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Microbial Biostimulants and Seaweed Extract Synergistically Influence Seedling Growth and Morphology of Three Onion Cultivars

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Abstract: Onion (*Allium cepa* L.), a globally cultivated vegetable crop, possesses a shallow root system, making it vulnerable to abiotic stresses. The increasing frequency of extreme weather events in recent years necessitates sustainable solutions to enhance onion growth. Biostimulants offer a promising and accessible approach to promote onion growth and quality in an environmentally friendly and sustainable manner. This study investigated the effects of nine commercial microbial biostimulants (LALRISE Mycorrhizae, LALRISE *Bacillus*, Mighty Mycorrhizae, MycoApply, Spectrum DS, Spectrum Myco, Spectrum, Tribus Original, and Tribus Continuum) and one non-microbial commercial biostimulant (Kelpak—seaweed extract) on the seedling growth of three onion cultivars: Carta Blanca (white), Don Victoro (yellow), and Sofire (red). The results indicated that biostimulants did not significantly affect onion seed germination, but germination rates did vary among the onion cultivars. These cultivars also exhibited significant morphological and biomass differences, with principal component analysis revealing a more obvious effect on root growth compared to shoot growth. Kelpak seaweed extract increased the plant height, leaf area, and shoot fresh weight and dry weight of onion seedlings but decreased the root-to-shoot dry-weight ratio. The effects of microbial biostimulants on onion seedling growth depended on both the onion cultivar and Kelpak seaweed extract. In general, LALRISE Mycorrhizae, Mighty Mycorrhizae, Spectrum Myco, Spectrum DS, and Tribus Continuum exhibited positive effects on seedling growth in certain onion cultivars. Furthermore, the benefits of microbial biostimulants were amplified when combined with Kelpak seaweed extract application. These findings suggest a synergistic interaction between microbial and non-microbial biostimulants, leading to enhanced onion seedling growth. Further research is required to evaluate the long-term effects of these biostimulants on onion plant growth after transplanting to fields.

Keywords: *Allium*; mycorrhizae; *Bacillus*; pseudomonas; seaweed extract; onion growth; root scan; morphology

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1. Introduction

Onion (*Allium cepa* L.) is the second-most-consumed fresh vegetable crop in the United States, after potato, with an annual per capita consumption of 8.7 kg in 2020 [1]. However, onion is one of the few vegetable crops that is highly sensitive to soil conditions due to its shallow root system with few branched lateral roots, which limits its ability to absorb water and nutrients from a large area [2,3]. Young onion plants are generally more vulnerable to environmental stress. Therefore, transplanting well-established and strong onion seedlings can reduce the risk of seedling loss in the early stages of plant growth, ultimately resulting in high yields [4,5]. Additionally, using onion transplants ensures uniform plant growth, precisely controls plant spacing, and accelerates the cultivation process in the field by allowing for an earlier start [6,7]. Therefore, a consistent supply of high-quality onion seedlings is essential for efficient onion production. While seed treatments like plant hormones, radiation, and chemicals may be effective in boosting germination and seedling quality, they often require specialized equipment or precise environmental conditions that may not be practical for most growers [8–11]. To achieve both high-quality onion seedling production and long-term benefits for post-transplant growth, we need sustainable and practical solutions.

Sustainable cultivation can improve plant growth, feed the rapidly increasing population, and preserve Earth's natural resources [12,13]. In the context of onion production, the application of biostimulants is a promising practice for sustainable production. Biostimulants are either microorganisms or non-microbial substances applied to plants or their surrounding rhizosphere, which can benefit plant growth or crop quality by enhancing nutrient availability and uptake [14]. Microbial biostimulants, such as arbuscular mycorrhizal fungi (AMF) and plant growth-promoting bacteria (PGPB), benefit plant growth through forming symbiotic relationships with plants, increasing nutrient availability, and producing growth-stimulating compounds [15]. Non-microbial biostimulant seaweed extracts are rich in phytohormones, minerals, and polysaccharides that promote plant growth [14]. On one hand, biostimulants can help onion plants resist abiotic stress caused by frequent extreme weather events, such as the prolonged high temperatures and drought in many regions in recent years [16]. On the other hand, biostimulants potentially can also enhance onion plant growth, especially root growth, which ultimately results in higher yields. Both seaweed extract and microbial biostimulants such as *Bacillus* sp. have been reported to promote onion plant growth and yield [17,18]. However, the effectiveness of biostimulants can vary among different crop genotypes and environmental conditions [13]. Therefore, it is necessary to determine the efficacy of different biostimulant products on specific crops before applying them on a large scale [19]. Additionally, there can be synergistic effects when combining different biostimulants, so it is also necessary to test the combined application method in advance before using it in onion production [20].

The germination and seedling stages are the most vulnerable to various stress factors due to their reliance on seed reserves and underdeveloped root systems [21]. Young plants are highly susceptible to stresses such as drought, high salinity, extreme temperatures, and nutrient deficiencies. Applying biostimulants at these stages can enhance seedlings' tolerance the various stresses encountered during this vulnerable period of plant growth [22]. Microbial biostimulants are particularly beneficial when inoculated early in germination or seedling stages, as this gives microbes more time to multiply independently and colonize the substrate and rhizosphere, resulting in greater effects on plant growth [23,24]. Previous studies have shown that microbial biostimulants inoculated in the 4- or 5-leaf seedling stage improved lettuce leaf health compared to both untreated controls and treatments where biostimulants were applied later, including one week post-transplant or at the head-formation stage [25]. Inoculating microbial biostimulants in the seedling stage can be a cost-effective strategy for growers, as the microbes establish themselves and propagate over time, reducing the need for repeated applications of large quantities. Therefore, investigating the effectiveness of biostimulants on onion seedlings is necessary. The objective of this study was to investigate the effectiveness of various biostimulant products on seedling production of three onion cultivars, and to investigate whether combining microbial biostimulants with seaweed extract can synergistically enhance onion seedling growth. To ensure a clear and focused presentation of the findings on onion seedlings, the carry-over effects of the biostimulants on the onion mini-bulb stage will be presented in a separate publication.

2. Materials and Methods

2.1. Plant Materials and Cultivation

The experiment was conducted in a greenhouse located at the Texas A&M AgriLife Research and Extension Center in Dallas, TX, USA, from 17 October 2022 to 12 December 2022. One onion seed was sowed in a cell of a 392-cell propagation tray (Speedling

Incorporated, Ruskin, FL, USA), which has 28 columns and 14 rows. Each propagation tray was divided into 3 experimental areas, each area with 8 columns and 14 rows. Two rows of onion seeds were planted between each experimental area, but these rows were excluded from data collection to minimize edge effects. BM2 seed germination mix (Berger, Saint-Modeste, QC, Canada) was used. Three onion cultivars (Nunhems USA Incorporated, Parma, ID, USA), including Carta Blanca (white), Don Victoro (yellow), and Sofire (red), were randomly assigned to the three experimental areas in each propagation tray. Onion seeds were generously provided by Dr. Daniel I. Leskovar from Texas A&M AgriLife. For data collection, each area within a tray (8 × 14 cells) with the same onion cultivar planted was treated as an experimental unit. An experimental unit represents the smallest indivisible unit to which a specific treatment combination (cultivar x seaweed extract x microbial biostimulant) can be applied. The onion seedlings were harvested 58 days after sowing (DAS). Onion seedlings were irrigated with tap water using a fine-mist hose as needed and fertilized weekly with ICL Peters Professional 20-10-20 (20% N-2.19% P-16.56% K, Everris, Dublin, OH, USA) at a rate of 150 mg/L nitrogen by subirrigation. Irrigation and fertilization were applied uniformly to each cell, ensuring that all cells received the same amount of water and nutrients.

2.2. Greenhouse Temperature and Light Conditions

The experimental greenhouse was equipped with a gas-heating system and a fan-and-pad evaporative cooling system for temperature control. The greenhouse air temperatures were measured using thermocouples, and the photosynthetically active radiation (PAR) was measured using a quantum sensor. All temperature and light sensors were connected to a datalogger (Campbell Scientific Inc., Logan, UT, USA). The daily average air temperature was 19.93 ± 0.33 °C (mean ± standard error), and the daily average light integral was 10.35 ± 0.78 mol m^{-2} d^{-1} throughout the experiment.

2.3. Biostimulants Application Treatments

Ten commercial biostimulant products, including nine microbial and one non-microbial, were evaluated for their effects on onion germination and seedling plant growth in this study. The nine microbial biostimulants included mycorrhizal fungi and bacterial products. The non-microbial biostimulant was Kelpak (Kelp Products International Ltd., Cape Town, South Africa), an extract derived from seaweeds. The major ingredients, manufacturer information, and application rate of each biostimulant product are shown in Table 1. The detailed biological ingredients of each biostimulant product are listed in Supplemental Material Table S1. All biostimulant products were first applied at 0 DAS. During this initial application, the biostimulants were thoroughly incorporated into the substrate. The second application, via subirrigation, occurred at 14 DAS. The application rate and frequency were determined according to the manufacturers' recommendations, as well as by matching the colony forming units (CFUs) or spore counts for similar bacterial or mycorrhizal fungi products, respectively, to ensure sufficient microbial inoculation to influence plant growth. In addition to the first and second applications, Tribus Original and Tribus Continuum were applied a third and fourth time at 0.52 mL/L every two weeks, as recommended by the manufacturer. The seaweed extract product, Kelpak, was treated as an independent experimental factor, as we wanted to test whether Kelpak could have synergistic effects when applied in combination with microbial biostimulant products.

Table 1. Biostimulant products information and application rates. The unit of application rate refers to the amount of biostimulant products applied per liter of the substrate.

Biostimulant Product	Abbreviation of Product	Major Ingredients	Manufacturer	1st Application	2nd Application
LALRISE Mycorrhizae	LALMyco	One species of mycorrhizal fungi	Lallemand, Montreal, Quebec, Canada	4 g/L	8 g/L
LALRISE *Bacillus velezensis*	LALb	One species of bacteria	Lallemand, Montreal, Quebec, Canada	0.25 g/L	0.5 g/L
Mighty Mycorrhizae	MightyMyco	Sixteen species of mycorrhizal fungi	Wildroot Organic, Marble Falls, TX, USA	0.5 g/L	1 g/L
MycoApply	MycoApply	Four species of mycorrhizal fungi	Mycorrhizal Applications, Grants Pass, OR, USA	1.67 mL/L	3.34 g/L
Spectrum DS	SpcDS	Twenty species of bacteria, for drought-stressed soils	Tainio Biology, Spokane, WA, USA	0.5 g/L	1 g/L
Spectrum Myco	SpcMyco	Nineteen species of bacteria, four species of mycorrhizal fungi	Tainio Biology, Spokane, WA, USA	1.55 g/L	3.1 g/L
Spectrum	Spctrum	Twenty species of bacteria	Tainio Biology, Spokane, WA, USA	0.5 g/L	1 g/L
Tribus Original	TribusO	Three species of bacteria	Impello Bioscience, Fort Collin, CO, USA	0.26 mL/L	0.52 mL/L
Tribus Continuum	TribusC	Four species of bacteria	Impello Bioscience, Fort Collin, CO, USA	0.26 mL/L	0.52 mL/L
Kelpak	Kelp	Extracted from kelp *Ecklonia maxima*	Kelp Products International, Cape Town, South Africa	1% (v/v)	1% (v/v)

For all biostimulant products except for Tribus Original and Tribus Continuum, the first application occurred 0 days after sowing (DAS), the second application occurred 14 DAS. Tribus Original and Tribus Continuum were additionally applied a third and fourth time at 0.52 mL/L 28 and 44 DAS, as recommended by the manufacturer.

2.4. Data Collection

Germination. Germination counts were recorded at 17 DAS. The germination rate was determined by the following equation, where the total number of seeds per experimental unit is 112 [26].

$$\text{Germination rate } \% = \frac{\text{number of germinated seeds}}{\text{the total number of seeds per experimental unit}} \times 100$$

The relative germination rate was calculated by comparing each treatment to a specific control group using the following equation. The specific control group matched the treatment in terms of onion cultivar (white, yellow, or red) and seaweed treatment (with or without Kelpak) but lacked microbial biostimulant application [27].

$$\text{Relative germination rate } \% = \frac{\text{number of germinated seeds in the treatment}}{\text{number of germinated seeds in specific control group}} \times 100$$

Plant morphology and biomass. Seedlings were harvested at 58 DAS. Ten plants randomly selected per experimental unit were collected to measure leaf number, plant height, pseudostem diameter, and leaf area. Shoot length was measured from the tip of the uppermost leaf to the base of the stem. The pseudostem diameter, a predictor of the bulb size during the seedling stage [28], was measured at the widest point of the stem using a caliper. Leaf area was calculated by doubling the value obtained from the leaf area meter as two layers of onion leaves present on one side of the cylindrical or cone structure. For the plant morphology parameters, including leaf number, plant height, pseudostem diameter, and leaf area, ten plants of each cultivar within each experimental unit were collected. As a result, twenty data points were collected for each treatment combination. For biomass parameters, including the fresh weight (FW) and dry weight (DW) of shoots and roots, thirty plants per cultivar within each experimental unit were randomly picked and harvested. These plants were pooled into three groups of ten for each weight measurement. Harvested onion seedlings were carefully rinsed by hand and separated into shoots and roots using a razor blade. The FW of shoots and roots was recorded individually. Subsequently, the samples were dried at 70 °C to a constant weight to determine the DW. In total, there were 60 plants involved in the biomass parameter measurement for each treatment combination, but 6 data points were collected because 10 plants were measured together as a group.

Root scan. Following FW measurement, root samples were scanned using a scanner (Epson Perfection V850, Epson America. Inc., Long Beach, CA, USA) before being placed in the oven for drying. A total of twenty roots from each treatment combination were included in the root scan. WinRhizo software (version 2022a, Regent Instruments Inc., Québec City, QC, Canada) was then employed to analyze the root scan images, quantifying various root architectural parameters including total root length, root area, root volume, and average root diameter.

2.5. Experiment Design and Statistical Analysis

The experiment was designed as a randomized complete block design, with onion cultivar, microbial biostimulants, and seaweed extract as three experimental factors. As previously noted, three distinct onion cultivars were randomly planted in three designated experimental areas (experimental units) within each tray. Each microbial biostimulant product was applied to four trays, with two trays receiving the application of seaweed extract (Kelpak) and two trays without it. With 10 levels of microbial biostimulants (including a control, Table 1), 3 levels of onion cultivar, and 2 levels of seaweed extract application, there were 60 treatment combinations in total. There were 2 replications for each treatment combination, with each replication containing 112 subsamples. All collected parameters were subjected to analysis of variance (ANOVA) using the PROC GLM procedure in SAS software (Version 9.4, SAS Institute Inc., Cary, NC, USA). Prior to ANOVA, data normality and homogeneity of variance were checked using the Shapiro–Wilk test and Levene's test, respectively. Additionally, principal component analysis (PCA) was performed using the prcomp function in R software (version 4.3.3, R Foundation for Statistical Computing, Vienna, Austria) to visualize the variance in different parameters across cultivars and microbial biostimulants with or without seaweed extract.

3. Results and Discussion

Table 2 indicates significant interactions among onion cultivars, microbial biostimulants, and seaweed extract for most measured parameters. This suggests that treatment effects vary depending on the specific combination of experimental factors. Consequently, a detailed analysis of each treatment combination is necessary. Kelpak seaweed extract had a less-pronounced impact on onion seedling growth compared to cultivars and microbial biostimulants as seaweed extract only had a significant impact on seven of the fifteen parameters measured, and it was applied at only two levels (with or without). Thus, we present the results for these two seaweed extract application conditions in separate tables to enhance clarity.

Table 2. Three-way ANOVA test results showing levels of significance of microbial biostimulants, seaweed extract, and cultivar on onion seedling morphology and growth parameters.

	Parameters	Micro	Kelp	CV	Micro × Kelp	Micro × CV	Kelp × CV	Micro × Kelp × CV
Germination	Germination rate	NS	NS	***	NS	NS	NS	NS
	Relative germination rate	NS	NS	*	NS	NS	*	NS
Shoot morphology	Leaf number	***	NS	***	**	NS	NS	NS
	Plant height	***	***	***	***	***	***	***
	Pseudostem diameter	***	NS	***	***	*	NS	*
	Leaf area	***	***	***	***	***	***	***
Biomass	Shoot fresh weight	***	***	NS	***	**	**	***
	Shoot dry weight	***	**	**	***	**	*	***
	Root fresh weight	***	NS	***	*	NS	NS	**
	Root dry weight	***	NS	***	**	*	NS	***
	Root/shoot ratio	***	**	***	***	NS	NS	NS
Root morphology	Total length	***	*	*	***	NS	NS	***
	Projected area	***	NS	***	***	NS	NS	***
	Root diameter	***	*	***	**	*	NS	**
	Root volume	***	NS	***	***	**	NS	***

Micro = microbial biostimulants, Kelp = non-microbial biostimulant seaweed extract—Kelpak, CV = cultivar. Level of significance: NS represents not significant; * represents $p < 0.05$; ** represents $p < 0.01$; *** represents $p < 0.001$.

3.1. Germination

Neither germination rate nor relative germination rate were affected by either microbial biostimulants or the non-microbial biostimulant—seaweed extract (Table 2). There was a significant difference in the germination rate and relative germination rate among the three cultivars. Therefore, germination data were pooled across different biostimulant treatments and analyzed solely by cultivar (Figure 1). The germination rate ranged from 87.28% in the yellow onion cultivar to 95.65% in the red onion cultivar. The germination rates observed in this study were at the higher end of the range reported in previous onion studies (53.55% to 96.52%), which were influenced by factors such as temperature, priming, water potential, and cultivar [9,29,30]. Given the high baseline germination rates in this study, there was limited potential for biostimulants to further enhance onion seed germination under ideal conditions in the absence of biotic and abiotic stresses. Muhie's study on carrots has demonstrated that while biostimulant priming can improve germination under stressful conditions like drought, high salinity, and high temperature, it did not significantly impact germination under ideal conditions when there was no drought, high salinity, or high temperature stress [31]. The relative germination rates ranged from 0.98 in the white cultivar to 1.01 in the red cultivar. The red onion cultivar showed the highest germination rate compared to both white and yellow cultivars. The red cultivar also exhibited the highest relative germination rate. Both red and yellow cultivars had relative germination rates higher than 1, which indicates that biostimulants might have stimulated seed germination. However, the main effects of biostimulants on germination are not significant, as indicated in Table 2. Studies have shown that seaweed extract made from *Macrocystis pyrifera*, *Gelidium robustum*, and *Ecklonia arborea* can increase germination rates in mung bean seeds [32]. In another study, a microbe, *Bacillus subtilis*, also contained in the Spectrum and Tribus series products in this study, was found to improve wheat germination under both normal and drought conditions [33]. While in this study no significant effect was observed on onions, biostimulants still hold promise for enhancing seed germination in other crops such as carrot, wheat, and mung bean.

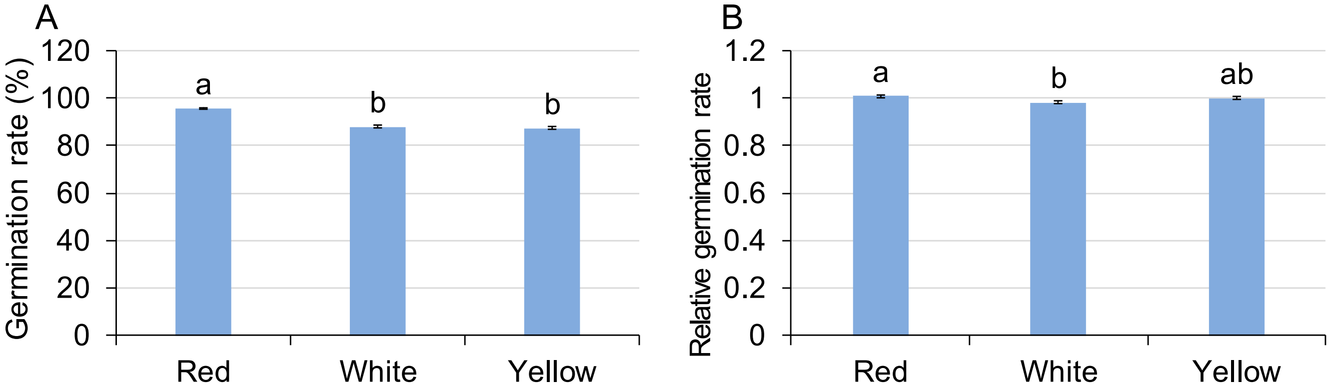

Figure 1. Germination rate (**A**) and relative germination rate (**B**) of three onion cultivars. Standard errors are presented at the top of each bar. Different letters suggest significant difference among onion cultivars indicated by Tukey's HSD test at $p < 0.05$.

3.2. Shoot Morphology

To distinguish the treatments with and without seaweed extract application, shoot morphological parameters, including leaf number, plant height, pseudostem diameter and leaf area, are presented in Tables 3 and 4, respectively. Microbial biostimulants generally did not affect the leaf number, though LALRISE Mycorrhizae reduced the leaf number in both red and white onion cultivars without seaweed extract. This result aligns with previous research on lettuce seedlings, where mycorrhizal biostimulants did not show significant positive effects on leaf number [34]. This could be attributed to the possibility that mycorrhizal biostimulants require a longer period to exhibit their influence on onion plant growth. The effect of microbial biostimulants on plant height varied with cultivar and the application of the Kelpak seaweed extract. For onion seedlings without seaweed extract application, Spectrum DS increased plant height in the yellow cultivar by 8%. For the white cultivar that received seaweed extract application, Mighty Mycorrhizae increased plant height by 14% compared to the control group.

Table 3. Morphology and growth of onion seedlings of three cultivars treated with microbial biostimulants without Kelpak seaweed extract.

Cultivar	Biostimulant	Leaf Number	Plant Height (cm)	Pseudostem Diameter (cm)	Leaf Area (cm^2)
Red	Control	3.05 ± 0.07 a	25.56 ± 0.42 a	0.49 ± 0.02 abc	12.90 ± 0.33 a
	LALMyco	2.60 ± 0.11 b	24.12 ± 0.62 ab	0.53 ± 0.03 a	11.34 ± 0.47 b
	LALb	3.00 ± 0.00 a	23.78 ± 0.31 bc	0.43 ± 0.01 bcd	10.65 ± 0.20 bc
	MightyMyco	2.90 ± 0.07 a	22.33 ± 0.35 cd	0.43 ± 0.01 cd	10.97 ± 0.32 bc
	MycoApply	3.00 ± 0.00 a	22.70 ± 0.31 bcd	0.37 ± 0.01 d	8.62 ± 0.21 d
	SpcDS	3.00 ± 0.00 a	23.11 ± 0.29 bc	0.51 ± 0.02 ab	10.66 ± 0.26 bc
	SpcMyco	3.00 ± 0.00 a	23.53 ± 0.37 bc	0.50 ± 0.02 abc	10.64 ± 0.39 bc
	Spctrum	3.00 ± 0.00 a	23.07 ± 0.19 bc	0.53 ± 0.03 a	11.37 ± 0.29 b
	TribusO	2.90 ± 0.07 a	21.24 ± 0.28 d	0.50 ± 0.02 abc	9.74 ± 0.26 cd
	TribusC	2.90 ± 0.07 a	23.48 ± 0.32 bc	0.49 ± 0.02 abc	10.73 ± 0.37 bc
	Mean	2.94 ± 0.02	23.29 ± 0.14	0.48 ± 0.01	10.76 ± 0.12
White	Control	2.85 ± 0.11 a	29.50 ± 0.32 a	0.56 ± 0.03 a	14.48 ± 0.30 a
	LALMyco	2.20 ± 0.09 b	23.30 ± 0.49 c	0.53 ± 0.02 a	11.18 ± 0.52 c
	LALb	2.75 ± 0.12 a	27.21 ± 0.52 b	0.51 ± 0.01 ab	12.96 ± 0.37 ab
	MightyMyco	2.60 ± 0.11 ab	23.71 ± 0.35 c	0.50 ± 0.01 ab	10.58 ± 0.25 cd
	MycoApply	2.65 ± 0.11 ab	23.16 ± 0.44 c	0.42 ± 0.02 b	9.28 ± 0.34 d
	SpcDS	2.75 ± 0.10 a	24.33 ± 0.39 c	0.58 ± 0.02 a	11.62 ± 0.35 bc
	SpcMyco	2.75 ± 0.12 a	23.12 ± 0.38 c	0.57 ± 0.02 a	10.76 ± 0.32 cd
	Spctrum	2.65 ± 0.11 b	24.80 ± 0.54 c	0.58 ± 0.03 a	11.72 ± 0.38 bc
	TribusO	2.45 ± 0.15 ab	24.74 ± 0.74 c	0.53 ± 0.01 a	10.51 ± 0.52 cd
	TribusC	2.45 ± 0.11 ab	24.01 ± 0.51 c	0.53 ± 0.02 a	11.25 ± 0.27 c
	Mean	2.61 ± 0.04	24.79 ± 0.20	0.53 ± 0.01	11.43 ± 0.15
Yellow	Control	2.80 ± 0.09 ab	25.96 ± 0.42 b	0.56 ± 0.02 a	12.26 ± 0.53 ab
	LALMyco	2.40 ± 0.11 b	25.00 ± 0.37 bc	0.53 ± 0.01 ab	10.22 ± 0.43 cd
	LALb	2.65 ± 0.11 ab	25.32 ± 0.43 bc	0.57 ± 0.01 a	13.36 ± 0.85 a
	MightyMyco	2.55 ± 0.11 ab	24.29 ± 0.32 bc	0.54 ± 0.02 ab	10.69 ± 0.27 bcd
	MycoApply	2.90 ± 0.07 a	25.08 ± 0.31 bc	0.47 ± 0.02 b	8.92 ± 0.25 d
	SpcDS	2.80 ± 0.09 ab	28.11 ± 0.36 a	0.61 ± 0.02 a	13.03 ± 0.32 a
	SpcMyco	2.75 ± 0.10 ab	25.42 ± 0.49 bc	0.56 ± 0.03 ab	10.89 ± 0.34 bc
	Spctrum	2.65 ± 0.11 ab	24.63 ± 0.46 bc	0.53 ± 0.01 ab	10.36 ± 0.22 cd
	TribusO	2.55 ± 0.11 ab	25.24 ± 0.33 bc	0.55 ± 0.02 ab	10.47 ± 0.24 bcd
	TribusC	2.70 ± 0.11 ab	23.74 ± 0.31 c	0.58 ± 0.02 a	9.54 ± 0.25 cd
	Mean	2.68 ± 0.03	25.28 ± 0.14	0.55 ± 0.01	10.97 ± 0.16
Overall Mean		2.74 ± 0.02	24.45 ± 0.10	0.52 ± 0.00	11.06 ± 0.08

Red, White, and Yellow represent onion cultivar Sofire, Carta Blanca, and Don Victoro, respectively. LALMyco, LALb, MightyMyco, SpcDS, SpcMyco, TribusO, and TribusC represent LALRISE Mycorrhizae, LALRISE *Bacillus velezensis*, Mighty Mycorrhizae, Spectrum DS, Spectrum Myco, Tribus Original, and Tribus Continuum, respectively. Different letters within a column of the same cultivar suggest significant difference among microbial biostimulants indicated by Tukey's HSD test at $p < 0.05$.

The application of biostimulants did not significantly affect the pseudostem diameter of all three onion cultivars, regardless of seaweed extract. The effects of different biostimulants on leaf area varied. Without seaweed extract (Kelpak) application, none of the biostimulants increased leaf area in the onion seedlings. However, when combined with seaweed extract, Spectrum Myco increased leaf area in the red cultivar by 18%. In the white cultivar, LALRISE Mycorrhizae, Mighty Mycorrhizae, Spectrum Myco, and Tribus Continuum all increased leaf area by 17% to 34% compared to the control group. While Kelpak seaweed extract did not affect the leaf number and pseudostem diameter, it led to a 4% increase in plant height and a 5% increase in leaf area compared to the control (Tables 2–4). Therefore, both microbial biostimulants (LALRISE Mycorrhizae, Mighty Mycorrhizae, Spectrum DS, Spectrum Myco, and Tribus Continuum) and Kelpak seaweed extract can improve plant height and leaf area in onion seedlings. However, the effects on leaf number and pseudostem diameter were not significant. Prolonged biostimulant application is often a promising approach to enhance plant growth benefits [35]. Therefore, onions may require a longer time to respond with changes in these two parameters.

Table 4. Morphology and growth of onion seedlings of three different cultivars treated with microbial biostimulants and Kelpak seaweed extract.

Cultivar	**Biostimulant**	**Leaf Number**	**Plant Height (cm)**	**Pseudostem Diameter (cm)**	**Leaf Area (cm^2)**
Red	Control	3.00 ± 0.00	23.89 ± 0.41 ab	0.48 ± 0.02 abc	11.31 ± 0.31 bc
	LALMyco	3.00 ± 0.00	25.26 ± 0.47 a	0.54 ± 0.02 ab	11.92 ± 0.37 b
	LALb	2.80 ± 0.09	23.07 ± 0.41 bc	0.47 ± 0.02 bc	10.30 ± 0.22 cd
	MightyMyco	2.70 ± 0.11	23.98 ± 0.43 ab	0.49 ± 0.01 abc	11.65 ± 0.28 b
	MycoApply	2.90 ± 0.07	24.56 ± 0.29 ab	0.43 ± 0.01 c	11.16 ± 0.32 bc
	SpcDS	2.90 ± 0.07	22.13 ± 0.22 c	0.48 ± 0.02 abc	9.05 ± 0.19 d
	SpcMyco	2.95 ± 0.09	25.32 ± 0.28 a	0.57 ± 0.03 a	13.34 ± 0.36 a
	Spctrum	3.00 ± 0.00	23.16 ± 0.20 bc	0.51 ± 0.02 abc	11.51 ± 0.28 bc
	TribusO	2.85 ± 0.08	25.20 ± 0.30 a	0.53 ± 0.03 ab	11.95 ± 0.25 b
	TribusC	2.90 ± 0.07	23.80 ± 0.35 ab	0.48 ± 0.02 abc	11.74 ± 0.35 b
	Mean	2.90 ± 0.02	24.04 ± 0.13	0.50 ± 0.01	11.39 ± 0.12
White	Control	2.65 ± 0.11	25.55 ± 0.48 bc	0.51 ± 0.02 abc	11.14 ± 0.39 efg
	LALMyco	2.50 ± 0.11	27.14 ± 0.55 abc	0.58 ± 0.02 a	14.27 ± 0.44 ab
	LALb	2.55 ± 0.11	24.61 ± 0.69 c	0.49 ± 0.01 bc	10.65 ± 0.28 fg
	MightyMyco	2.60 ± 0.11	29.10 ± 0.56 a	0.52 ± 0.01 abc	14.94 ± 0.50 a
	MycoApply	2.60 ± 0.13	24.84 ± 0.51 c	0.46 ± 0.01 c	10.23 ± 0.37 g
	SpcDS	2.70 ± 0.11	25.78 ± 0.43 bc	0.53 ± 0.02 abc	12.26 ± 0.31 cdef
	SpcMyco	2.45 ± 0.11	26.88 ± 0.47 abc	0.59 ± 0.02 a	13.06 ± 0.28 bcd
	Spctrum	2.85 ± 0.08	27.18 ± 0.47 abc	0.56 ± 0.02 ab	12.68 ± 0.38 bcde
	TribusO	2.40 ± 0.11	26.73 ± 0.51 abc	0.53 ± 0.02 abc	11.93 ± 0.23 defg
	TribusC	2.85 ± 0.15	28.08 ± 0.99 ab	0.56 ± 0.02 ab	13.95 ± 0.74 abc
	Mean	2.62 ± 0.04	26.59 ± 0.20	0.53 ± 0.01	12.51 ± 0.17
Yellow	Control	2.65 ± 0.11 ab	27.91 ± 0.38 a	0.58 ± 0.02 ab	12.71 ± 0.45 a
	LALMyco	2.50 ± 0.11 ab	26.30 ± 0.35 b	0.60 ± 0.02 a	11.54 ± 0.47 abc
	LALb	2.65 ± 0.11 ab	23.80 ± 0.33 de	0.58 ± 0.02 ab	10.36 ± 0.33 bcde
	MightyMyco	2.75 ± 0.10 ab	24.97 ± 0.35 bcd	0.48 ± 0.01 c	10.23 ± 0.30 bcde
	MycoApply	2.80 ± 0.09 ab	25.48 ± 0.36 bc	0.48 ± 0.02 c	10.16 ± 0.47 cde
	SpcDS	2.95 ± 0.05 a	23.27 ± 0.28 e	0.50 ± 0.01 bc	9.10 ± 0.21 e
	SpcMyco	2.80 ± 0.09 ab	26.08 ± 0.37 b	0.57 ± 0.02 ab	11.78 ± 0.33 ab
	Spctrum	2.60 ± 0.11 ab	24.32 ± 0.30 cde	0.58 ± 0.02 ab	9.78 ± 0.26 de
	TribusO	2.45 ± 0.11 b	26.19 ± 0.32 b	0.60 ± 0.02 a	11.01 ± 0.29 bcd
	TribusC	2.70 ± 0.11 ab	24.94 ± 0.40 bcd	0.54 ± 0.02 abc	11.17 ± 0.36 abcd
	Mean	2.69 ± 0.03	25.32 ± 0.14	0.55 ± 0.01	10.78 ± 0.13
Overall mean		2.73 ± 0.02	25.32 ± 0.10	0.53 ± 0.00	11.56 ± 0.09

Red, White, and Yellow represent onion cultivar Sofire, Carta Blanca, and Don Victoro, respectively. LALMyco, LALb, MightyMyco, SpcDS, SpcMyco, TribusO, and TribusC represent LALRISE Mycorrhizae, LALRISE *Bacillus velezensis*, Mighty Mycorrhizae, Spectrum DS, Spectrum Myco, Tribus Original, and Tribus Continuum, respectively. Different letters within a column of the same cultivar suggest significant difference among microbial biostimulants indicated by Tukey's HSD test at $p < 0.05$.

3.3. Biomass

To distinguish the treatments without and with the application of seaweed extract, onion seedlings' shoot and root biomass parameters, including shoot fresh weight (shoot FW), shoot dry weight (shoot DW), root fresh weight (root FW), root dry weight (root DW), and root-to-shoot dry-weight ratio (root/shoot), are presented in Tables 5 and 6, respectively. Biostimulants from the Spectrum series consistently produced some of the highest shoot FWs compared to other biostimulants. For example, Spectrum DS resulted in the highest shoot FW for yellow onion cultivars without seaweed extract and for white onion cultivars with seaweed extract. Shoot DW generally followed similar trends to shoot FW. Notably, when combined with seaweed extract, Mighty Mycorrhizae increased shoot DW by 25% compared to the control group in the red onion cultivar. With seaweed extract application, Mighty Mycorrhizae and Spectrum DS resulted in the highest root FW in red and white onion cultivars, respectively. Regarding root DW, LALRISE Mycorrhizae showed the highest value in the red cultivar without seaweed extract. The positive effects of microbial biostimulants on root DW became more pronounced when combined with seaweed extract. Mighty Mycorrhizae increased the root DW by 28% in the red cultivar, and Spectrum DS increased the root DW by 20% in the white cultivar, compared to their respective control groups. It is also worth mentioning that MycoApply consistently reduced both shoot and root biomass across all studied onion cultivars, regardless of seaweed extract application. This was caused by the high application rate of MycoApply during the second application. We confirmed this fact in an independent experiment with different MycoApply application rates (data not published). There was a misguidance that exceeding recommended application rates for biostimulant products will not negatively impact plant growth [36]. Our results indicated that the high application rate of MycoApply at 3.34 g/L resulted in tip burn in the onion seedlings. The electrical conductivity (EC) and pH of the MycoApply treatment solution at 3.34 g/L were 0.96 and 7.07, respectively, both falling within the normal range. Therefore, it is unlikely that EC or pH contributed to the problem. Tip burn in seedlings might be caused by competition for nutrients between plants and mycorrhizal fungi, particularly during the initial stages of inoculation [37]. In this study, MycoApply was applied at 0 and 14 DAS (Table 1). Since onion seedlings have underdeveloped root systems at this time, the mycorrhizal fungi can compete for essential nutrients like nitrogen and carbon to establish themselves. However, as the fungi develop hyphae, they ultimately enhance root activity, promoting nutrient and water uptake by the plant [38]. Therefore, a very high inoculation rate of MycoApply with underdeveloped root systems might initially exacerbate competition for nutrients.

The root/shoot ratio is an important factor in assessing onion seedling quality, since strong roots and compact seedlings contribute to reducing transplant shock and facilitating early stand establishment [5]. In the white onion cultivar, the application of LALRISE Mycorrhizae and Spectrum without seaweed extract increased the root/shoot ratio by 27–28% compared to the control group. When seaweed extract was included, Spectrum DS boosted the ratio by 12% in the red cultivar, with LALRISE Mycorrhizae, Spectrum DS, and Spectrum increasing the root/shoot ratio by 18% to 27% in the yellow cultivar. Seaweed extract application increased shoot FW and DW by 6% and 5%, respectively, and decreased the root/shoot ratio by 3%, but it did not affect the root FW and DW (Tables 2, 5 and 6). While no measurements of physiological responses were performed, applying seaweed extract normally enhances nutrient uptake and photosynthetic efficiency [13]. A previous study on tomato seedlings found that the Kelpak seaweed extract did not increase the shoot DW [19]. Another study on onions demonstrated a positive correlation between the number of Kelpak applications and onion yield [39]. Our findings align with the onion study, indicating a positive impact of Kelpak on onion seedling growth. Seaweed extracts are known to be rich in plant growth-promoting substances such as plant hormones, potassium, and bacteriostatic polysaccharides [40,41]. While these compounds may contribute to Kelpak's positive effects on onion seedling growth, more frequent applications might be necessary to maximize its benefits.

Table 5. Biomass parameters of onion seedlings of three different cultivars treated with microbial biostimulants without Kelpak seaweed extract.

Cultivar	Biostimulant	Shoot FW (g)	Shoot DW (mg)	Root FW (g)	Root DW (mg)	Root/Shoot
Red	Control	1.25 ± 0.04 a	79.06 ± 3.02 a	0.40 ± 0.02 a	20.14 ± 0.69 ab	25.56 ± 0.82
	LALMyco	1.01 ± 0.07 b	73.98 ± 6.52 a	0.39 ± 0.02 ab	21.06 ± 0.94 a	29.10 ± 1.55
	LALb	0.97 ± 0.02 b	70.02 ± 1.66 a	0.35 ± 0.00 ab	18.68 ± 0.63 ab	26.66 ± 0.54
	MightyMyco	0.99 ± 0.02 b	69.83 ± 2.45 a	0.35 ± 0.01 ab	18.18 ± 0.49 ab	26.12 ± 0.72
	MycoApply	0.75 ± 0.02 c	49.11 ± 1.67 b	0.27 ± 0.01 c	14.22 ± 0.44 c	29.00 ± 0.54
	SpcDS	1.03 ± 0.02 b	66.50 ± 1.68 a	0.35 ± 0.01 ab	18.04 ± 0.50 b	27.14 ± 0.53
	SpcMyco	0.99 ± 0.04 b	68.63 ± 3.85 a	0.34 ± 0.02 b	19.07 ± 0.46 ab	28.11 ± 1.25
	Spctrum	1.08 ± 0.01 b	71.27 ± 1.31 a	0.37 ± 0.01 ab	20.35 ± 0.68 ab	28.54 ± 0.70
	TribusO	0.96 ± 0.05 b	65.80 ± 2.07 a	0.39 ± 0.01 ab	19.50 ± 0.26 ab	29.76 ± 0.92
	TribusC	0.98 ± 0.03 b	71.55 ± 1.77 a	0.37 ± 0.01 ab	19.91 ± 0.87 ab	27.86 ± 1.17
	Mean	1.00 ± 0.02	68.57 ± 1.30	0.36 ± 0.01	18.92 ± 0.30	27.79 ± 0.32
White	Control	1.25 ± 0.05 a	82.52 ± 1.37 a	0.36 ± 0.01 a	17.30 ± 0.51 a	20.95 ± 0.35 b
	LALMyco	0.89 ± 0.08 bc	62.30 ± 7.20 bc	0.32 ± 0.03 a	16.22 ± 0.96 a	26.91 ± 1.68 a
	LALb	1.10 ± 0.05 ab	77.22 ± 5.61 ab	0.32 ± 0.02 a	17.40 ± 1.10 a	22.71 ± 0.94 ab
	MightyMyco	0.93 ± 0.02 bc	64.97 ± 1.82 abc	0.29 ± 0.01 ab	15.49 ± 0.20 a	23.90 ± 0.48 ab
	MycoApply	0.76 ± 0.02 c	46.99 ± 2.56 c	0.22 ± 0.01 b	11.39 ± 0.68 b	24.32 ± 0.95 ab
	SpcDS	1.05 ± 0.05 ab	68.24 ± 2.75 ab	0.30 ± 0.02 ab	15.95 ± 0.52 a	23.47 ± 0.78 ab
	SpcMyco	0.99 ± 0.04 bc	68.01 ± 4.12 ab	0.31 ± 0.01 ab	17.24 ± 0.68 a	25.57 ± 0.88 ab
	Spctrum	1.04 ± 0.09 ab	69.20 ± 5.18 ab	0.34 ± 0.03 a	18.34 ± 1.36 a	26.66 ± 1.22 a
	TribusO	0.94 ± 0.05 bc	64.98 ± 1.82 abc	0.33 ± 0.01 a	16.67 ± 0.73 a	25.72 ± 1.26 ab
	TribusC	0.96 ± 0.02 bc	66.52 ± 1.92 ab	0.34 ± 0.01 a	17.86 ± 0.58 a	26.99 ± 1.31 a
	Mean	0.99 ± 0.02	67.09 ± 1.62	0.31 ± 0.01	16.38 ± 0.34	24.72 ± 0.39
Yellow	Control	1.23 ± 0.09 ab	71.97 ± 5.00 ab	0.42 ± 0.03 a	19.44 ± 0.74 a	27.40 ± 1.28
	LALMyco	0.90 ± 0.06 c	62.13 ± 5.31 abc	0.38 ± 0.03 ab	18.95 ± 1.17 a	30.89 ± 1.26
	LALb	1.08 ± 0.09 abc	72.83 ± 4.75 ab	0.40 ± 0.02 a	20.02 ± 0.77 a	27.80 ± 1.11
	MightyMyco	1.03 ± 0.06 abc	71.28 ± 6.33 ab	0.39 ± 0.02 a	20.29 ± 1.26 a	28.85 ± 0.97
	MycoApply	0.82 ± 0.03 c	49.48 ± 3.53 c	0.30 ± 0.02 b	14.94 ± 0.67 b	30.64 ± 1.64
	SpcDS	1.26 ± 0.10 a	78.31 ± 5.22 a	0.41 ± 0.01 a	21.03 ± 0.54 a	27.43 ± 1.91
	SpcMyco	1.05 ± 0.04 abc	69.65 ± 1.98 ab	0.42 ± 0.01 a	20.79 ± 0.58 a	29.89 ± 0.64
	Spctrum	0.95 ± 0.02 bc	59.71 ± 1.43 abc	0.38 ± 0.01 ab	19.30 ± 0.69 a	32.38 ± 1.14
	TribusO	1.00 ± 0.04 abc	66.62 ± 3.02 abc	0.41 ± 0.01 a	19.65 ± 0.63 a	29.72 ± 1.35
	TribusC	0.82 ± 0.02 c	57.33 ± 1.50 bc	0.37 ± 0.01 ab	18.09 ± 0.25 ab	31.68 ± 1.07
	Mean	1.01 ± 0.03	65.93 ± 1.62	0.39 ± 0.01	19.25 ± 0.31	29.67 ± 0.43
Overall mean		1.00 ± 0.01	67.20 ± 0.88	0.35 ± 0.00	18.18 ± 0.21	27.39 ± 0.27

Red, White, and Yellow represent onion cultivar Sofire, Carta Blanca, and Don Victoro, respectively. LALMyco, LALb, MightyMyco, SpcDS, SpcMyco, TribusO, and TribusC represent LALRISE Mycorrhizae, LALRISE *Bacillus velezensis*, Mighty Mycorrhizae, Spectrum DS, Spectrum Myco, Tribus Original, and Tribus Continuum, respectively. Different letters within a column of the same cultivar suggest significant difference among microbial biostimulants indicated by Tukey's HSD test at $p < 0.05$.

Table 6. Biomass parameters of onion seedlings of three different cultivars treated with microbial biostimulants and Kelpak seaweed extract.

Cultivar	Biostimulant	Shoot FW (g)	Shoot DW (mg)	Root FW (g)	Root DW (mg)	Root/Shoot
Red	Control	1.03 ± 0.02 abcd	69.12 ± 2.36 bcd	0.35 ± 0.01 abc	18.07 ± 0.63 bc	26.23 ± 0.90 ab
	LALMyco	1.11 ± 0.06 abc	75.20 ± 2.97 abc	0.35 ± 0.01 abc	18.09 ± 0.39 bc	24.17 ± 0.64 b
	LALb	0.95 ± 0.02 bcd	67.67 ± 2.04 bcd	0.34 ± 0.01 bc	17.74 ± 0.59 bc	26.29 ± 0.95 ab
	MightyMyco	1.16 ± 0.05 ab	86.33 ± 4.82 a	0.41 ± 0.03 a	23.21 ± 1.94 a	27.05 ± 2.14 ab
	MycoApply	0.84 ± 0.04 d	54.86 ± 3.07 d	0.28 ± 0.01 c	15.16 ± 0.81 c	27.76 ± 1.17 ab
	SpcDS	0.91 ± 0.04 cd	62.16 ± 2.73 cd	0.33 ± 0.01 abc	18.18 ± 0.98 bc	29.28 ± 1.23 a
	SpcMyco	1.24 ± 0.07 a	79.50 ± 4.43 ab	0.39 ± 0.02 ab	20.67 ± 1.19 ab	26.04 ± 0.78 ab
	Spctrum	1.08 ± 0.04 abc	72.48 ± 3.91 abc	0.39 ± 0.02 ab	19.97 ± 0.86 ab	27.68 ± 0.81 ab
	TribusO	1.12 ± 0.04 abc	75.53 ± 2.35 abc	0.39 ± 0.01 ab	20.11 ± 0.71 ab	26.67 ± 0.84 ab
	TribusC	1.15 ± 0.07 ab	78.18 ± 3.35 ab	0.38 ± 0.01 ab	20.62 ± 0.67 ab	26.45 ± 0.48 ab
	Mean	1.06 ± 0.02	72.10 ± 1.48	0.36 ± 0.01	19.18 ± 0.39	26.76 ± 0.36

Table 6. *Cont.*

Cultivar	Biostimulant	Shoot FW (g)	Shoot DW (mg)	Root FW (g)	Root DW (mg)	Root/Shoot
White	Control	1.11 ± 0.07 ab	71.98 ± 5.66 ab	0.33 ± 0.02 abc	17.05 ± 0.89 bc	23.99 ± 0.98
	LALMyco	1.13 ± 0.04 ab	77.56 ± 3.17 ab	0.32 ± 0.02 abc	17.33 ± 0.44 bc	22.44 ± 0.53
	LALb	0.91 ± 0.03 bc	64.48 ± 2.81 bc	0.30 ± 0.01 c	15.75 ± 0.30 c	24.70 ± 1.33
	MightyMyco	1.17 ± 0.07 ab	78.29 ± 3.91 ab	0.34 ± 0.01 abc	18.16 ± 0.25 abc	23.42 ± 0.97
	MycoApply	0.81 ± 0.03 c	49.13 ± 2.50 c	0.23 ± 0.01 d	11.63 ± 0.34 d	23.82 ± 0.59
	SpcDS	1.30 ± 0.06 a	85.08 ± 3.48 a	0.39 ± 0.01 a	20.51 ± 0.59 a	24.21 ± 0.74
	SpcMyco	1.24 ± 0.03 a	83.01 ± 0.79 a	0.37 ± 0.02 ab	19.30 ± 0.43 ab	23.27 ± 0.60
	Spctrum	1.17 ± 0.03 ab	75.65 ± 3.46 ab	0.32 ± 0.01 bc	17.73 ± 0.62 bc	23.57 ± 0.86
	TribusO	1.06 ± 0.03 abc	71.10 ± 0.95 ab	0.33 ± 0.02 abc	16.63 ± 0.59 c	23.40 ± 0.86
	TribusC	1.10 ± 0.12 ab	72.03 ± 6.83 ab	0.32 ± 0.01 abc	16.89 ± 0.54 bc	24.32 ± 1.95
	Mean	1.10 ± 0.02	72.83 ± 1.68	0.32 ± 0.01	17.10 ± 0.33	23.71 ± 0.31
Yellow	Control	1.27 ± 0.05 a	82.15 ± 4.60 a	0.43 ± 0.02 a	21.12 ± 0.72 a	25.87 ± 0.63 c
	LALMyco	1.03 ± 0.04 bcd	69.23 ± 2.77 ab	0.39 ± 0.01 a	19.22 ± 0.54 ab	27.91 ± 1.01 bc
	LALb	0.91 ± 0.04 cd	61.70 ± 3.37 bcd	0.37 ± 0.01 ab	18.65 ± 0.45 ab	30.50 ± 1.03 ab
	MightyMyco	1.01 ± 0.06 bcd	69.13 ± 4.12 ab	0.39 ± 0.02 a	20.05 ± 1.11 ab	29.07 ± 0.59 abc
	MycoApply	0.85 ± 0.03 d	50.73 ± 2.79 d	0.30 ± 0.01 b	14.06 ± 0.50 c	27.89 ± 0.82 bc
	SpcDS	0.85 ± 0.02 d	52.99 ± 1.36 cd	0.36 ± 0.01 ab	17.39 ± 0.28 b	32.92 ± 0.95 a
	SpcMyco	1.13 ± 0.03 ab	71.69 ± 1.85 ab	0.40 ± 0.02 a	20.92 ± 1.10 a	29.23 ± 1.51 abc
	Spctrum	0.99 ± 0.03 bcd	64.92 ± 1.23 bc	0.39 ± 0.02 a	20.06 ± 0.50 ab	30.91 ± 0.70 ab
	TribusO	1.06 ± 0.02 bc	70.55 ± 2.45 ab	0.43 ± 0.01 a	20.59 ± 0.74 a	29.23 ± 0.80 abc
	TribusC	1.04 ± 0.05 bc	68.53 ± 2.02 b	0.39 ± 0.01 a	19.93 ± 0.30 ab	29.20 ± 0.92 abc
	Mean	1.02 ± 0.02	66.16 ± 1.41	0.39 ± 0.01	19.20 ± 0.33	29.27 ± 0.36
Overall mean		1.06 ± 0.01	70.36 ± 0.91	0.36 ± 0.00	18.49 ± 0.21	26.58 ± 0.26

Red, White, and Yellow represent onion cultivar Sofire, Carta Blanca, and Don Victoro, respectively. LALMyco, LALb, MightyMyco, SpcDS, SpcMyco, TribusO, and TribusC represent LALRISE Mycorrhizae, LALRISE *Bacillus velezensis*, Mighty Mycorrhizae, Spectrum DS, Spectrum Myco, Tribus Original, and Tribus Continuum, respectively. Different letters within a column of the same cultivar suggest significant difference among microbial biostimulants indicated by Tukey's HSD test at $p < 0.05$.

3.4. Root Morphology

Root length, root area, average root diameter, and root volume are presented in Tables 7 and 8, to distinguish between treatments without and with the application of seaweed extract, respectively. Without seaweed extract application, microbial biostimulants either had no effect on root length or even decreased it. The reduction in root length in microbial biostimulant-treated groups could potentially be attributed to competition for nutrients between the onion seedlings and the establishing microbial communities [37]. However, the trend changed when seaweed extract was applied. In the white onion cultivar, Tribus Continuum increased root length by 17% compared to the control. These results agreed with previous research suggesting that the combination of microbial biostimulants (*Bacillus* sp. and *Pseudomonas* sp.) and Kelpak seaweed extract has a more positive effect on *Amaranthus hybridus* root length compared to the sole application of either microbial biostimulants or Kelpak [42]. Microbial biostimulants' influence on root area varied with cultivar and seaweed extract application. Without seaweed extract, biostimulants generally had no effect or even decreased root area, with the exception that Mighty Mycorrhizae, which resulted in the largest root area among the biostimulants in the yellow cultivar. When seaweed extract was included, the results became more positive. In the red cultivar, Spectrum Myco yielded the highest root area. In the white cultivar, several microbial biostimulants, including Mighty Mycorrhizae, Spectrum DS, Spectrum Myco, and Tribus Continuum, showed the highest root area numerically. The highest average root diameter across cultivars was observed in treatments with microbial biostimulants, except for the yellow cultivar with seaweed extract application. Notably, LALRISE Mycorrhizae resulted in the highest average root diameter in all cultivars without seaweed extract. When seaweed extract was included, Spectrum and Tribus Original showed the highest average root diameter in the red cultivar, while LALRISE Mycorrhizae and Spectrum Myco resulted in the highest values in the white cultivar. When it comes to root volume, some mycorrhizal biostimulants achieved the highest values among microbial biostimulants. Specifically, Mighty Mycorrhizae led to the highest root volume in the yellow cultivar without seaweed extract application. Additionally, Spectrum Myco yielded the highest root volume in both the red and white cultivars when seaweed extract was included. Regarding the effect of seaweed extract, it decreased the root length by 2% and increased the average root diameter by 1%, but it did not affect the root area and root volume (Tables 2, 7 and 8). The effects of combining seaweed extract and microbial biostimulants on root morphology were similar to the trends observed in biomass parameters, although the difference compared to the control group was less significant. Specifically, Tribus Continuum, LALRISE Mycorrhizae, Mighty Mycorrhizae, Spectrum Myco, Spectrum, Spectrum DS, and Tribus Original showed the potential to enhance root growth compared to other microbial biostimulants.

Table 7. Root morphology of onion seedlings of three different cultivars treated with microbial biostimulants without Kelpak seaweed extract.

Cultivar	Biostimulant	Length (cm)	Area (cm^2)	AvgDiam (mm)	RootVolume (cm^3)
Red	Control	64.14 ± 1.82 a	5.07 ± 0.19 a	0.79 ± 0.02 abc	0.32 ± 0.02 a
	LALMyco	55.83 ± 2.35 abc	4.74 ± 0.16 ab	0.86 ± 0.02 a	0.32 ± 0.01 a
	LALb	56.67 ± 1.40 abc	4.36 ± 0.09 b	0.77 ± 0.01 bc	0.26 ± 0.01 b
	MightyMyco	54.82 ± 2.08 bc	4.37 ± 0.14 b	0.80 ± 0.02 abc	0.28 ± 0.01 ab
	MycoApply	51.62 ± 1.54 c	3.35 ± 0.12 c	0.65 ± 0.02 d	0.17 ± 0.01 c
	SpcDS	56.53 ± 2.12 abc	4.27 ± 0.15 b	0.76 ± 0.02 bc	0.26 ± 0.01 b
	SpcMyco	52.98 ± 1.81 bc	4.18 ± 0.09 b	0.80 ± 0.02 abc	0.26 ± 0.01 b
	Spctrum	57.90 ± 1.94 abc	4.59 ± 0.12 ab	0.80 ± 0.02 abc	0.29 ± 0.01 ab
	TribusO	53.60 ± 2.03 bc	4.38 ± 0.11 b	0.83 ± 0.02 ab	0.28 ± 0.01 ab
	TribusC	61.52 ± 2.38 ab	4.41 ± 0.11 b	0.73 ± 0.03 cd	0.25 ± 0.01 b
	Mean	56.56 ± 0.66	4.37 ± 0.05	0.78 ± 0.01	0.27 ± 0.00
White	Control	66.07 ± 1.97 a	4.52 ± 0.13 a	0.69 ± 0.02 ab	0.25 ± 0.01 a
	LALMyco	56.15 ± 1.78 b	4.21 ± 0.11 abc	0.76 ± 0.02 a	0.25 ± 0.01 a
	LALb	55.41 ± 1.67 b	4.04 ± 0.11 abc	0.73 ± 0.02 ab	0.23 ± 0.01 a
	MightyMyco	54.61 ± 1.51 b	3.93 ± 0.10 bc	0.72 ± 0.02 ab	0.22 ± 0.01 a
	MycoApply	51.92 ± 1.79 b	3.38 ± 0.12 d	0.66 ± 0.02 b	0.18 ± 0.01 b
	SpcDS	54.04 ± 2.18 b	3.84 ± 0.10 cd	0.72 ± 0.02 ab	0.22 ± 0.01 ab
	SpcMyco	54.00 ± 1.37 b	4.01 ± 0.11 abc	0.74 ± 0.02 a	0.24 ± 0.01 a
	Spctrum	59.84 ± 2.38 ab	4.42 ± 0.17 ab	0.74 ± 0.02 a	0.26 ± 0.01 a
	TribusO	54.56 ± 1.66 b	3.87 ± 0.12 cd	0.71 ± 0.02 ab	0.22 ± 0.01 ab
	TribusC	56.60 ± 1.67 b	4.10 ± 0.10 abc	0.73 ± 0.01 ab	0.23 ± 0.01 a
	Mean	56.32 ± 0.62	4.03 ± 0.04	0.72 ± 0.01	0.23 ± 0.00
Yellow	Control	62.22 ± 1.99 a	4.98 ± 0.20 ab	0.80 ± 0.01 abcd	0.31 ± 0.02 ab
	LALMyco	57.06 ± 2.49 ab	4.89 ± 0.14 abc	0.87 ± 0.02 a	0.33 ± 0.01 ab
	LALb	57.05 ± 2.32 ab	4.71 ± 0.18 abc	0.83 ± 0.01 ab	0.31 ± 0.01 abc
	MightyMyco	60.58 ± 1.49 a	5.16 ± 0.13 a	0.85 ± 0.02 ab	0.35 ± 0.01 a
	MycoApply	51.74 ± 1.16 b	3.74 ± 0.08 d	0.73 ± 0.02 d	0.21 ± 0.01 d
	SpcDS	61.61 ± 2.01 a	4.91 ± 0.15 abc	0.80 ± 0.02 abcd	0.31 ± 0.01 ab
	SpcMyco	61.22 ± 1.46 a	5.04 ± 0.14 ab	0.83 ± 0.02 abc	0.33 ± 0.02 ab
	Spctrum	61.68 ± 1.55 a	4.77 ± 0.11 abc	0.78 ± 0.02 bcd	0.29 ± 0.01 abc
	TribusO	56.34 ± 1.62 ab	4.44 ± 0.12 bc	0.79 ± 0.02 abcd	0.28 ± 0.01 bc
	TribusC	58.06 ± 1.60 ab	4.28 ± 0.10 cd	0.74 ± 0.02 cd	0.25 ± 0.01 cd
	Mean	58.76 ± 0.60	4.69 ± 0.05	0.80 ± 0.01	0.30 ± 0.00
Total Mean		57.21 ± 0.37	4.36 ± 0.03	0.77 ± 0.00	0.27 ± 0.00

Red, White, and Yellow represent onion cultivar Sofire, Carta Blanca, and Don Victoro, respectively. LALMyco, LALb, MightyMyco, SpcDS, SpcMyco, TribusO, and TribusC represent LALRISE Mycorrhizae, LALRISE *Bacillus velezensis*, Mighty Mycorrhizae, Spectrum DS, Spectrum Myco, Tribus Original, and Tribus Continuum, respectively. Length, Area, AvgDiam, and RootVolume represent total root length, root area, average root diameter, and root volume, respectively. Different letters within a column of the same cultivar suggest significant difference among microbial biostimulants indicated by Tukey's HSD test at $p < 0.05$.

Table 8. Root morphology of onion seedlings of three different cultivars treated with microbial biostimulants and Kelpak seaweed extract.

Cultivar	Biostimulant	Length (cm)	Area (cm^2)	AvgDiam (mm)	RootVolume (cm^3)
Red	Control	55.09 ± 1.47 ab	4.48 ± 0.14 abc	0.81 ± 0.02 ab	0.29 ± 0.01 ab
	LALMyco	56.79 ± 1.50 ab	4.46 ± 0.11 abc	0.79 ± 0.02 ab	0.28 ± 0.01 abc
	LALb	52.93 ± 1.96 b	4.12 ± 0.08 bc	0.79 ± 0.02 ab	0.26 ± 0.01 abc
	MightyMyco	56.30 ± 1.79 ab	4.42 ± 0.11 abc	0.79 ± 0.02 ab	0.27 ± 0.01 abc
	MycoApply	55.07 ± 1.57 ab	3.97 ± 0.11 c	0.73 ± 0.02 b	0.23 ± 0.01 c
	SpcDS	52.89 ± 2.25 b	4.08 ± 0.13 bc	0.78 ± 0.02 ab	0.25 ± 0.01 bc
	SpcMyco	62.00 ± 2.00 a	4.90 ± 0.14 a	0.80 ± 0.02 ab	0.31 ± 0.01 a
	Spctrum	51.66 ± 1.73 b	4.28 ± 0.12 bc	0.83 ± 0.02 a	0.28 ± 0.01 ab
	TribusO	55.62 ± 1.93 ab	4.54 ± 0.11 ab	0.83 ± 0.03 a	0.30 ± 0.01 ab
	TribusC	57.96 ± 1.51 ab	4.60 ± 0.14 ab	0.80 ± 0.03 ab	0.29 ± 0.02 ab
	Mean	55.63 ± 0.59	4.39 ± 0.04	0.80 ± 0.01	0.28 ± 0.00
White	Control	52.83 ± 1.22 bc	3.96 ± 0.09 ab	0.75 ± 0.02 ab	0.24 ± 0.01 abc
	LALMyco	52.13 ± 1.66 c	4.03 ± 0.11 ab	0.78 ± 0.02 a	0.25 ± 0.01 abc
	LALb	54.30 ± 1.56 bc	3.87 ± 0.08 b	0.72 ± 0.01 ab	0.22 ± 0.01 c
	MightyMyco	58.87 ± 1.62 abc	4.41 ± 0.09 a	0.75 ± 0.01 ab	0.26 ± 0.01 ab
	MycoApply	51.69 ± 1.92 c	3.18 ± 0.14 c	0.62 ± 0.02 c	0.16 ± 0.01 d
	SpcDS	59.51 ± 1.39 ab	4.38 ± 0.09 a	0.74 ± 0.02 ab	0.26 ± 0.01 abc
	SpcMyco	56.94 ± 1.85 abc	4.44 ± 0.14 a	0.78 ± 0.02 a	0.27 ± 0.01 a
	Spctrum	58.25 ± 1.66 abc	4.10 ± 0.14 ab	0.70 ± 0.02 b	0.23 ± 0.01 bc
	TribusO	56.57 ± 1.42 abc	4.06 ± 0.11 ab	0.72 ± 0.02 ab	0.23 ± 0.01 bc
	TribusC	61.78 ± 1.53 a	4.39 ± 0.11 a	0.71 ± 0.01 ab	0.25 ± 0.01 abc
	Mean	56.29 ± 0.54	4.08 ± 0.04	0.73 ± 0.01	0.24 ± 0.00
Yellow	Control	58.01 ± 1.86	5.26 ± 0.24 a	0.91 ± 0.03 a	0.38 ± 0.03 a
	LALMyco	59.45 ± 1.30	4.75 ± 0.10 ab	0.80 ± 0.01 bc	0.30 ± 0.01 b
	LALb	52.88 ± 1.76	4.24 ± 0.11 bc	0.81 ± 0.02 b	0.27 ± 0.01 bc
	MightyMyco	54.66 ± 1.82	4.26 ± 0.09 bc	0.79 ± 0.02 bc	0.26 ± 0.01 bc
	MycoApply	55.56 ± 1.53	3.97 ± 0.10 c	0.72 ± 0.02 c	0.23 ± 0.01 c
	SpcDS	52.85 ± 1.41	4.45 ± 0.12 bc	0.85 ± 0.02 ab	0.30 ± 0.01 b
	SpcMyco	57.66 ± 1.59	4.59 ± 0.15 b	0.80 ± 0.02 bc	0.29 ± 0.01 b
	Spctrum	54.56 ± 1.71	4.56 ± 0.14 bc	0.84 ± 0.02 ab	0.30 ± 0.01 b
	TribusO	59.64 ± 2.05	4.85 ± 0.11 ab	0.82 ± 0.02 ab	0.31 ± 0.01 b
	TribusC	59.98 ± 2.04	4.74 ± 0.14 ab	0.80 ± 0.02 bc	0.30 ± 0.01 b
	Mean	56.53 ± 0.56	4.57 ± 0.05	0.81 ± 0.01	0.29 ± 0.00
Total mean		56.15 ± 0.33	4.35 ± 0.03	0.78 ± 0.00	0.27 ± 0.00

Red, White, and Yellow represent onion cultivar Sofire, Carta Blanca, and Don Victoro, respectively. LALMyco, LALb, MightyMyco, SpcDS, SpcMyco, TribusO, and TribusC represent LALRISE Mycorrhizae, LALRISE *Bacillus velezensis*, Mighty Mycorrhizae, Spectrum DS, Spectrum Myco, Tribus Original, and Tribus Continuum, respectively. Length, Area, AvgDiam, and RootVolume represent total root length, root area, average root diameter, and root volume, respectively. Different letters within a column of the same cultivar suggest significant difference among microbial biostimulants indicated by Tukey's HSD test at $p < 0.05$.

3.5. Synergistic Effects of Microbial Biostimulants and Kelpak Seaweed Extract

Summarizing the shoot morphology, biomass, and root morphology results, it can be found that when applied alone, microbial biostimulants increased plant height and the root/shoot ratio compared to the control. However, the combined application of microbial biostimulants and Kelpak seaweed extract resulted in further improvements in plant height, leaf area, shoot DW, root DW, root/shoot ratio, and root length, compared to the control. Moreover, the interaction between microbial biostimulants and seaweed extract application was significant for shoot and root DW (Table 2). An enhanced synergistic impact was found on LALRISE Mycorrhizae, Mighty Mycorrhizae, and Spectrum DS when applied alongside seaweed extract. Studies on cucumber and tomato crops have also shown similar synergistic effects when combining microbial biostimulants like mycorrhizal fungi and *Trichoderma* spp. with seaweed extracts derived from *Ascophyllum* sp. and *Macrocytis* sp. [43,44]. The polysaccharides in seaweed extract can serve as a nutrient source for microbial biostimulants [45], which might be one of the reasons of the synergistic effects. Therefore, to amplify the positive effects of microbial biostimulants and Kelpak seaweed extract on onion growth, a combined application is recommended.

3.6. Principal Component Analysis (PCA)

To visualize the morphology and biomass parameters in two dimensions, we performed principal component analysis (PCA). The results, presented in Figures 2–4, respectively, separate data points based on the level of seaweed extract application, the type of microbial biostimulants, and the onion cultivars. The principal components (PC1 and PC2) explained a total of 55.7% of the variance in morphology and biomass parameters. The PCA biplot (Figures 2–4) reveals a spatial separation between root and shoot growth parameters. Most root growth-related parameters, including root FW, root DW, root length, root area, root volume, and average root diameter, clustered in the second quadrant, while shoot growth-related parameters, including shoot FW, shoot DW, leaf area, and plant height, tended towards the third quadrant. This suggests a positive correlation between PC2 (*y*-axis) and root growth-related parameters, and a negative correlation between PC2 and most shoot growth-related parameters. PC1, represented on the *x*-axis, exhibits high negative loadings on several biomass variables, including shoot FW, shoot DW, root FW, and root DW. Additionally, PC1 shows high negative loadings on root and shoot morphology variables like root volume, root area, and leaf area. Figures 2–4 utilize large colored data points to represent the average values for each level of the independent variables (onion cultivar, Kelpak seaweed extract, and microbial biostimulants).

Figure 2. Principle component analysis (PCA) biplot of morphology and biomass parameters of onion seedlings without seaweed extract application (No) or with seaweed extract application (Yes). Root.shoot, FW, and DW represent root-to-shoot dry-weight ratio, fresh weight, and dry weight, respectively.

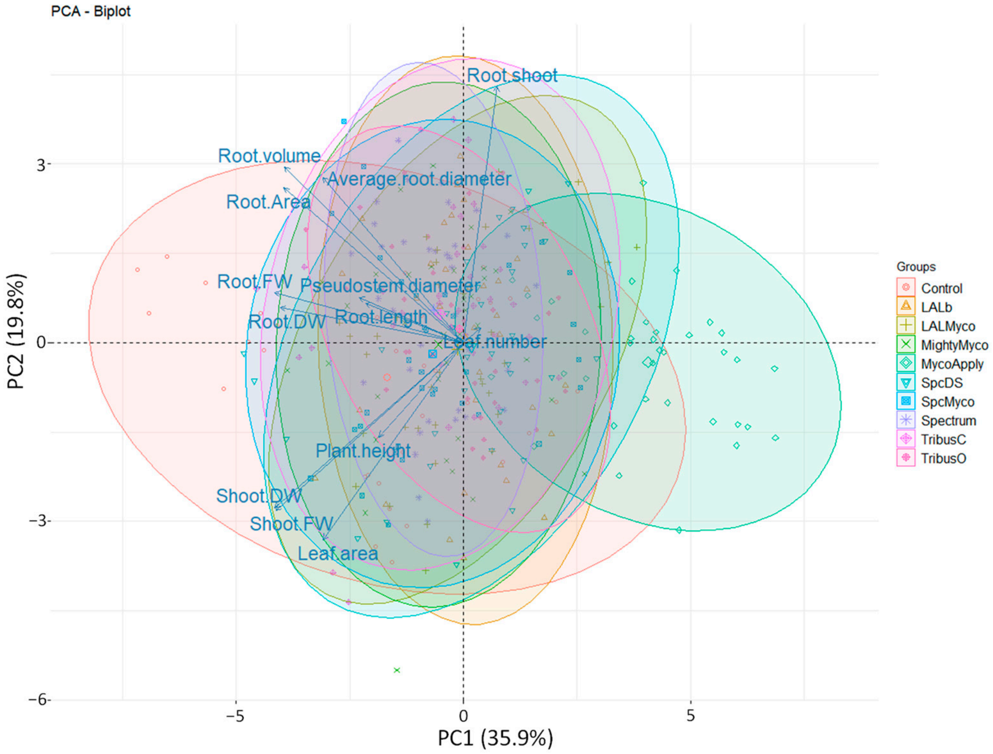

Figure 3. Principle component analysis (PCA) biplot of morphology and biomass parameters of onion seedlings treated with different microbial biostimulants. LALMyco, LALb, MightyMyco, SpcDS, SpcMyco, TribusO, and TribusC represent LALRISE Mycorrhizae, LALRISE Bacillus velezensis, Mighty Mycorrhizae, Spectrum DS, Spectrum Myco, Tribus Original, and Tribus Continuum, respectively. Root.shoot, FW, and DW represent root-to-shoot dry-weight ratio, fresh weight, and dry weight, respectively.

In Figure 2, for instance, the large-red-circle and large-green-triangle points near the origin represent the average results for treatments with and without seaweed extract application, respectively. While Figure 2 does not show distinct clusters separating data points based on situations with or without seaweed extract application, the position of the larger average points suggests that seaweed extract increased shoot growth in onion seedlings compared to the control group, with minimal impact on root growth. Notably, our study utilized subirrigation, where the seaweed extract was applied to the substrate instead of the foliage. Despite this difference in the application method, our findings align with previous tomato research, where the foliar application of seaweed extract also showed a more significant impact on shoot growth compared to root growth [46]. This consistent response across application methods suggests a strong overall benefit of seaweed extract on plant shoot growth compared to root growth.

In Figure 3, the overlapping data points for microbial biostimulants indicate that pooling data across different cultivars and seaweed extract applications hinders the effective separation of variance among the microbial biostimulants. It can be found that the control group exhibited a lower root/shoot ratio compared to all microbial biostimulants. Looking at the positive extent of PC1 (x-axis), however, MycoApply stands out as it reduced both shoot and root growth in onion seedlings due to the high application rate, as discussed previously.

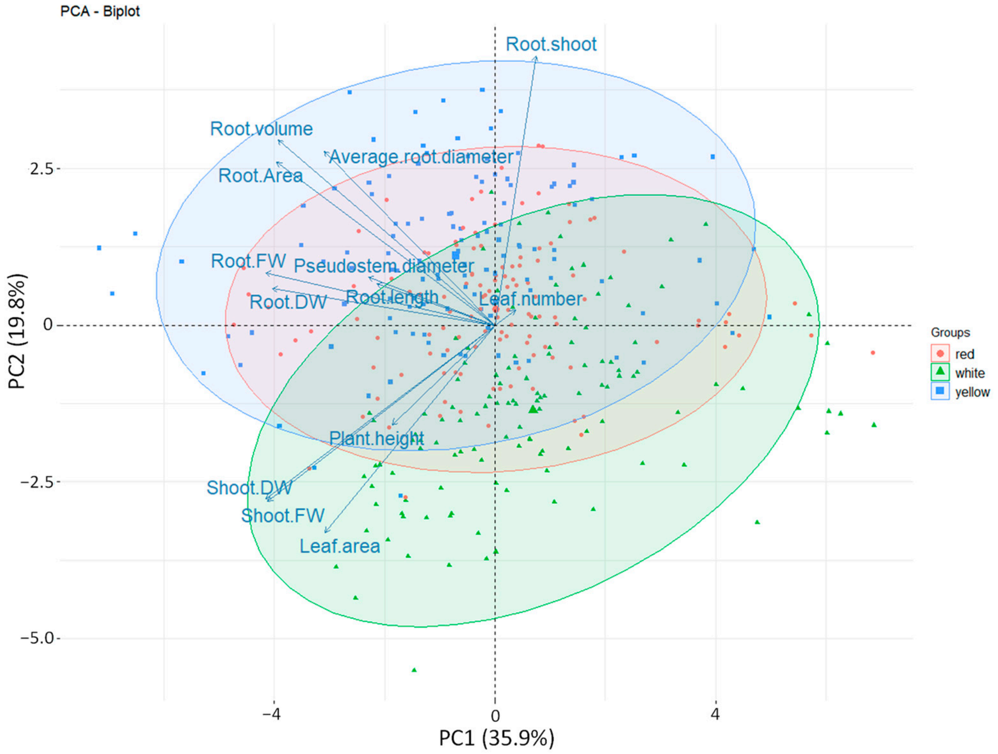

Figure 4. Principle component analysis (PCA) biplot of morphology and biomass parameters in different onion cultivars. Red refers to onion cultivar Sofire, white refers to onion cultivar Carta Blanca, and yellow refers to onion cultivar Don Victoro. Root.shoot, FW, and DW represent root-to-shoot dry-weight ratio, fresh weight, and dry weight, respectively.

Figure 4 highlights a more substantial influence of onion cultivar on morphology and biomass parameters compared to seaweed extract application (Figure 2) and microbial biostimulants (Figure 3). This is evident from the well-separated clusters of data points representing the different cultivars in the PCA biplot. Notably, the separation between cultivars appears to be driven primarily by root growth-related parameters. The yellow cultivar clusters in a region associated with the best root growth, while the white cultivar clusters in a region associated with the poorest root growth. The red cultivar occupies an intermediate position on the PCA biplot, suggesting root growth performance between the other two cultivars.

4. Conclusions

This study investigated the effects of nine microbial biostimulant products and one non-microbial biostimulant product made from seaweed extract, and their interactions, on the germination, morphological, and biomass parameters of three different cultivars of onion seedlings. We observed substantial cultivar-dependent differences in these parameters, regardless of biostimulant treatment. Several microbial biostimulants (LALRISE Mycorrhizae, Might Mycorrhizae, Spectrum DS, Spectrum Myco, Spectrum, and Tribus Continuum), along with the seaweed extract (Kelpak), showed positive effects on onion seedling growth in certain cultivars. Combining seaweed extract and microbial biostimulants resulted in even more pronounced positive effects on onion seedling growth. Based on these findings, Kelpak, the six microbial biostimulants mentioned above, and their combinations warrant further investigation in terms of their effects onion plant growth. To gain a more comprehensive understanding of these biostimulant products' impact, further investigations are necessary. This includes evaluating their long-term effects on yield and quality after transplanting seedlings to field conditions. Additionally, characterizing plant physiological responses and the mineral profiles of onion plants and bulbs will provide insights into the biostimulants' mode of action, ultimately leading to a more targeted application.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/horticulturae10080800/s1, Table S1: Detailed ingredients of biostimulant products.

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