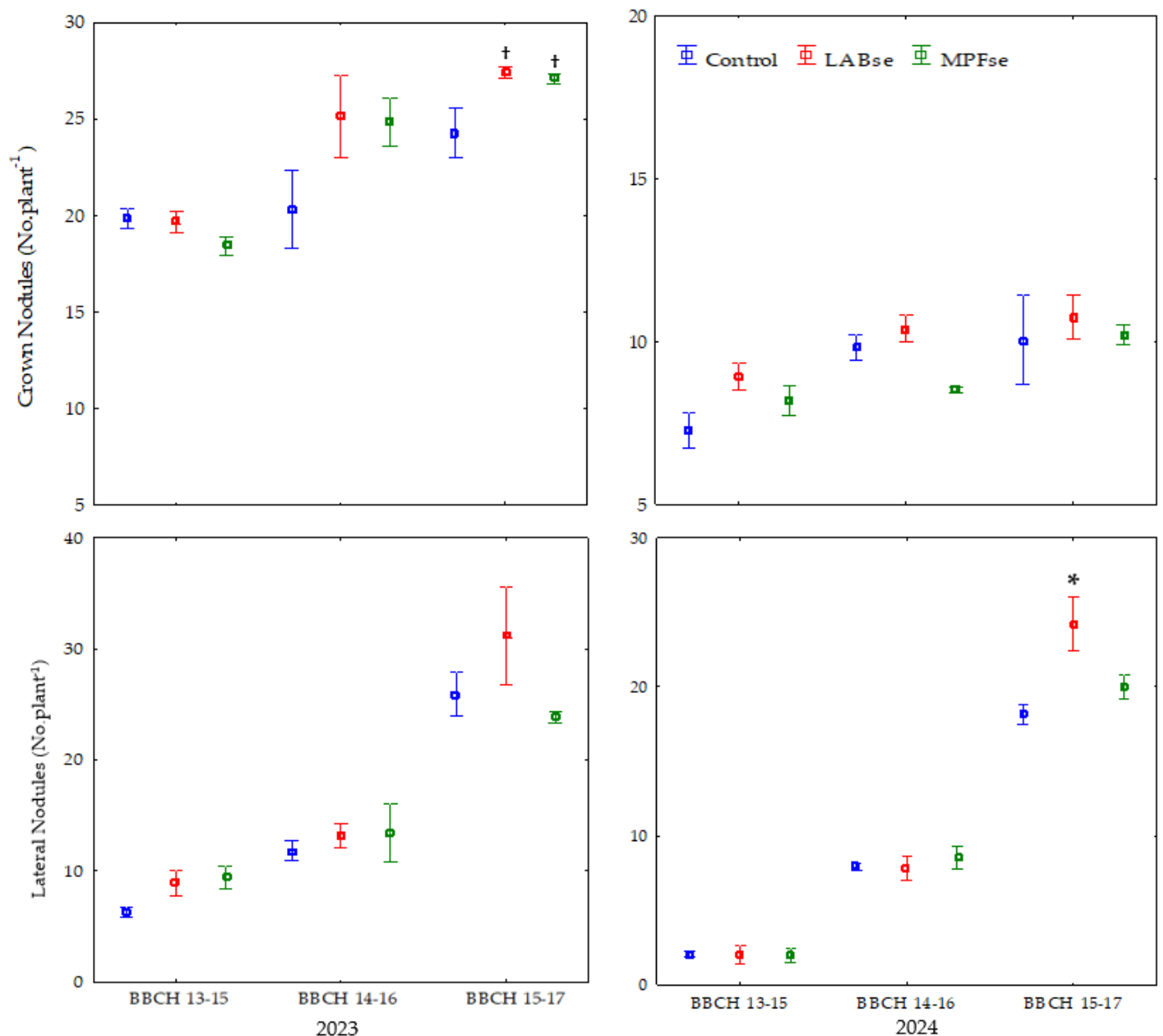


Figure 3. Effect of seed treatment on crown nodules and lateral nodules of pea at three samplings times in 2023 and 2024. Error bar indicates standard error ($n = 3$). Symbols indicate a significant difference from untreated control at * $p < 0.05$, † $p < 0.1$ by Dunnett's test. The abbreviations are indicated in Table 2.

The root nodules of the seed treatment of LAB were significantly higher than (31.59 nodules. plant⁻¹) in the untreated control (27.24 nodules. plant⁻¹) and seed treatment of MPF (29.06 nodules. plant⁻¹) (Table 3). Notably, at the BBCH15–17 stage in the 2023 season, seed treatment resulted in a higher number of crown nodules compared to the untreated control (Dunnett's test, $p < 0.1$). Seed treatment of LAB recorded nodules per plant and lateral nodules were higher than the untreated control (Dunnett's test, $p < 0.05$) (Figure 3).

The nodule position of the seed treatment with MPF was significant for the untreated control ($p = 0.0326$). However, there was no difference in field emergence, root length and plant vigour among the seed treatments (Table 3).

Principal component analysis based on the correlation of growth parameters and root nodules. The first two PCs explained 94.51% of the total variability in the data (Figure 4).

Shoot and root length, shoot dry weight, root dry weight and the number of nodules were positioned near each other and on the side of PCA1, indicating a significant positive correlation ($r = 0.960, 0.0885, 0.934$, and 0.941 , respectively). The number of crown nodules and lateral nodules were also strongly positively correlated with the total number of nodules ($r = 0.840, 0.894$, respectively) (Table 4 and Figure 4). This PCA result supports the earlier evidence of association between shoot and root length, root dry weight, and nodule number of treatments compared to untreated controls. The longer the shoot and root, the greater the number of nodules and the higher the root dry weight (Table 3, Figures 2 and 3).

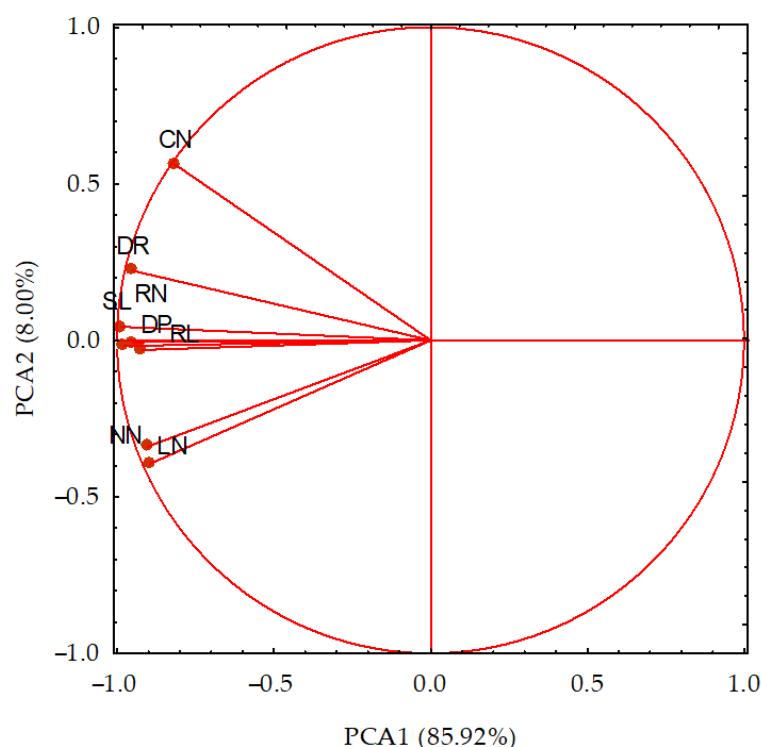


Figure 4. Principal components analysis (PCA) 1 and 2 demonstrating relationships among shoot length (SL), root length (RL), dry shoot weight (DS), dry root weight (DR), root nodules (RN), crown nodules (CN), lateral nodules (LN) of seed treatment of pea grown under field conditions in 2023 and 2024.

Table 4. Correlation between shoot and root parameters of pea.

Variation	Shoot Length (cm)	Root Length (cm)	Dry Shoot Weight (g)	Dry Root Weight (g)	Root Nodules (No. Plant ^{−1})	Crown Nodules (No. Plant ^{−1})	Lateral Nodules (No. Plant ^{−1})
SL	1.000	0.856	0.977	0.913	0.960	0.780	0.879
RL		1.000	0.789	0.881	0.885	0.731	0.804
DS			1.000	0.891	0.934	0.753	0.861
DR				1.000	0.941	0.881	0.770
RN					1.000	0.840	0.894
CN						1.000	0.509
LN							1.000

SL, shoot length; RL, root length; DWS, dry weight shoot; DWR, dry weight root; RN, root nodules; CN, crown nodules; LN, lateral nodules. Correlations are significant at $p < 0.05$.

The findings of chlorophyll content assessment in pea leaves at 7 and 14 days after foliar application during 2023 and 2024 are presented in Figure 5.

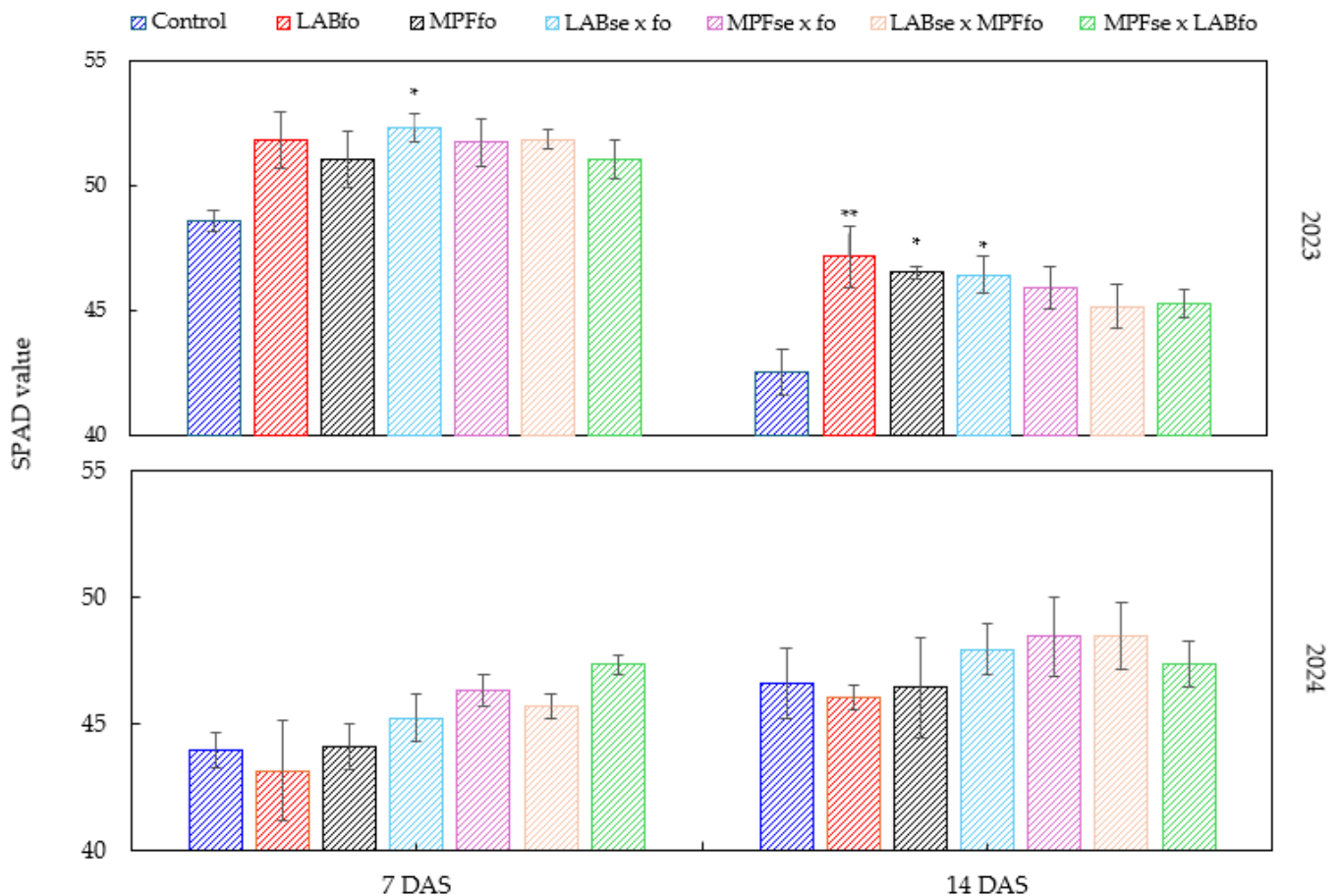


Figure 5. Effect of treatment on Chlorophyll content of pea grown at 7 and 14 days after spray under field conditions in 2023 and 2024. DAS: day after spray. Error bar indicates the mean $\pm$ standard error ($n = 3$). Symbols indicate a significant difference from untreated control at * $p < 0.05$, ** $p < 0.01$ by Dunnett's test. The abbreviations are indicated in Table 2.

In 2023, at 7 days post-foliar application, SPAD values in the treated plants increased by approximately 5–8% compared to the untreated control. Notably, a statistically significant difference was observed in the seed treatment combined with foliar application of LAB compared to the untreated control (Dunnett's test, $p < 0.05$). Similarly, at 14 days post-application, SPAD values increased by 6.1–10.8% compared to the untreated control. The result of Dunnett's test also showed that the foliar and seed treatment (Dunnett's test, $p < 0.01$), foliar application with LAB and MPF (Dunnett's test, $p < 0.05$) showed significant differences from the control. In contrast, no significant differences were observed between foliar application treatments and the control at 7 or 14 days after application in 2024 (Figure 5).

3.2. Effects of Treatments on Yield Components, Seed Yield, and Protein Content

The effects of the treatments on yield components, yield and protein content measured in the two seasons are summarized in Tables 5 and 6. The results generally indicated a positive trend in several yield components and yield in 2024 compared to 2023. Specifically, the number of seeds per plant, number of pods per plant, pod setting ratio, number of seeds per pod, overall yield, and protein content were all higher in 2024, except for pod weight per plant, one thousand kernel weight and hectoliter weight in 2024 were lower than in 2023. Detailed results for individual years are presented as Supplementary Material in Tables S2 and S3.

Table 5. Effects of treatments on agronomic and reproductive parameters of pea under field conditions in 2023 and 2024.

Variation	Plant Height (cm)	Node Number (No. Plant ⁻¹)	Pod Number Branch ⁻¹ (No.)	Pods Plant ⁻¹ (No.)	Pod Weight (g Plant ⁻¹)	Pod Setting Ratio (%)
Season						
2023	85.55	18.09 ^b	1.74	5.82 ^b	8.42 ^a	60.61 ^b
2024	88.41	19.31 ^a	1.69	8.47 ^a	7.47 ^b	82.88 ^a
Treatment						
Control	83.23	18.07	1.68	6.42	7.04 ^{ab}	63.35 ^b
LABse	87.45	18.90	1.77	7.50	6.86 ^{ab}	82.23 ^a
MPFse	89.25	19.03	1.70	7.77	8.55 ^{ab}	80.30 ^{ab}
LABfo	84.46	18.70	1.69	6.70	6.70 ^b	65.61 ^{ab}
MPFfo	87.71	18.80	1.69	7.77	8.00 ^{ab}	71.78 ^{ab}
LABse × fo	89.25	19.20	1.78	7.73	8.32 ^{ab}	76.64 ^{ab}
MPFse × fo	88.65	18.43	1.78	6.70	8.81 ^{ab}	66.55 ^{ab}
LABse × MPFfo	86.59	18.57	1.73	6.53	8.16 ^{ab}	63.58 ^{ab}
MPFse × LABfo	86.23	18.60	1.61	7.20	9.07 ^a	75.71 ^{ab}
Season	ns	***	ns	***	**	***
Treatment	ns	ns	ns	ns	**	**
Season × Treatment	ns	ns	ns	ns	ns	ns

Different letters within the column show a statistical difference at p -Value < 0.05, Tukey HSD test. ns (non-significant); ** p < 0.01; *** p < 0.001. The abbreviations are indicated in Table 2.

Table 6. Effects of treatments on yield components, yield and protein content of pea under field conditions in 2023 and 2024.

Variation	Seeds Pod ⁻¹ (No.)	Seed Number (No. Plant ⁻¹)	Seed Weight (g Plant ⁻¹)	TKW (g)	Seed Yield (t ha ⁻¹)	HW (kg hL ⁻¹)	Protein Content (%)
Season							
2023	3.50	19.65 ^b	4.34 ^b	211.07 ^a	1.93	79.53 ^a	20.96 ^b
2024	3.37	28.16 ^a	5.70 ^a	198.94 ^b	2.13	74.11 ^b	22.01 ^a
Treatment							
Control	3.27 ^{ab}	18.77 ^b	4.31 ^{ab}	201.34	2.07	76.58	21.24
LABse	3.03 ^b	22.53 ^{ab}	3.95 ^b	202.10	2.03	76.57	21.55
MPFse	3.56 ^{ab}	27.07 ^a	5.45 ^{ab}	204.83	2.27	76.87	21.46
LABfo	3.15 ^{ab}	20.30 ^{ab}	4.14 ^{ab}	204.43	1.51	76.83	21.56
MPFfo	3.34 ^{ab}	26.20 ^{ab}	5.05 ^{ab}	195.01	1.73	77.02	21.33
LABse × fo	3.44 ^{ab}	26.50 ^{ab}	5.51 ^{ab}	209.33	2.41	76.65	21.53
MPFse × fo	3.59 ^{ab}	23.80 ^{ab}	5.86 ^a	210.04	1.97	76.98	21.54
LABse × MPFfo	3.65 ^{ab}	23.00 ^{ab}	4.97 ^{ab}	205.16	2.04	76.77	21.54
MPFse × LABfo	3.89 ^a	27.00 ^a	5.95 ^a	212.79	2.26	77.08	21.61
Season	ns	***	***	**	ns	***	***
Treatment	*	**	**	ns	ns	ns	ns
Season × Treatment	ns	ns	ns	ns	ns	ns	ns

TKW, thousand kernel weight; HW, hectoliter weight. Different letters within the column show a statistical difference at p -Value < 0.05, Tukey HSD test. ns (non-significant); * p < 0.05; ** p < 0.01; *** p < 0.001.

Analysis of variance indicated that different treatments with LAB and MPF applied independently and in combination (seed treatment, foliar treatment, and combination seed + foliar treatment) had significant effects (p < 0.01) on pod setting ratio and the number of seeds per plant in both two years (Tables 5 and 6). The plants whose seeds were treated with LAB had the highest pod setting ratio (82.23%), whereas the lowest number of seeds per pod and seed weight per plant. The combination of the seed treatment with MPF and LAB foliar application showed the highest values, with 9.07 pods plant⁻¹, 3.89 seeds pod⁻¹, and 5.95 g plant⁻¹ of seed weight (Tables 5 and 6).

Although eight different treatments with LAB and MPF applied independently and in combination (seed treatment, foliar treatment, and combination seed + foliar treatment)

showed no significant differences between the treatments and the control, they showed positive increasing trends for plant height (1.6–7.4%), the number of nodes per plant (2.0–6.2%), the number of pods per plant (1.7–21%), and protein content (0.4–1.7%) compared to the control.

Yield was not different among treatments in both 2023 and 2024. Pea yield ranged from 1.51 t ha⁻¹ to 2.27 t ha⁻¹ for the treatments. Likewise, pods per branch ranged from 1.61 to 1.78; one thousand kernel weight ranged from 195.01 to 212.79 g; and hectoliter weight ranged from 76.57 to 77.08 kg hL⁻¹ across all treatments.

Principal component analysis was used to illustrate the correlation of yield components, seed yield, and protein content by simplifying many traits, with PCA1 and 2 describing 60.4% and 11.4% of the overall variance, respectively (Figure 6). The analysis results showed that protein content, plant height, number of seeds per plant, weight of pods per plant, weight of seeds per plant, seeds per plant, and thousand kernel weight were positively correlated with yield with *r* values of 0.711, 0.440, 0.503, 0.556, 0.520, 0.499, and 0.522, respectively (Table 7 and Figure 6).

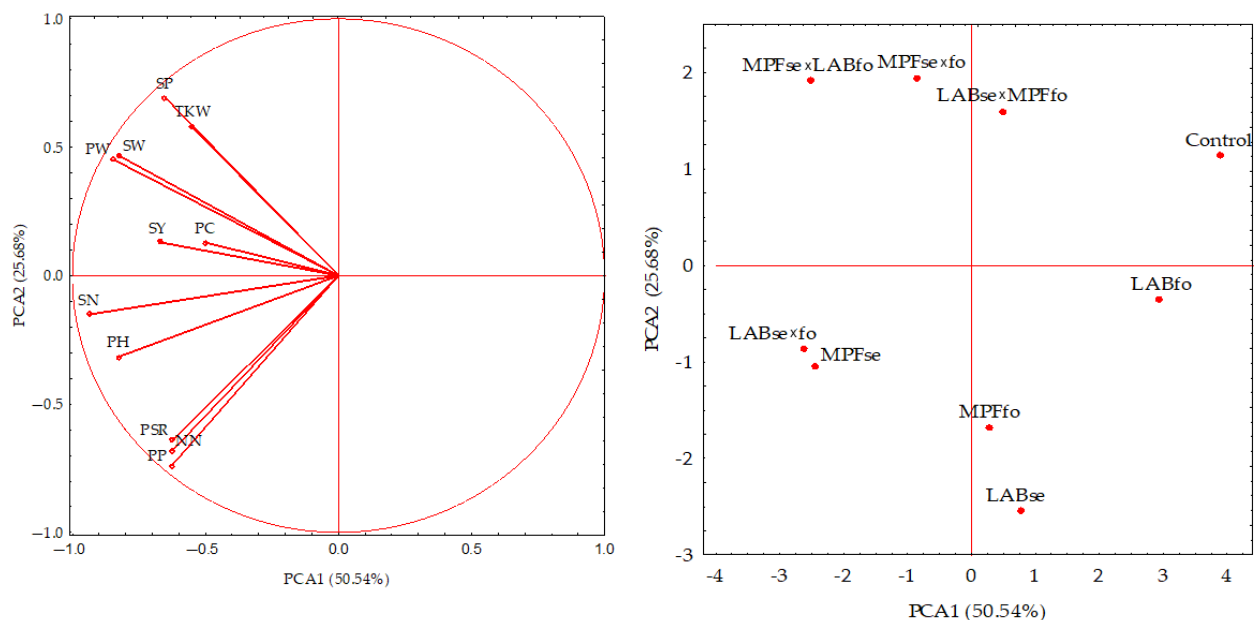


Figure 6. Principal components analysis (PCA) 1 and 2 demonstrating relationships among plant height (PH), node number (NN), pod setting ratio (PSR), pod weight (PW), pods plant⁻¹ (PP), seed number (SN), seed weight plant⁻¹ (SW), seed pods⁻¹ (SP), thousand kernel weight (TKW), seed yield (SY), protein content (PC) of nice different treatments of pea grown under field conditions in 2023 and 2024.

The number of nodes per plant, pod setting ratio, and pod number per plant were clustered near each other in the same quadrant, indicating their positive correlated association, which agreed with the positive association obtained through correlation analysis (Table 7). Meanwhile, the number of seeds per pod was negatively correlated with the number of nodes per plant, pods per plant, and pod setting ratio (Figure 6).

In the multivariate analysis of different treatments, the seed treatment with MPF, the seed and foliar application with LAB, and the combined MPF seed treatment with LAB foliar application were positively correlated with seed yield and protein content. In contrast, the untreated control, seed treatment with LAB, foliar application with LAB, and MPF were negatively correlated with seed yield and protein content (Figure 6).

Table 7. Correlation between yield components, seed yield and protein content of pea.

Variation	Plant Height (cm)	Node Number (No. plant ^{−1})	Pods Plant ^{−1} (No.)	Pod Setting Ratio (%)	Pod Weight (g plant ^{−1})	Seeds Pod ^{−1} (No.)	Seed Number (No. plant ^{−1})	Seed Weight (g plant ^{−1})	TKW (g)	Seed Yield (t ha ^{−1})	Protein Content (%)
PH	1	0.729	0.705	0.607	0.603	0.268	0.794	0.580	0.208	0.440	0.346
NN		1	0.863	0.752	0.162	−0.085	0.659	0.156	0.049	0.271	0.411
PP			1	0.853	0.236	−0.072	0.758	0.229	−0.152	0.284	0.091
PSR				1	0.181	−0.052	0.627	0.136	0.086	0.488	0.292
PW					1	0.910	0.794	0.982	0.571	0.556	0.306
SP						1	0.583	0.875	0.636	0.499	0.320
SN							1	0.765	0.279	0.503	0.319
SW								1	0.614	0.520	0.293
TKW									1	0.522	0.137
SY										1	0.711

PH, plant height; NN, node number; PP, pods plant^{−1}; PSR, pod setting ratio; PW, pod weight; SP, seed pods^{−1}; SN, seed number; SW, weight seed, TKW, thousand kernel weight; SY, seed yield.

4. Discussion

In our study, pre-sowing treatment of pea seeds with LAB and MPF improved plant growth parameters, especially the significant increase in shoot length and dry root, as compared to control plants (Table 1 and Figure 1). These findings align with prior research demonstrating that LAB exerted a substantial influence on plant growth characters, including plant height, root length, and the number of soybean branches [21]. The effect of LAB in seed treatment was also reported to positively influence the development of plantlets, as reflected by significant differences in root and shoot length and weight compared to untreated controls [36]. Several studies have demonstrated that the plant growth-promoting attributes of LAB species predominantly stimulate root development, better root growth, higher germination rates, and increased availability of essential nutrients such as nitrogen, phosphorus, and potassium, contributing to enhanced root and shoot lengths [37,38]. Especially, seeds treated with a coating of *T. vires* has been demonstrated a positive impact on parameters of growth and increased the rate of emergence of plants in field conditions [18]. Seed treatment of chickpeas with some *Trichoderma* species has been demonstrated to promote plant growth; notably, the use of seven isolates of *T. vires* as a seed treatment has been shown to improve germination rates and increase root biomass of soybean [39,40].

However, several studies have indicated that while LAB seed treatment resulted in a significant increase in shoot length compared to the control, there was no obvious increase in root length [41]. The results by Gwiazdowski et al. (2023) reported that certain strains significantly promoted plant growth, whereas others exhibited no observable effect on plant development [42]. Our results also showed no significant differences in field emergence rate, root length, or plant vigour compared to the control (Table 1). The experiment conducted in the field provided a more accurate assessment of the potential of the tested microorganisms; however, environmental factors and plant host selection significantly influenced the growth promotion effects, frequently resulting in inconsistent field performance [12,43,44]. Data revealed that foliar application of LAB and MPF during the flowering stage of pea plants had no statistically significant influence on leaf chlorophyll content. Fluctuations in leaf chlorophyll content (SPAD) could be associated with a plant adaptation to environmental stressors encountered during the flowering phase. Less rainfall and higher temperatures in June 2023, relative to the same timeframe in 2024, showed that SPAD values decreased by 11% at 14 days post-foliar application (Figures 1 and 4).

The interactions between plant roots and the soil microbiota play a critical role in plant nutrition, and the application of lactic acid bacteria may facilitate the establishment

of favorable conditions for nodulation [21,45]. In this study, seed treatment with LAB resulted in a higher number of nodules per plant, including an increased presence of lateral nodules, compared to the untreated control (Table 2 and Figure 4). PCA results also revealed a significant positive correlation between shoot and root length, shoot and root dry weight, and root nodules (Table 2 and Figure 4). This indicates that the biomass stimulation concerned is not only the aerial parts but also the root system, demonstrating a significant increase [46].

Various studies have indicated that the use of LAB or any single biological treatment is still ineffective in practical applications; therefore, an integrated approach combining LAB with other biological treatments is necessary [38]. In our results, the combined application of seed treatment with MPF and foliar application of LAB showed a general trend of increasing seeds per pod and per plant, as well as pod and seed weight per plant, compared to LAB applied separately as a seed or foliar treatment and the untreated control (Tables 4 and 5). Previous studies have also indicated that combined inoculation with *Rhizobium*, *Bacillus* strains, and *Trichoderma* spp. not only inhibited soil-borne fungi but also significantly improved chickpea yield compared to using either separately or the control [47]. Similarly, the combined application of *Rhizobium leguminosarum* and *Trichoderma* species proved more effective than the use of either alone [48]. The results of Abd-El-Khair et al. (2018) also revealed that *T. harzianum* or *Bacillus subtilis* combined with *Rhizobium* greatly increased pods per plant and pod fresh and dry weight of faba bean plants compared to each treatment applied independently [49]. In contrast, some studies have found that combining *B. subtilis*, *Bradyrhizobium japonicum*, and *T. harzianum* in peanut plants did not result in increased plant growth and development compared to using them separately [43]. The treatments with LAB and MPF applied independently and in combination on pea under field conditions showed an enhancement of 1.4–7.2% in plant height, 1.7–21.0% in the number of pods per plant, and 0.4–1.74% in protein content; however, no significant differences were observed between the treatments and the untreated control (Tables 4 and 5). In our study, protein content in 2024 (22.01%) was higher than in 2023 (20.96%). This outcome aligns with previous findings, which suggest that protein content in peas is influenced not only by genetic factors but also by environmental conditions, particularly temperature and rainfall [50].

The inconsistencies in the field performance, viability, and environmental adaptability of introduced bioagents present significant challenges to their adoption [51]. Field studies on rye and wheat seeds treated with LAB demonstrated variable effects on yield. A significant decrease in rye grain yield was observed during the first season, while in the following year, some strains led to an increase in grain yield and others had no impact. In contrast, the treatment of wheat seeds with LAB consistently showed a positive effect on yield throughout both field experiments [42]. Yield variation during our two-year experiment ranged from 72.9 to 114.4% compared to the control. Although treatments significantly affected shoot/root growth and nodulation, these responses did not result in statistically significant yield improvements. This is due to environmental stress during sensitive reproductive stages, such as flowering and pod formation, where high temperatures, limited rainfall, or nutrient fluctuations can impair pollination, reduce pod set, or limit seed filling, masking earlier vegetative gains. In our study, the lower prolonged rainfall during May and June 2023 had an unfavorable effect on pea plant flowering and pod-setting processes, influencing yield components, seed yield, and protein content (Figure 1). Several studies have demonstrated that pea is particularly sensitive to elevated temperatures and limited precipitation during the reproductive phase [52,53]. The impact of high temperature on reproductive development varies depending on its intensity and duration. For instance, moderate heat stress has been associated with a reduction in seed number, primarily due to

a decreased growth rate between flowering and seed-filling stages [54]. In contrast, a short period of extremely high temperature can cause the abscission of reproductive organs in pea and direct damage to yield [55]. As similarly reported by Strejckova et al. (2018), the yield was influenced by markedly different climatic conditions, with unfavorable weather potentially leading to a reduction in yield [18]. The analysis results showed that protein content, plant height, number of seeds per plant, weight of pods per plant, weight of seeds per plant, seeds per plant, and thousand kernel weight were positively correlated with yield. Meanwhile, the number of seeds per pod was negatively correlated with the number of nodes per plant, pods per plant, and pod setting ratio (Figure 5). In general, an increase in the number of pods per plant can lead to a reduction in the number of seeds per pod due to competition for assimilates and nutrient allocation during the reproductive phase, particularly under environmental stress. In our study, this trade-off was evident, where the increase in pod number was likely counterbalanced by a decrease in seeds per pod. This negative correlation between pod number and seed number per pod is consistent with our findings and has also been reported in previous studies [55,56]. According to Sadras et al. (2013), yield was correlated with seed number and was unrelated to seed size; seed size was negatively associated with seeds per pod and seed number [57].

These findings demonstrate that while LAB and MPF enhance plant growth through distinct mechanisms, their combined application does not inherently ensure consistent benefits to crops under field conditions. This underscores the need for further investigations into the interactions among plants, biocontrol agents, and the environment.

5. Conclusions

In summary, seed treatments with lactic acid bacteria *L. plantarum* and mycoparasitic *T. virens* significantly enhanced early vegetative growth parameters, particularly shoot length and dry root weight. Notably, LAB seed treatment also increased the number of root nodules. The treatments demonstrated positive increasing trends in several yield components for plant height, the number of nodes per plant, and the number of pods per plant compared to the control. The combined application of MPF seed treatment and LAB foliar application at the flowering stage significantly enhanced pod weight per plant, seed number per pod and per plant, and seed weight compared to treatments with LAB applied separately as either foliar or seed treatment, as well as the untreated control. However, no clear effects of LAB and MPF applied independently or in combination were observed on chlorophyll content and yield over the two years of the experiment. Seed yield exhibited variability under field conditions, ranging from 1.51 to 2.27 t ha⁻¹ and inconsistency over the two years of the experiment. Protein content ranged from 21.24% to 21.61%, with no statistically significant differences detected among treatments.

These findings highlight the potential of applications of LAB and MPF in organic pea cultivation while also emphasizing the complexity of responses under field conditions. Therefore, further studies are needed to elucidate the mechanisms driving plant growth promotion and to evaluate the long-term effects of LAB and MPF treatments on pea seed yield when applied independently and in combination.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/agronomy15081792/s1>, Table S1: Effect of seed treatment to field emergence, growth parameters of pea under field condition in 2023 and 2024; Table S2. Effects of treatment on yield components of pea under field conditions in 2023 and 2024. Table S3. Effects of treatment on yield and protein content of pea under field conditions in 2023 and 2024.

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investigation, T.G.N., M.K. (Marek Kopecký), K.P., P.D., T.N.H., J.L., D.K., Y.T.M. and D.K.T., resources, P.K., data curation, T.G.N., writing—original draft preparation, T.G.N., writing—review and editing, T.G.N., P.K., I.C., K.P. and A.B., visualization, T.G.N.; supervision, P.K.; project administration, P.K.; funding acquisition, I.C. All authors have read and agreed to the published version of the manuscript.

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